

T.R.
GEBZE TECHNICAL UNIVERSITY
GRADUATE SCHOOL

ISOLATION, PURIFICATION AND CHARACTERIZATION OF
NITROGEN-FIXING BACTERIA FROM POTATO
RHIZOSPHERE SOILS IN TÜRKİYE

SENA USTA

A THESIS OF MASTER OF SCIENCE
DEPARTMENT OF MOLECULAR BIOLOGY AND GENETICS

ADVISOR: ASSOC. PROF. DR. MİNE GÜL ŞEKER

JULY 2025

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T.C.
GEBZE TEKNİK ÜNİVERSİTESİ
LİSANSÜSTÜ EĞİTİM ENSTİTÜSÜ

TÜRKİYE'DEKİ PATATES RİZOSFER TOPRAKLARINDAN
AZOT BAĞLAYICI BAKTERİLERİN İZOLE EDİLMESİ,
SAFLAŞTIRILMASI VE KARAKTERİZASYONU

SENA USTA

YÜKSEK LİSANS TEZİ
MOLEKÜLER BİYOLOJİ VE GENETİK
ANABİLİM DALI

DANIŞMAN: DOÇ. DR. MİNE GÜL ŞEKER

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MASTER of SCIENCE/ JURY APPROVAL FORM

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SIGNATURE/SEAL



*I dedicate this thesis to myself
and my family, my greatest
supporters.*

ABSTRACT

With the Green Revolution, the continuous increase in human population and food demand has transformed agriculture into a global industry, leading to the widespread use of chemical fertilizers and pesticides to improve product yield and quality. However, the excessive use of chemical fertilizers has led to serious environmental issues, including soil degradation, water pollution, and greenhouse gas emissions. As a sustainable alternative, biofertilizers offer a promising solution to reduce environmental impact of biofertilizers while maintaining crop productivity.

In this study, a total of 45 soil samples were collected from the rhizosphere of potato plants across major potato-growing regions in Türkiye. From these samples, 542 bacterial colonies were isolated using serial dilution and spread plate methods, and selected based on distinct morphological characteristics such as pigmentations, surface, and etc. The isolates were screened on nitrogen-free bromothymol blue (NFB) agar medium to identify potential nitrogen-fixing bacteria, resulting in the selection of approximately 150 promising candidates. These were further tested using Nessler's reagent to detect and quantify ammonium (NH_4^+) production, and 30 isolates which reached to highest ammonium levels were selected for detailed characterization.

Based on 16S rRNA gene sequence analysis, the isolates were identified as belonging to several genera including *Pseudomonas*, *Acinetobacter*, *Stutzerimonas*, *Enterobacter*, and *Citrobacter*. These taxa are known to inhabit plant rhizospheres and include strains with potential nitrogen-fixation and plant growth-promoting traits, supporting their relevance for further characterization.

For further characterization biochemical tests were conducted to assess the metabolic properties of the 30 selected isolates and 16S rRNA gene sequencing is carried out for these 30 isolates.

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Keywords: Potato rhizosphere, nitrogen fixation, soil bacteria, characterization

ÖZET

Yeşil Devrim ile, nüfusun ve gıda talebinin hızla artması, tarımı küresel bir endüstriye dönüştürmüş ve kimyasal gübreler ile pestisitlerin yaygın şekilde kullanımına yol açmıştır. Ancak, kimyasal gübrelerin aşırı kullanımı, toprak bozulması, su kirliliği ve sera gazı emisyonları gibi ciddi çevresel sorunlara neden olmuştur. Sürdürülebilir bir alternatif olarak biyogübreler, çevresel etkiyi azaltırken ürün verimliliğini korumak için umut verici bir çözüm sunmaktadır.

Bu çalışmada, Türkiye'nin başlıca patates yetiştirme bölgelerinden, patates bitkilerinin rizosferinden toplam 45 toprak örneği toplanmıştır. Bu örneklerden, seri sulandırma ve yayma plakası yöntemleri kullanılarak toplam 542 bakteriyel koloni izole edilmiş ve belirgin morfolojik özelliklerine göre seçilmiştir. İzolatlar, potansiyel azot fikseden bakterileri belirlemek amacıyla azot içermeyen bromotimol mavili (NFB) agar ortamında taranmış ve yaklaşık 150 umut verici izolat seçilmiştir. Bu izolatlar daha sonra Nessler reaktifi kullanılarak amonyum (NH_4^+) üretimi açısından test edilmiş ve en yüksek amonyum seviyesine sahip 30 izolat detaylı karakterizasyon için seçilmiştir. Seçilen 30 izolatın metabolik özelliklerini değerlendirmek amacıyla biyokimyasal testler yapılmıştır. Bu 30 izolatlar tür düzeyinde tanımlanmak üzere 16S rRNA gen dizilemesine gönderilmiştir.

16 S rRNA gen dizi analizleri sonucunda izolatların *Pseudomonas*, *Acinetobacter*, *Stutzerimonas*, *Enterobacter* ve *Citrobacter* cinslerine ait olduğu belirlenmiştir. Bu grupların rizodferde yaygın olarak bulunması ve bitki gelişimini destekleyici potansiyele sahip olmaları çalışmanın amacı destekler niteliktedir.

Anahtar Kelimeler: Patates rizosferi, azot fiksasyonu, toprak bakterisi, karakterizasyon

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LIST OF SYMBOLS AND ABBREVIATIONS

%	: Percentage
°C	: Centigrade Degree
µg	: Microgram
µl	: Microliter
µm	: Micrometer
µM	: Micromolar
A	: Absorbance
bp	: base pair
DNA	: Deoxyribonucleic Acid
EDTA	: Ethylene Diamine Tetraacetic Acid
g	: Gram
h	: hour
HCl	: Hydrogen chloride
Kg	: Kilogram
KOH	: Potassium hydroxide
M	: Molar
m	: minute
mg	: Miligram
MJ	: Megajoule
ml	: Mililitre
mM	: Milimolar
MR	: Methyl Red
Mt	: Million ton
NaCl	: Sodium chloride
NFB	: Nitrogen-free medium
ng	: Nanogram
nm	: Nanometer
ONPG	: o-nitrophenyl-β-D-galactopyranoside
PBS:	: Phosphate Buffered Solution
PCR	: Polymerase Chain Reaction
Pg	: Picogram
Pg	: Picogram
PGP	: Plant Growth Promoting
pH	: Power of hydrogen
rDNA	: Ribosomal Deoxyribonucleic Acid
rpm	: Rotation per minute
TAE	: Tris Acetate EDTA
V	: Volt
VP	: Voges-Proskauer
w/v	: Weight/Volume
x g	: Times the acceleration due to gravity

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1. INTRODUCTION

The global demand for food is increasing more rapidly than many agricultural systems can provide. To maintain productivity, cultivators today utilize significantly greater quantities of synthetic fertilizers, particularly those comprising nitrogen (N), phosphorus (P), and potassium (K). From 1961 to 2020, global utilization of nitrogen fertilizers increased nearly tenfold [Jelliffe et al., 2024]. Crops generally utilize only around one-third of the nitrogen that is applied; the remainder is lost through runoff, leaching, volatilization, or denitrification, which pollutes the air and water and raises greenhouse gas emissions [Liu et al., 2024]. The energy-intensive Haber-Bosch process used to make these fertilizers also releases a significant amount of CO₂ [Krietsch et al., 2019]. These losses increase expenses and have an adverse effect on the environment.

According to Zhang et al. (2017), potatoes (*Solanum tuberosum* L.), the fourth most important food crop in terms of calories provided for human consumption after rice, wheat, and maize, are extremely sensitive to nutrient balance. According to field research, in some regions, like the Ethiopian highlands, adding nitrogen can more than double tuber yield; however, too much nitrogen degrades quality and encourages nitrate leaching [Craswell et al., 2021]. Turkey is currently one of the top fifteen producers of potatoes due to its diverse climate [FAOSTAT, 2023]. In order to achieve the best yields, Turkish farmers usually apply 50–60 kg N da⁻¹ because of the shallow roots of potatoes and their early nitrogen requirements. This costly practice greatly increases off-farm nutrient losses [Çolakoğlu, 2016].

A sustainable approach involves utilizing biological agents that enhance or mobilize nutrients present in the soil. Plant-growth-promoting (PGP) microorganisms are promising options. Direct advantages include biological nitrogen fixation, solubilization of phosphate, zinc, and potassium, as well as the synthesis of growth hormones. PGB bacteria can indirectly chelate iron using siderophores, inhibit diseases, and degrade insecticides [Timofeeva et al., 2023]. Strains extracted from local soils are inherently adapted to particular conditions, hence they often endure and exhibit optimal performance when developed as biofertilizers.

Among the actions of PGPB, biological nitrogen fixation (BNF) provides the most evident pathway for diminishing synthetic nitrogen inputs. Only diazotrophic bacteria possess the nitrogenase enzyme, which transforms atmospheric N₂ into ammonia used by plants. Incorporating effective diazotrophs into non-leguminous crops such as potatoes could enhance nitrogen-use efficiency while reducing fertilizer expenses and emissions.

1.1. Aim of the Study

Maintaining potato (*Solanum tuberosum* L.) output while reducing dependence on synthetic NPK fertilizers requires alternatives that preserve yield without incurring long-term soil and water quality detriments. Numerous field and greenhouse investigations have demonstrated that biofertilizers—particularly diazotrophic plant-growth-promoting (PGPB) bacteria—can enhance tuber yields and reduce chemical nitrogen inputs by 30–50%, while also improving soil nutrient levels and water-use efficiency [Abdullah et al., 2020]. Although the concept of isolating PGPB strains from local potato production soils holds promise, it remains underexplored and represents a key area of novelty in our research. Diazotrophs, including *Azospirillum* spp. and *Bacillus licheniformis*, as well as associated rhizobacteria, not only fix atmospheric N₂ but also produce phytohormones and facilitate the mobilization of phosphate and potassium, hence improving crop performance under diminished fertilizer applications [Naqqash et al., 2022]. This study investigates a soil-centered approach for potato cultivation in Turkey, based on the existing facts. Soil samples will be obtained from five prominent potato-cultivating areas; nitrogen-fixing bacteria will be isolated on LB agar media, purified, and characterized visually and biochemically. Isolates will be evaluated using a two-step screening process: (i) qualitative assessment of NFb color change, and (ii) quantitative measurements of ammonium release (Nessler's reagent) The thirty highest scoring strains will thereafter undergo 16S rDNA sequencing for conclusive phylogenetic identification. This research seeks to find indigenous, high-efficiency diazotrophs adapted to local soils and climate, establishing the foundation for a region-specific biofertilizer that sustains potato yields while decreasing synthetic nitrogen inputs, hence reducing costs and pollution in Türkiye's primary potato-producing regions.

Within the present COST-linked MSc project, these native nitrogen-fixing isolates have been purified and banked as the core active ingredient of a prototype potato fertiliser. Ongoing work will optimize consortia composition and carrier formulation to cut synthetic N inputs without sacrificing yield, thereby aligning agronomic efficiency with environmental stewardship.



2. LITERATURE

2.1. Fertilizers

Synthetic nitrogen fertilisers (e.g., urea, NPK) supply modern potato systems, yet global field data show crops take up only about one-third of what is applied; the remainder is lost through volatilisation or leaching. Volatilised nitrogen is converted to nitrous oxide, a greenhouse gas with a 100-year global-warming potential 298 times that of CO₂ [Omara et al., 2019; Skiba et al., 2014]. Leached nitrate, on the other hand, contributes to groundwater contamination and aquatic eutrophication, posing serious environmental and public health risks. These losses not only waste costly inputs but also intensify pressure for stricter emission limits in Europe's intensive potato belts.

Biofertilizers formulated with PGP microbes offer a complementary strategy. Field studies demonstrate that replacing up to 70 % of mineral N with a bio-organic inoculum increased tuber yields by 10–155 % while improving nitrogen-use efficiency [Tan et al., 2024]. Crucially, performance depends on matching strains to the local crop and soil microbiome: a recent survey of Turkish potato rhizospheres recovered 59 indigenous diazotrophic isolates—mainly *Pseudomonas*, *Massilia* and *Variovorax*—each exhibiting multiple PGP traits, including acetylene-confirmed N₂ fixation [Devkota et al., 2024].

2.2. Nitrogen

Nitrogen constitutes around 78% of Earth's atmosphere, however its dinitrogen (N₂) form is chemically inert and inaccessible to most organisms [Mehta, 2021]. Despite this inaccessibility, every living cell relies on nitrogen to synthesize DNA and RNA, amino acid chains, chlorophyll, ATP, and numerous other metabolites that facilitate cell division, energy transfer, and genetic inheritance. Consequently, the ongoing provision of biologically available nitrogen supports the stability of natural ecosystems and the productivity of contemporary agriculture.

2.2.1. Natural Pools and Chemical Forms

Approximately 4 Pg of nitrogen exist in the atmosphere, while terrestrial soils contain about 150 Pg of organic matter and 0.5 Pg of inorganic nitrogen in the form of ammonium and nitrate [Battye et al., 2017]. Reactive nitrogen is also present in the hydrosphere (0.3 Pg of NO_3^-) and in terrestrial plant biomass (0.2 Pg). In well-aerated agricultural soils, nitrifying bacteria rapidly convert ammonium (NH_4^+) into nitrate (NO_3^-), making nitrate the dominant inorganic nitrogen form. In contrast, estuaries and aquaculture ponds tend to have more dissolved organic nitrogen, nitrite (NO_2^-), and gaseous forms like N_2O . Meanwhile, submerged paddy soils and acidic forest floors typically contain higher levels of ammonium and organic amides.

2.2.2. Biogeochemical Cycling

The atmospheric N_2 cannot be assimilated directly by plants, it must first be “fixed” into reactive compounds—mainly ammonia (NH_3) or its ionised form ammonium (NH_4^+). Nature performs most of this work: almost 90 % of global natural fixation is mediated by diazotrophic bacteria and cyanobacteria that convert N_2 to NH_3 in soils, root nodules and surface waters [Mehta, 2021]. Additional, smaller inputs arise from lightning and photochemical reactions in the atmosphere. Once reactive nitrogen enters ecosystems, it circulates through four key transformations known collectively as the nitrogen cycle. In ammonification, decomposer microbes release NH_4^+ from organic residues; nitrifying bacteria then oxidize NH_4^+ to nitrite (NO_2^-) and nitrate (NO_3^-); denitrifiers finally reduce NO_3^- back to N_2 or nitrous oxide (N_2O), completing the loop [Galloway et al., 2015].

2.2.3. Anthropogenic Perturbation

During the last century human activity added a new, powerful entry point to that cycle: the energy-intensive Haber–Bosch process, which produces synthetic fertiliser to sustain high-yield cropping. Current industrial output exceeds 180 Tg N yr^{-1} , roughly equal to all natural terrestrial fixation [Sutton et al., 2013]. While indispensable for feeding a growing population, large fertiliser surpluses now escape as ammonia volatilisation, nitrate leaching and N_2O emissions, driving soil acidification, water eutrophication and greenhouse-gas release [Anas et al., 2020]. These fertilisers have

greatly boosted food production, but they are costly to manufacture and often used inefficiently in the field; field studies show that in high-input crops such as sugar cane up to 60–80 % of the nitrogen applied can volatilise into the air or leach into water, wasting the money farmers spent on fertiliser while the escaped nitrogen fuels greenhouse-gas emissions, soil acidification and the eutrophication of rivers and lakes [Mariano et al., 2015]. Global fertiliser use already matches natural fixation on an annual basis, highlighting the urgency of more efficient nitrogen management [Mehta, 2021].

2.2.4. Environmental Consequences

From 1960 to 2020, the global nitrogen surplus increased nearly fivefold, with Asia currently leading in both total and per-hectare nutrient surplus, followed by Europe and the Americas. Although Europe showed a notable decline after the 1990s, overall nutrient use efficiency (NUE) decreased globally—from values over 100% in the 1960s to around 50% today [Ludermann et al, 2024].

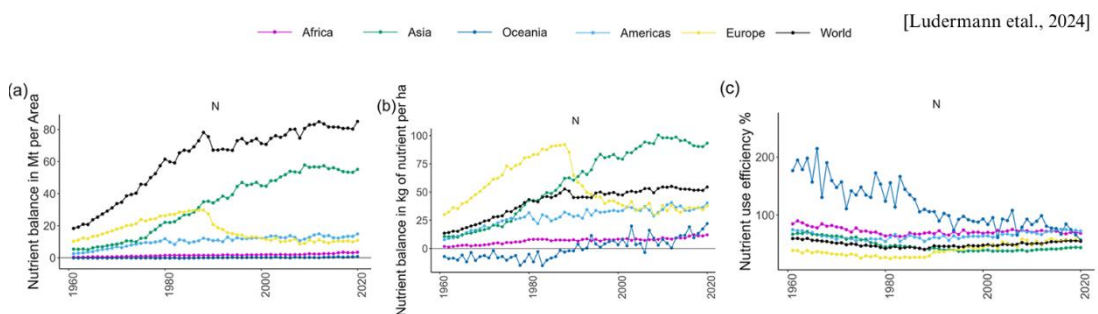


Figure 2.1: Global trends in nitrogen balance and use efficiency between 1960 and 2020 [Luderman, et al. 2024].

This decline is mainly driven by excessive nitrogen fertilizer application, which contributes to serious environmental consequences. Runoff and leaching of nitrate (NO_3^-) into water bodies can trigger eutrophication and algal blooms, creating hypoxic “dead zones” in coastal regions such as the Gulf of Mexico. Additionally, volatilization of ammonia (NH_3) from surface-applied urea can form fine particulate matter ($\text{PM}_{2.5}$) when it reacts with acidic aerosols, posing risks to air quality and human health. Moreover, nitrous oxide (N_2O), a byproduct of nitrogen transformations in soil, has a

global warming potential 298 times greater than CO₂ and contributes approximately 6% to total radiative forcing [IPCC, 2022].

2.2.5. Nitrogen in Plant Metabolism

In plants, nitrate (the dominant soil form in aerated fields) is transported into roots, reduced sequentially to nitrite and then ammonium, and finally incorporated into the amino acids glutamine and glutamate before flowing into proteins and nucleic acids [Novoa et al., 1981]. Adequate nitrogen therefore widens leaves, deepens chlorophyll colour and accelerates biomass accumulation, whereas deficiency slows growth, triggers early senescence and limits yield [Anas et al., 2020]. Yet oversupply can be equally damaging: excess nitrogen delays crop maturity, weakens stems and lowers nitrogen-use efficiency (NUE), the fraction of supplied nitrogen that is actually converted into harvestable produce [Anas et al., 2020].

2.2.6. Importance of Controlled Fertilizer Application

Improving NUE has become a central theme of sustainable agriculture. Meta-analyses indicate that well-timed fertiliser reductions of about 20 % often maintain yields while cutting nitrogen losses dramatically; sensor-guided site-specific application, drip fertigation, higher plant densities and legume rotations can lower total losses by 15–30 % and raise recovery efficiency beyond 50 % in some systems [Anas et al., 2020]. Complementary advances in crop genetics, such as breeding for enhanced nitrate uptake or ammonium assimilation, promise further gains, but field success ultimately depends on integrating molecular innovations with sound agronomy.

Nitrogen lies at the heart of cellular biochemistry, global food production and several major pollution challenges. A balanced approach that values biological fixation, moderates synthetic inputs and boosts plant uptake efficiency is therefore essential for supporting human nutrition while safeguarding planetary health.

2.3. Potato

The potato (*Solanum tuberosum* L.) is a cool-season member of the night-shade family whose large underground stems—*tubers*—store starch that fuels the next generation of shoots. Because tubers can be boiled, baked, fried, mashed or converted to starch, chips, ethanol and even biodegradable plastics, the crop meets culinary, industrial and food-security needs simultaneously. With a median energy yield of $\approx 18 \text{ MJ m}^{-2} \text{ yr}^{-1}$, potatoes now rank fourth in global calorie supply after maize, wheat and rice, while delivering useful vitamin C, potassium and complete protein in far less time than cereals require [FAOSTAT, 2024].

2.3.1. Potato Production

Most recent statistics place 2023 world output at ≈ 383 million t, up 2 % on 2022; China (≈ 96 Mt) and India (≈ 58 Mt) account for more than 40 % of that volume [FAOSTAT, 2024]. In Türkiye, potatoes are equally strategic: the Turkish Statistical Institute's second 2024 estimate predicts 6.5 Mt, a 14 % year-on-year rise driven by certified-seed schemes and pressure-irrigation in Niğde, Kayseri and Afyonkarahisar [TUIK, 2024]. Such volumes matter because per-capita fresh consumption in Türkiye already exceeds 45 kg yr^{-1} , and the frozen-fries and starch industries are expanding.

2.3.2. Essential Elements for Potato

Potatoes need all primary macronutrients (N, P, K) plus Ca, Mg and S, yet nitrogen (N) is usually the first limiting element. A 38 t ha^{-1} crop typically removes $\sim 120 \text{ kg N ha}^{-1}$, $\sim 200 \text{ kg K ha}^{-1}$ and $\sim 20 \text{ kg P ha}^{-1}$ from the field; these figures rise almost linearly with yield [Beukema and van der Zaag, 1990]. Physiologically, N drives chlorophyll synthesis, canopy expansion and the amino-acid pool that later reallocates into tubers. Field experiments in temperate regions show that marketable yields of $34\text{--}45 \text{ t ha}^{-1}$ need crops to take up $130\text{--}160 \text{ kg N ha}^{-1}$ over the season, most of it between stolon formation and early bulking [Westermann and Kleinkopf, 1985].

2.3.3. Growth Conditions of Potato

Potato thrives in mild, spring-like weather: shoots work most efficiently at air temperatures of roughly 15–22 °C, while the hormonal switch that enlarges stolons into tubers is fastest when daytime highs stay below 20 °C and nights hover around 12–17 °C; in contrast, consecutive nights above 22 °C almost entirely hold tuber set, causing yield loss [Lal et al., 2022]. The crop is also sensitive to light length: it behaves as a short-day plant, so when late-summer day-length falls to about 12 h, a signal reaches the stolon tips and tuberisation proceeds; long days keep the canopy in leaf-building mode, which is why tropical farmers favour cooler highlands or cultivars bred for a longer critical photoperiod [Kittipadukal et al., 2012]. Highest yields are obtained in loose, well-drained sandy-loam soils kept slightly acidic (pH 5.0 – 6.5); pH values above 7 restrict micronutrient uptake, whereas values below 5 slow soil microbes yet help suppress common scab disease [Schuh, 2022]. Over the growing season the crop transpires and evaporates about 450–650 mm of water, so keeping soil moisture near 70–80 % of field capacity—neither drought-dry nor waterlogged—prevents drought-induced knobiness and the internal blackheart that follows flooding [Djaman et al., 2021].

2.3.4. Nitrogen Effect on Potato

When the soil cannot supply enough nitrate or ammonium, the plant forms fewer, thinner leaves, sunlight capture drops and tubers remain small or misshapen. Too much N has the opposite—but equally harmful—effect: vines stay vegetative, delaying tuber initiation; harvested tubers have lower dry-matter (specific gravity), fry to a darker colour and store poorly because diseases such as soft rot exploit the lush tissues. Getting the dose right is therefore as much a quality decision as a yield decision [Beukema and van der Zaag, 1990].

2.3.5. Economic and Environmental Stakes

Because potatoes are irrigated on light, sandy soils, the nitrate ion (highly water-soluble) leaches easily below the rooting zone or is lost as nitrous oxide. Reviews of commercial systems indicate that 60–80 % of applied N fertiliser can escape capture, turning fertiliser bills into sunk costs and contaminating groundwater or the

atmosphere [Anas et al., 2020]. Improving nitrogen-use efficiency thus protects both farm profit and ecosystems. Tools already on the market include split applications, controlled-release urea, nitrification inhibitors and biofertilizers based on nitrogen-fixing bacteria.

Potatoes deliver more calories per litre of water or per day in the field than any major cereal, but the crop's high nitrogen demand makes it vulnerable to mis-management. Supplying the *right* amount of N, in synchrony with its rapid mid-season uptake, underpins yield, tuber quality and environmental stewardship alike. For Türkiye—where new processing plants, seed-potato export markets and climate-change pressures converge—modern nitrogen strategies are no longer optional but essential.

2.4. Plant Growth Beneficial Bacteria

2.4.1 Definition and Ecological Niche

Plant-growth-beneficial (PGB) bacteria—internationally known as plant-growth-promoting rhizobacteria (PGPR), are a physiologically diverse group of rhizosphere and endophytic microbes that enhance plant performance without causing disease. They inhabit the narrow soil zone influenced by roots—the rhizosphere—or colonise internal tissues as endophytes, where bacterial densities can reach 10^5 – 10^7 CFU g⁻¹ fresh weight [Dawwan et al., 2013]. In contrast to symbiotic rhizobia that form nodules on legumes, many PGB bacteria associate loosely with a broad range of crops, including potato (*Solanum tuberosum* L.), and express their benefits through biochemical rather than structural partnerships [Hayat et al., 2010].

2.4.2 Mechanisms of Plant Growth Promotion

The PGPB facilitate crop productivity through a range of mechanisms that influence nutrient dynamics, root development, and plant resilience. These mechanisms are generally categorized as direct or indirect, depending on whether their effects are exerted through nutrient acquisition and hormonal regulation, or via enhanced plant defense and stress tolerance.

Direct mechanisms through which plant-growth-promoting bacteria (PGPB) enhance crop productivity include nutrient acquisition and phytohormone production. Free-

living diazotrophs such as *Azospirillum*, *Klebsiella*, and *Burkholderia* can convert atmospheric nitrogen (N₂) into plant-available ammonium via the nitrogenase enzyme, thereby supplementing chemical fertiliser inputs and achieving agronomically relevant nitrogen supply in non-leguminous crops [Timofeeva et al., 2023]. In addition, species like *Bacillus* and *Pseudomonas* contribute to phosphorus and iron availability by secreting organic acids and siderophores—high-affinity Fe³⁺ chelating compounds that mobilize otherwise inaccessible nutrient reserves in the soil [Oleńska et al., 2020]. Furthermore, approximately 80% of rhizobacteria are capable of synthesising phytohormones such as indole-3-acetic acid (IAA), cytokinins, and gibberellins, which reshape root system architecture and increase the absorptive surface area [Babalola, 2010]. Collectively, these direct mechanisms support improved nutrient uptake, earlier canopy closure, and enhanced shoot biomass, ultimately contributing to better plant growth and productivity.

Indirect mechanism refers to the ways in which plant-growth-promoting bacteria (PGPB) enhance plant health by suppressing pathogens and alleviating environmental stress. These bacteria may produce antibiotics, lytic enzymes, or hydrogen cyanide that inhibit phytopathogenic organisms, while also outcompeting harmful microbes for micronutrients through siderophore production [Ma, 2017]. Additionally, PGPB can activate plant systemic resistance pathways, thereby strengthening the plant's innate immunity. Another important indirect mechanism involves ACC-deaminase-containing strains, which lower ethylene levels—a stress-related hormone—and help plants better tolerate salinity, drought, and flooding conditions [Oleńska et al., 2020].

2.4.3. Value of Using Local, Host-Adapted Isolates

Field success hinges on rhizosphere competence—rapid root colonisation, persistence and metabolic activity under local edaphic and climatic conditions. Indigenous strains are pre-adapted to these selective pressures and often outperform exotic inoculants in competitiveness and trait expression [Souza et al., 2020]. Host specificity also matters: potato-derived isolates of *Bacillus cereus* and *Achromobacter xylosoxidans* exhibited stronger IAA production and phosphate-solubilising capacity on potato than non-host strains [Dawwan et al., 2013]. Therefore, screening native collections is a strategic prerequisite for consistent on-farm responses.

2.4.4. PGPB–Potato Interactions

Early field demonstrations showed that seed-piece bacterisation with selected rhizobacteria increased stolon elongation, advanced tuber set and raised marketable yield of potato by 5–33 % in replicated plots [Suslow et al., 1979]. In greenhouse experiments, inoculated plants displayed significant gains in shoot/root length and dry matter compared with uninoculated controls [Dawwan et al., 2013].

Local potato isolates producing IAA and solubilising rock phosphate elevated leaf N, P and K concentrations relative to controls, indicating improved nutrient acquisition efficiency [Dawwan et al., 2013]. Although potato lacks nodules, associative diazotrophs can contribute biologically fixed N directly to the crop or via mineralisation of microbial biomass, enhancing fertiliser-N recovery.

2.4.5. Nitrogen and Potato Productivity

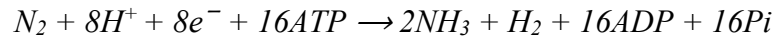
Potato is among the most N-demanding field crops; insufficient supply compromises canopy duration, tuber bulking and ultimately yield. Yet N fertiliser recovery seldom exceeds 50 %, leaving large losses to the environment. Diazotrophic PGPB offer a dual opportunity: (i) provision of additional N through BNF and (ii) modulation of root architecture via phytohormones, which enhances uptake of applied N. Reviews of soil beneficial bacteria predict that strategic inoculation could allow 25–30 kg N ha⁻¹ fertiliser savings without yield penalties in N-intensive systems [Timofeeva et al., 2023].

2.5. Nitrogen Fixation

Nitrogen (N) is the most abundant element in Earth’s atmosphere, yet it is also the single most growth-limiting nutrient in both managed and natural ecosystems. Plants cannot directly assimilate dinitrogen (N₂); thus, they rely on either industrially manufactured fertilizer or on biological nitrogen fixation (BNF)—the enzymatic conversion of N₂ into ammonium (NH₄⁺) by specialized prokaryotes. Because BNF currently contributes roughly half of the reactive nitrogen entering the biosphere each year, second only to the Haber-Bosch process, its ecological and agronomic importance can hardly be overstated [Sun et al., 2020].

2.5.1. The Chemical Fundamental

At the core of BNF lies nitrogenase, a two-component metallo-enzyme that breaks the triple bond of N₂:



This reaction requires at least 16 moles of ATP per mole of N₂ reduced, making BNF one of the most energy-demanding processes in biology [Sun et al., 2020]. Three homologous isoforms are known—Mo-nitrogenase (Nif), V-nitrogenase (Vnf) and Fe-only nitrogenase (Anf)—whose catalytic cores are encoded by highly conserved *nif*/*vnf*/*anf* gene clusters [Koirala and Brözel, 2021]. Mo-nitrogenase is by far the most widespread, while the alternative forms provide functional back-ups under molybdenum-limited conditions and expand the ecological breadth of diazotrophs.

2.5.2. Biological Diversity of Diazotrophs

All known nitrogen-fixing organisms are prokaryotes—bacteria or archaea—collectively termed diazotrophs. They fall into three ecological guilds:

Free-living fixers (e.g., *Azotobacter*, *Clostridium*, many cyanobacteria) derive all energy for BNF from photosynthesis, respiration, or fermentation, but generally contribute modest quantities of fixed N at ecosystem scale.

Associative (rhizosphere or endophytic) diazotrophs live on or within roots of cereals and other non-legumes; notable genera include *Azospirillum*, *Herbaspirillum*, and *Gluconacetobacter* [Guo et al., 2023].

Symbiotic diazotrophs trigger the formation of specialised root or stem nodules in legumes (*Rhizobium*, *Bradyrhizobium*), actinorhizal shrubs (*Frankia*), and certain cycads or *Gunnera* that host cyanobacteria. Within the micro-aerobic nodule matrix, oxygen diffusion is finely regulated, and leghemoglobin delivers O₂ to bacterial cbb₃ oxidases, resolving the paradox between the O₂-sensitivity of nitrogenase and the high respiratory demand of the reaction. Recent phylogenomic surveys extend the diazotrophic repertoire to at least 14 bacterial and archaeal phyla, emphasising horizontal gene transfer as a major driver in the evolutionary spread of *nif* genes [Koirala and Brözel, 2021].

2.5.3. Ecological and Agronomic Significance

BNF provides 21–60 Tg N yr⁻¹ in natural and agricultural systems and underpins protein supply from major grain legumes such as soybean, groundnut, and common bean [Lindström and Mousavi, 2020]. In forage systems, perennial legumes like alfalfa or clovers contribute up to 300 kg N ha⁻¹ yr⁻¹ to grassland nitrogen budgets, decreasing fertiliser inputs, improving soil organic matter, and supporting pollinator resources. Beyond legumes, cereal-associated diazotrophs can satisfy 20–25 % of rice or maize N demand under favourable conditions, and elite landraces such as Sierra Mixe maize derive up to 82 % of their N from associative fixation within mucilage-coated aerial roots [Guo et al., 2023].

2.5.4. Symbiotic Nitrogen Fixation

Symbiotic nitrogen fixation (SNF) is a mutually beneficial partnership in which specialised prokaryotes supply plants with biologically usable nitrogen while receiving carbon and shelter in return [Lindström and Mousavi, 2020]. The best-known agents are *Rhizobium*, *Bradyrhizobium*, *Mesorhizobium* and related “rhizobia,” which infect legumes [Mahmud et al., 2020]; however, actinobacteria of the genus *Frankia* form nodules on woody actinorhizal hosts [Mahmud et al., 2020], and certain cyanobacteria establish nitrogen-fixing alliances with ferns, cycads and even mosses [Koirala and Brözel, 2021]. Together these diverse symbioses convert an estimated 200 million tonnes of atmospheric N₂ to ammonium each year [Mahmud et al., 2020], making SNF a cornerstone of the global nitrogen cycle and a natural counter-balance to synthetic fertiliser use.

In a typical legume–rhizobium association, compatible bacteria enter root hairs, stimulate the formation of nodule primordia in the cortex, and ultimately become enclosed within plant-derived “symbiosomes.” Inside these micro-aerobic nodules the bacterial nitrogenase enzyme reduces N₂ to NH₃, while the host supplies photosynthetically derived malate to meet the high energy cost of the reaction [Mahmud et al., 2020]. A similar division of labour characterises *Frankia* nodules on alder, casuarina and other non-leguminous shrubs, as well as the cyanobacterial colonies that inhabit specialised coralloid roots of cycads and other hosts [Mahmud et al 2020]. Although the anatomical details differ, all symbioses share three broad

features: intimate physical contact, creation of an oxygen-regulated niche, and bidirectional nutrient exchange [Lindström and Mousavi, 2020].

2.5.5. Engineering Nitrogen Fixation for Non-Legume Crops

Three complementary routes are being explored to endow cereals with biological nitrogen-fixing capacity. First, *microbiome optimization* focuses on assembling (or engineering) endophytic bacterial consortia that can colonise internal plant tissues, reduce atmospheric N₂ and, under host-derived signals such as rhizopines, release ammonium directly to the plant. Second, *synthetic-biology approaches* aim to transplant complete *nif* gene clusters into plant organelles; proof-of-concept studies have already produced functional NifH in chloroplasts or mitochondria, yet rebuilding the entire oxygen-sensitive nitrogenase system *in planta* remains a major technical hurdle. Finally, *signalling grafts* seek to introduce legume-style nodulation pathways into cereals so that carefully engineered rhizobia can form controlled intracellular infections, creating nodule-like microenvironments where nitrogen fixation can proceed efficiently.

Collectively, these approaches aim to create “N-self-fertilising” crops that curtail the agricultural N footprint and enhance small-holder resilience in low-input systems.

2.5.6. Detection and Quantification of Biological Nitrogen Fixation

Reliable measurement of biological nitrogen fixation (BNF) is essential both for basic research and for selecting elite diazotrophic inoculants. Because no single technique is universally appropriate, contemporary laboratories combine complementary gas-phase, isotopic, and colorimetric tools to capture activity across time-scales and sample types.

Gas-phase surrogate assays

The acetylene-reduction assay (ARA) remains the work-horse for rapid screening. Nitrogenase reduces the inert surrogate substrate acetylene (C₂H₂) to ethylene (C₂H₄) with the same electron and ATP demand as the physiological N₂ → NH₃ reaction, allowing activity to be quantified by gas chromatography within minutes [Cordova-Rodriguez et al., 2022]. Modern portable GC systems and solid-phase microextraction have pushed detection limits into the pico- to nanomole range, enabling in-situ root-

or nodule-level measurements. Despite its speed, ARA cannot distinguish gross from net N input, and any hydrogenase-mediated H₂ recycling or changes in internal acetylene partial pressure introduce error.

Stable-isotope approaches

For absolute N balances, incorporation of ¹⁵N₂ remains the gold standard. Either direct enrichment (exposure of plants or cultures to 10–98 atom % ¹⁵N₂) or natural-abundance methods (exploiting the slight ¹⁴N preference of nitrogenase) can be applied, the latter allowing non-perturbative field studies over entire cropping seasons [Lindström and Mousavi, 2020]. Mass-spectrometric precision has now reached <0.03 ‰, permitting ecosystem-scale budgets, but high capital cost and sample preparation time still limit routine use.

pH-indicator and spectrophotometric media

In microbiology laboratories, semi-solid N-free (NFb) medium supplemented with bromothymol blue offers an inexpensive qualitative screen: ammonium excreted during BNF alkalises the medium, shifting the indicator from green to blue within 24–48 h [Cordova-Rodriguez et al., 2022]. Recent refinements translate this colour change into a quantitative read-out by recording absorbance at 610 nm throughout growth; calibration against known NH₄⁺ standards yields linear responses down to ~5 µg N mL⁻¹, rivalling Kjeldahl digestion yet avoiding hazardous acids [Cordova-Rodriguez et al., 2022].

The Nessler reagent test – targeted ammonium detection

For samples where optical density or medium pigmentation obscure bromothymol shifts, the century-old Nessler reaction offers a sensitive alternative. Potassium tetraiodomercurate(II) (the Nessler reagent) reacts specifically with ammonium ions liberated by nitrogenase to form a reddish-brown colloidal iodide complex (HG₂NI•NH₂OH), the intensity of which is measured at 420–450 nm. In microplate format it detects <1 µg NH₄⁺ N per well, making it ideal for high-throughput ranking of bacterial consortia or monitoring ammonium release from engineered endophytes. Because mercuric salts are toxic, assays are run in microlitre volumes behind a fume hood and effluents are collected for Hg²⁺ recovery. Interference from primary amines or high phosphate is alleviated by prior sample dilution or by passing the culture supernatant through a cation-exchange cartridge.

Integrating methods for robust estimates

A common workflow is to (i) prescreen isolates on NFb plates, (ii) quantify promising strains in liquid NFb by bromothymol absorbance or the Nessler micro-assay, and (iii) validate field performance with replicated ^{15}N natural-abundance or ARA measurements on inoculated plants. Cross-validation minimises artefacts such as acetylene inhibition of plant respiration or under-estimation of tightly assimilated NH_4^+ . Employing this tiered strategy, recent studies have discriminated diazotrophs that excrete $>40 \mu\text{g N mL}^{-1} \text{ day}^{-1}$ from those that merely fix for their own growth [Cordova-Rodriguez et al., 2022].

By weaving together these complementary techniques—and by choosing the Nessler assay when precise, low-cost ammonium quantification is required—researchers can chart BNF across molecular, organismal, and ecosystem scales with confidence.

2.6. 16S rDNA Sequencing in Identification of Bacteria

The bacterial 16S ribosomal RNA gene is $\approx 1,500$ bp long and contains nine hypervariable segments (V1–V9) that mutate fast enough to separate taxa, yet they are framed by highly conserved regions that serve as anchor points for “universal” PCR primers. Full-length amplification with the classic primer pair 27F (5'-AGAGTTTGATCMTGGCTCAG-3') / 1492R (5'-GGTTACCTTGTTACGACTT-3') yields an ≈ 1.4 kb product that, once Sanger-sequenced - while 16S rRNA gene sequencing is a widely used and powerful tool for bacterial identification, its reliability at the species level is limited. A sequence identity of $\geq 97\%$ is typically sufficient for genus-level classification, whereas $\geq 98.7\text{--}99\%$ is generally required for species-level assignment [Stackebrandt and Ebers, 2006]. However, due to high conservation among closely related species and horizontal gene transfer events, 16S-based species assignments should [Buetas et al 2024; Chen et al 2015].

3. MATERIALS

3.1. Microorganisms

Staphylococcus aureus ATCC 29213, *Klebsiella pneumoniae* (laboratory isolate), *Proteus vulgaris* (laboratory isolate), *Pseudomonas putida* KT244, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* MCTC 8213, and *Pseudomonas aeruginosa* ATCC 15692 were used as control strains, obtained from the Microbiota Laboratory of the Department of Molecular Biology and Genetics at Gebze Technical University.

3.2. Solutions and Media

Table 3.1: Solutions that are used

Solutions	Preparation
0,04% (w/v) Methyl Red	0,05 g of methyl red dissolved in 300 mL of 96%~ ethanol. Completed to 500 mL of volume with dH ₂ O.
0,5% (w/v) Bromothymol Blue	50 mg of bromothymol blue is dissolved in 10 mL of 0,2 N KOH
5% (w/v) Malachite Green	5 g of malachite green is dissolved in dH ₂ O.
1,64% Ferric EDTA (Fe-EDTA)	1,64 g of Fe-EDTA dissolved in 100 ml dH ₂ O.
Solutions	Preparation
3% Hydrogen Peroxide (H ₂ O ₂)	10 ml of 30% H ₂ O ₂ is diluted completing the volume to 100 mL with dH ₂ O.
40% (w/v) Potassium Hydroxide (KOH)	20 g of KOH dissolved in 50 ml volume with dH ₂ O.
0,2 N KOH	0,112 g of KOH dissolved in 10 mL volume with dH ₂ O.
0,5 M EDTA	18,61 g of disodium EDTA dihydrate salt dissolved in 80 mL of dH ₂ O. pH is adjusted to 8.0 with NaOH (pellet). Volume is completed to 100 ml with dH ₂ O.

Table 3.1: Continued	
Fe-EDTA	3,8 g of disodium EDTA dihydrate dissolved in 10 ml of 1M NaOH. 2,5 g of ferrous chloride dissolved in 5 ml of dH ₂ O. Both of the solutions mixed and incubated for 15 min. at room temperature. Precipitate is collected by filter paper and washed with 95% of ethanol. Fe-EDTA is left to dry at room temperature. Collected in powder form.
Micronutrient Solution	For 100 ml; 0,004 g, CuSO ₄ .5H ₂ O; 0, 012 g, ZnSO ₄ .7H ₂ O; 0,140 g, H ₃ BO ₃ ; 0,1 g, Na ₂ MoO ₄ .2H ₂ O; 0,1175 g, MnSO ₄ .H ₂ O complete volume to 100 mL with dH ₂ O.
1X PBS	8 g NaCl; 1,42 g Na ₂ HPO ₄ ; 0,24 g KH ₂ PO ₄ ; 0,2 g KCl is dissolved in 1L of dH ₂ O. pH adjusted to 7.2.
50X TAE Buffer	121 g of Tris-base dissolved in 350 ml of dH ₂ O. 28,55 mL of glacial acid (acetic acid) and 100 ml of 0,5 M EDTA is added. Volume is completed to 500 ml.
1X TAE Buffer	20 mL of 50X TAE buffer is diluted by completing volume to 1L with dH ₂ O.

Table 3.2: Media that are used.

Media Name	Preparation
Urea Broth (CONDA)	3,87 grams of the medium is suspended in 100 ml of distilled water without heating. When the powder is dissolved filter sterilization is performed.
Phenylalanine Agar	2 g/L, DL-Phenylalanine; 3 g/L, Yeast Extract; 5 g/L, NaCl; 1 g/L, K ₂ HPO ₄ ; 15 g/L, agar is weighed and dissolved in dH ₂ O according to the required volume of media. Finally, pH is adjusted to 7.3 ± 0.2 and the media is autoclaved at 121°C for 15 minutes. Distributed into sterile tubes and cooled down in the position for slant agar.
Phenol Red Broth (MERCK)	1,5 g of the media is dissolved in 100 ml of dH ₂ O and autoclaved at 121°C for 15 minutes.
OF Basal Medium (CONDA)	9,8 g/L is weighed and dissolved according to the required volume of media. Autoclaved at 121°C for 15 minutes. 10 ml of 10% glucose solution (filter sterilized) is added to 100 ml of media after.
Moller Decarboxylase Broth (BD)	10.5 g/L Moller Decarboxylase Broth Base media and 1% of the amino acid is weighed and dissolved in dH ₂ O for every amino acid according to required media. Autoclaved at 121°C for 15 minutes.
LB Broth (CAISSON)	25 g/L LB broth media is weighed and dissolved in dH ₂ O according to required media. Autoclaved at 121°C for 15 minutes.
ONPG Broth (HIMEDIA)	13.1 g/L is weighed and dissolved in dH ₂ O according to the required media. Heated to boiling point, no autoclaving.
Peptone Water	15 g/L is weighed and dissolved in dH ₂ O according to the required media. Autoclaved at 121°C for 15 minutes.
SIM Agar (MERCK)	30 g/L is weighed and dissolved in dH ₂ O according to the required media and heated until powder is completely dissolved and distributed into tubes. Autoclaved at 121°C for 15 minutes. After autoclaving tubes are let to be cooled down vertically.

Table 3.2: Continued.

Media Name	Preparation
Simmon Citrate Agar (ACUMEDIA)	24.2 g/L is weighed and dissolved in dH ₂ O according to the required media. Autoclaved at 121°C for 15 minutes.
TSI Agar (HIMEDIA)	64.52 g/L is weighed and dissolved according to the required volume of media. Autoclaved at 121°C for 15 minutes.

3.3. Chemicals, Devices, Kits, DNA Marker, Primers

Table 3.3: Chemicals (Reagents, sugars, stains, etc.) that are used.

Chemicals	Manufacturer
Ethanol	Düzey Lab
6X Loading Dye	Thermo Scientific
Acetic Acid	Merck
Agar	Conda
Agaroz	Prona
Ammonium Sulphate ((NH ₄) ₂ SO ₄)	Merck
Biotin	Sigma Aldrich
Boric Acid (H ₃ BO ₃)	Sigma Aldrich
Bromothymol Blue	Sigma Aldrich
Copper (II) Sulphate Pentahydrate	Sigma Aldrich
D(-)-Arabinose	Fluka
D(-)-Fructose	Riedel-De Haen
D(-)-Mannitol	Merck
D(+)-Cellobiose	Fluka
D(+)-Galactose	Fluka

Table 3.3: Continued.

Chemicals	Manufacturer
D(+)-Glucose	Riedel-De Haen
D(+)-Lactose	Neofroxx
D(+)-Mannose	Fluka
D(+)-Sucrose	Fluka
D(+)-Trehalose	Fluka
D(+)-Xylose	Fluka
Disodium EDTA Dihydrate ($\text{Na}_2\text{H}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$)	Sigma Aldrich
Dipotassium Phosphate (K_2HPO_4)	Merck
DL-Malic Acid	Sigma Aldrich
Ethylenediaminetetraacetic Acid (EDTA)	Sigma Aldrich
Ferrous (III) Chloride Hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$)	Sigma Aldrich
Glycerol	Neofroxx
Gram Staining Kit	Sigma Aldrich
Hydrochloric Acid	Sigma Aldrich
Hydrogen Peroxide (H_2O_2)	Sigma Aldrich
Immersion Oil	Merck
Kovac's Reagent	Biolife
L-Arginine	Neofroxx
L-Lysine	Neofroxx
L-Ornithine	Neofroxx
L-Phenylalanine	Neofroxx
Magnesium Sulfate Heptahydrate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$)	Merck

Table 3.3: Continued.

Chemicals	Manufacturer
Malachite Green	Merck
Manganese (II) Sulfate Monohydrate (MnSO ₄ ·H ₂ O)	Sigma Aldrich
Methyl Red	Merck
Mineral Oil	Sigma Aldrich
Phenol Red	Neofroxx
Potassium Hydroxide (KOH)	Zag
Pyridoxin	Sigma Aldrich
SafeRed Gel Dye	EcoTech
Sodium Chloride (NaCl)	Merck
Sodium Hydroxide (NaOH)	Sigma Aldrich
Sodium Molybdate (Na ₂ MoO ₄)	Merck
Tris-base	Sigma Aldrich
Zinc Sulphate Heptahydrate (ZnSO ₄ ·7H ₂ O)	Sigma Aldrich
α-naphthol	Fluka

Table 3.4: Devices that are used.

Devices	Manufacturer
+4 °C Refrigerator	Vestel
+4 °C Refrigerator	Profilo
−20 °C Deep Freezer	Vestel
−20 °C Deep Freezer	Profilo
−80 °C Deep Freezer	Thermo Scientific, Forma 86°C
Biosafety Cabinet	Thermo Scientific Herasafe KS 8

Table 3.4:Continued.

Orbital Shaker	TOS30 Orbital Shaker-Incubator
Fume Hood	Tezsan
Incubator	Heraeus, B6
Incubator	VWR, INCU-Line IL 53
Gel Electrophoresis Tank	BioRad, Mini-Sub Cell GT
Magnetic Stirrer	Wisd, WiseStir MSH-20A
Microwave Oven	Bluesky
Microplate Reader	BMG LABTECH, FLUOstar Omega
Microcentrifuge	Beckman Coulter, Microfuge 18 Centrifuge
Refrigerated Microcentrifuge	LABOGENE, Scanspeed 1730R
Light Microscope	Nikon, Eclipse E100
Stereoscope	Leica
Autoclave	NÜVE OT-90L
Autoclave	HIRAYAMA HV-110L
pH Meter	Hanna Instruments, pH 211 Microprocessor pH Meter
Centrifuge	Gyrozen, 1248R
Microvolume Spectrophotometer	Thermo Scientific
UV Imaging System	Vilber Lourmat Infinity - 3026 WL/26M
Vortex Mixer	Daihan

Table 3.5: Kits that are used.

Oxidase Test Strips	ChemBio Laboratory Research
PCR kit	Thermo Scientific Dream Taq Polymerase Green
DNA isolation kit	ZYMO- Quick-DNA Miniprep Plus Kit

Table 3.6: DNA marker.

1Kb DNA Ladder	New England Biolabs
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Table 3.7: Primers.

27F	5'-AGAGTTTGATCMTGGCTCAG-3'
1492R	5'-GGTTACCTTGTTACGACTT-3'

4. METHODS

4.1. Soil Sampling

Soil sampling was conducted in five major Turkish potato-growing provinces (Niğde, Afyonkarahisar, Kayseri, Konya and İzmir) between August and October 2024 by Prof. Dr. Mehmet Parlak and their team from Çanakkale 18 Mart University (ÇOMÜ) in the scope of COST project (124O159), when tuber bulking coincides with peak rhizosphere activity.

4.1.1. Rhizospheric Soil Collection

In each province, three commercial agricultural fields (≥ 5 ha) were geo-referenced. Within each field, a zig-zag transect was walked, and five soil cores were collected from the 0–20 cm depth—the root zone richest in exudates and diazotrophic activity—using a sterilised stainless-steel auger ($\varnothing$ 2 cm). The five subsamples (approximately 100 g each) were combined into a single composite sample (~ 500 g), thoroughly mixed in a sterile polyethylene bag, and labelled with the site code, GPS coordinates, potato cultivar, and sampling date (ÇOMÜ/COST Project no. 124O159).

For rhizobacteria isolation and statistical robustness, this sampling scheme was applied to three independent fields per province and three subsamples per field, yielding a total of nine composite soil samples per province. All tools were disinfected between sampling sites using 70% ethanol followed by flame sterilisation to prevent cross-contamination. At the end of each day, a field control ('blank' sample) was obtained by handling an ethanol-rinsed auger without soil contact, as described by Hou and Williams (2013).

Immediately after collection, all soil samples were placed in insulated coolers containing ice packs. To prevent wicking and contamination by meltwater, samples were double-bagged. Samples reached the laboratory within 8 hours and were processed the same day. In cases of unforeseen delay, samples were stored at 4 °C for no more than 48 hours, a timeframe that maintains both culturable diazotroph viability and DNA integrity [Hou and Williams, 2013].

4.1.2. Pre-Processing in the Laboratory

Rhizospheric soil is collected from the surface of potato tubers and roots. Under aseptic conditions, large root fragments and stones were removed. Soil samples are prepared to be used in bacterial isolation and long-term storage. Initially, the collected soils are air-dried at room temperature in a clean environment to remove excess moisture. To detach rhizospheric soil, roots and tubers were gently shaken by hand and tapped against a sterile surface to dislodge tightly adhering soil particles. In some cases, a sterile brush or spatula was used to aid detachment while minimizing damage to the root surface. Once air-dried, the soil samples were gently crushed and passed through a sterile 2 mm mesh sieve to obtain a uniform texture and to remove plant debris and stones. Following this step, subsamples from each field were combined to create a statistically representative composite sample. The processed soils were then stored in sterile, airtight containers under cool and dry conditions. While these samples were primarily used for rhizobacterial isolation in this thesis, a portion was also reserved for metagenomic and physicochemical analyses carried out as part of the broader research project.

4.1.3. Preparation of Soil Samples for Rhizobacteria Isolations

Approximately 10 grams of each soil sample were suspended in 15 mL of sterile 1X PBS to wash and thoroughly mixed by vortexing for 2–3 minutes. The resulting soil–buffer suspension was centrifuged at 4000 rpm (approximately $3068 \times g$) for 20 minutes at 4 °C to sediment the heavier soil particles and minimize physical impurities. Supernatant is removed and remaining cleansed soil is stored at 4 °C for short-term preservation until further use in rhizobacterial isolation procedures. This pretreatment step was essential to enhance bacterial recovery and reduce non-biological debris during the isolation phase.

4.3. Isolation of Rhizobacteria

For each field sample, two separate enrichment cultures were prepared, each incubated at a different temperature (30 °C and 37 °C). One gram of pretreated soil was suspended in 9 mL of sterile Peptone Water. To promote the revival and proliferation

of soil bacteria, the suspensions were incubated for 24 hours at the respective temperatures.

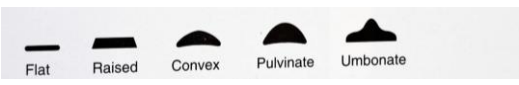
Serial Dilution

To obtain an optimal number of distinct isolates, serial dilutions were performed using PBS in sterile microcentrifuge tubes, starting from the 24-hour Peptone Water–soil enrichment culture and continuing down to a 10^{-6} dilution. Based on preliminary trials, cultures were vortexed after incubation and, without allowing sedimentation, dilutions were immediately prepared. Dilution levels of 10^{-4} , 10^{-5} , and 10^{-6} were selected for the spread plate method. These dilutions were inoculated onto LB agar plates using a sterile Drigalski spatula. Plates were incubated and observed at both 48 hours and 14 days to recover the maximum diversity of culturable isolates.

4.3.1. Colony Morphology

The isolates were examined under a stereomicroscope and selected based on their colony morphologies, including pigmentation, shape, margin, elevation, size, texture, surface appearance, and optical properties.

Table 4.1: Colony morphology descriptions.

Aspects of Bacterial Colony Morphology	Characteristics
Pigmentation	White, yellow, yellowish white, pink, pinkish white, brownish, orange, etc.
Shape	 Circular Rhizoid Irregular Filamentous
Margin	 Entire Undulate Lobate Curled Rhizoid Filamentous
Elevation	 Flat Raised Convex Pulvinate Umbonate

basic assays provide initial insight into bacterial physiology and taxonomy, and help distinguish between morphologically similar isolates. For each preliminary test, fresh 24-hour cultures grown on solid LB agar were used to ensure physiological consistency and increase the accuracy and reproducibility of the results.

4.4.1. Gram Staining

The Gram staining method is a differential staining technique used to classify bacteria into two main groups—Gram-positive and Gram-negative—based on the structure of their cell walls. Gram-positive bacteria have a thick peptidoglycan layer, whereas Gram-negative bacteria have a thinner peptidoglycan wall and an additional outer membrane rich in lipopolysaccharides [Madigan et al., 2018].

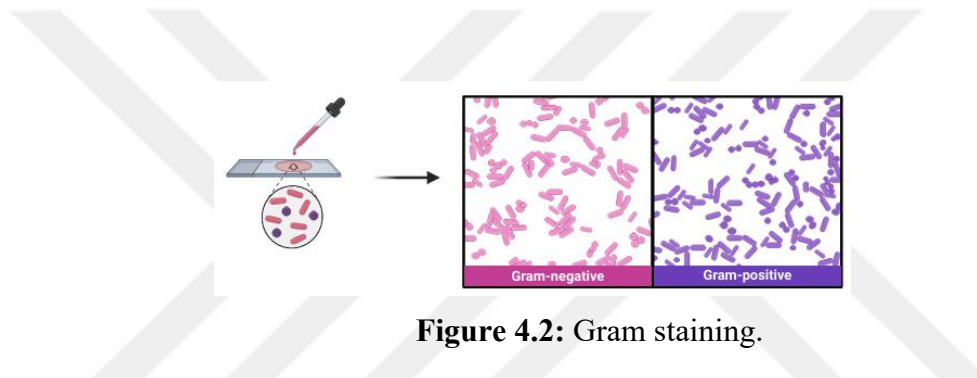


Figure 4.2: Gram staining.

For staining, a fresh colony from a 24-hour LB agar culture was selected and suspended in a drop of sterile distilled water (dH₂O) on a clean glass slide using a sterile inoculation loop. The smear was air-dried and heat-fixed by gently passing the slide through a flame. Sigma Aldrich Gram Staining Kit is used in this process. Crystal violet was applied for 1 minute, followed by rinsing with distilled water. Then, Lugol was added for 1 minute to form a crystal violet-iodine complex. After washing off, the slide was decolorized with Decolorization Reagent for 10–15 seconds, immediately washed again, and counterstained with safranin for 30 seconds. The stained slides were examined under a light microscope at 1000x magnification using immersion oil. Gram-positive bacteria appeared purple due to adherence of the crystal violet stain, while Gram-negative bacteria appeared pink due to counter staining by safranin [Beveridge, 2001; Cappuccino and Welsh, 2017].

4.4.2. Oxidase Test

The oxidase test is a rapid biochemical assay used to determine the presence of cytochrome c oxidase enzyme, which is a component of the bacterial electron transport chain. This enzyme catalyzes the final step of aerobic respiration, facilitating electron transfer from cytochrome c to molecular oxygen, ultimately producing water [Leboffe and Pierce, 2011].

The presence of cytochrome c oxidase is a key diagnostic feature that differentiates certain Gram-negative non-enteric bacteria (e.g., *Pseudomonas aeruginosa*) from oxidase-negative enteric bacteria such as *Escherichia coli*. When present, cytochrome c oxidase catalyzes the oxidation of cytochrome c, which subsequently oxidizes the chromogenic oxidase reagent. This oxidation causes a rapid color change from pink to deep purple within 10–30 seconds, indicating a positive result.

A bacterial colony grown on LB agar (24 h incubation) was picked using a sterile wooden applicator stick and gently rubbed onto an Oxidase Test Strip™ that had been stored in a sealed, dry container. Observation for color change was made within the first 30 seconds. Any color change after 60 seconds was disregarded, in accordance with standard protocol.



Figure 4.3: Oxidase test.

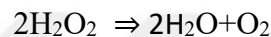
Positive result: Formation of a dark purple coloration on the Oxidase Test Strip™ within 30 seconds indicates the presence of cytochrome c oxidase (e.g., *Pseudomonas aeruginosa*).

Negative result: No color change or a colorless response suggests absence of the enzyme (e.g., *Escherichia coli*).

4.4.3. Catalase Test

Catalase test is a simple and quick biochemical assay used to detect the existence of the catalase enzyme, which catalyses hydrogen peroxide (H_2O_2) into water and oxygen. This enzyme is produced by most aerobic and facultatively anaerobic bacteria as a defense mechanism against the toxic effects of accumulated hydrogen peroxide, a byproduct of aerobic metabolism [Leboffe and Pierce, 2011].

The catalase test distinguishes catalase-positive bacteria such as *Staphylococcus* spp. and *Micrococcus* spp. from catalase-negative genera including *Streptococcus* and *Enterococcus*. The enzyme catalyzes the reaction:



Oxygen is released as gas bubbles upon contact with hydrogen peroxide, indicating a positive result.

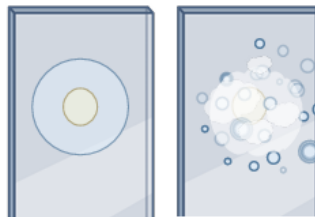


Figure 4.4: Catalase test.

From a 24-hour culture grown on LB agar, a small amount of bacterial biomass was transferred onto a clean glass slide using a sterile toothpick. A few drops of freshly prepared 3% hydrogen peroxide (H_2O_2) were added directly onto the cells. The appearance of bubbles within 5–10 seconds was noted.

Positive result: Immediate and visible formation of oxygen bubbles (e.g., *Staphylococcus aureus*).

Negative result: No bubble formation (e.g., *Enterococcus faecalis*).

4.4.4. Endospore Staining

The endospore stain is a differential staining method used to detect bacterial endospores. Endospores can survive extreme environmental conditions. They are highly resistant and are typically found in genera such as *Bacillus* and *Clostridium* [Leboffe and Pierce, 2011].

Endospores are composed of a dense protein coat and peptidoglycan layers, making them impermeable to most stains. Hence, a special method involving heat is required to facilitate dye penetration. The Schaeffer-Fulton method uses malachite green as the primary stain and safranin as a counterstain. Endospores appear green within pink/red vegetative cells.

A bacterial smear was prepared from a 48–72 h LB agar culture (reduced in nutrients to promote sporulation), air-dried, and heat-fixed. A piece of filter paper was placed over the smear, and the slide was flooded with 5% malachite green solution. The slide was steamed over hot water for 5 minutes, keeping the stain moist. After cooling, the filter paper was removed, and the slide was rinsed gently with water. Counterstaining was performed using safranin from Gram Staining Kit for 30 seconds, followed by a final rinse and drying.

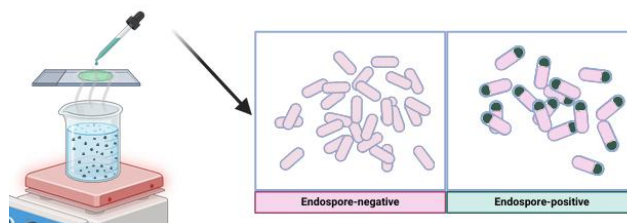


Figure 4.5: Endospore staining.

Positive result: Green-stained oval or spherical endospores visible within or outside red-stained vegetative cells (e.g., *Bacillus cereus*).

Negative result: Only red-stained vegetative cells observed; no green structures detected.

4.5. Screening of Isolates in Nitrogen-Free Medium

To assess the nitrogen-fixing ability of bacterial isolates, a solid nitrogen-free bromothymol blue (Nfb) medium was used, following the formulation described by Baldani et al. (2014). This medium contains no combined nitrogen source, malic acid as only carbon source and includes bromothymol blue as a pH-sensitive indicator dye. The color of the medium serves as a visual marker for bacterial metabolic activity. As the result of nitrogen fixation, ammonia is released into the medium and accumulated, this leads medium pH to increase and become alkaline, which shifts the color of the bromothymol blue indicator from greenish to blue, signaling potential diazotrophic activity.

The screening was carried out in sterile 24-well culture plates. Bacterial isolates were first cultivated on solid medium for 24 hours in their isolation temperatures. Small amounts of actively growing biomass were transferred into Nfb medium using sterile wooden toothpicks, with care taken to inoculate the center of each well containing approximately 1.5 mL of solid Nfb medium [Voronina et al., 2023]. The plates were incubated at 30°C and 37 °C temperatures (isolation temperature) observed on the 1st, 2nd, 3rd, and 7th days post-inoculation. The screening focused on color change as an indirect measure of nitrogen fixation. Wells were examined for shifts in the medium's color from green to blue, indicating pH increase due to ammonia accumulation .

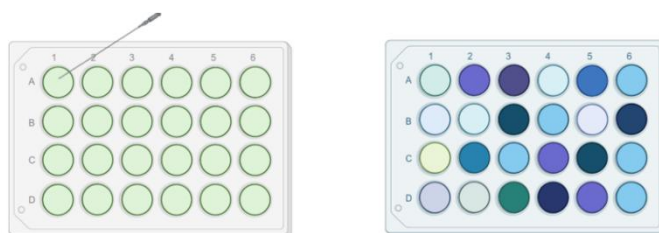


Figure 4.6: Nitrogen fixation screening on NFB agar medium on 24-well plates.

To validate the screening system, *Staphylococcus aureus* ATCC 29213 was included as a negative control, due to its inability to fix nitrogen, and *Klebsiella pneumoniae*, a known free-living diazotroph, was used as a positive control. Isolates showing color transitions similar to or faster than the positive control were prioritized for further analysis, while those resembling the negative control were excluded from later on nitrogen-fixation assessments. Approximately 150 best nitrogen fixing isolates

according to the color change on Nfb agar medium are selected to carry out Nessler's reagent.

4.6. Quantification of Ammonia Production by Nessler's Reagent

Nessler's reagent is a widely used chemical solution for the detection of ammonia, particularly in aqueous environments. It reacts with ammonium ions to form a yellow to brown-colored complex, depending on the concentration of ammonia present. This colorimetric change can be quantified spectrophotometrically. Although its use is well-documented in environmental water testing, its application in the quantitative analysis of bacterial nitrogen fixation is relatively limited. Therefore, optimization steps were required to adapt the protocol for accurate quantification of ammonia production by bacterial isolates.

4.6.1. Optimization of the Protocol

To enable spectrophotometric quantification, a standard curve was required. First, the optimal detection wavelength for the Nessler-ammonia complex was determined, along with the ideal volume of Nessler's reagent to be used in reactions. In addition, the necessity of a chelating reagent was assessed, as metal ions such as Mg^{2+} and Fe^{3+} can interfere with the assay.

For the preparation of standard solutions, ammonium sulfate $((\text{NH}_4)_2\text{SO}_4)$ was used as the ammonium source. A 100 mg/L $\text{NH}_4^+\text{-N}$ stock solution was prepared, from which the following concentrations were derived in Nfb broth (without pH indicator): (1, 2, 3, 4, 5, 6, 7, 8, 9, and 10) mg/L. Using Nfb broth instead of water minimized color interference from media components and provided compatibility with later bacterial culture assays.

4.6.1.1. EDTA and Nessler's Reagent Ratio Optimization

Each ammonium concentration was treated with varying volumes of Nessler's reagent. Typical working concentrations of Nessler's reagent range between 10–20%. For example, 100–200 μL of reagent was added to 1 mL of standard solution. Since Nessler's reagent contains mercury, minimizing volume was prioritized for safety

without compromising detection efficiency. Reagent concentrations of 5%, 10%, 15%, and 20% (v/v) were tested.

Due to potential interference from metal ions, a chelating agent was used to enhance ammonium detection. Giovanni's protocol recommends Rochelle salt; however, 0.1 M EDTA was used as an alternative at a final concentration of 2-3 mM.

Spectrophotometric measurements were carried out at 420 nm, wavelengths recommended in previous studies and protocols [Giovanni et al., 2011]. The optimized conditions—10% Nessler's reagent, 0.1 M EDTA, and 420 nm wavelength—were then used to construct a standard curve.

4.6.2. Ammonium Sulfate Standard Curve

Ammonium standard solutions were prepared as described above. For each concentration, two biological replicates and three technical replicates were analyzed.

Each replicate was prepared by transferring 1 mL of the prepared standard solution into a 2 mL Eppendorf tube. Then, 25 μ L of 0.1 M EDTA was added, followed by vortex mixing and incubation at room temperature for 2 to 3 minutes. Subsequently, 100 μ L of Nessler's reagent was added inside a fume hood. The mixture was vortexed again and incubated in the dark for 15 minutes before spectrophotometric analysis.

After incubation, samples were transferred to a microplate, and absorbance was measured at 420 nm. Absorbance values were used to generate a standard curve, with the resulting regression equation (R^2) later used to calculate unknown ammonia concentrations.

4.6.3. Ammonia Detection in Isolate Cultures

Each bacterial isolate was first cultured in LB broth for 16–18 hours. Following incubation, optical density (OD_{600}) was measured, and cells were pelleted by centrifugation at 4000 g for 10 minutes at +4 °C. To eliminate residual nitrogen sources, the pellet was washed three times with PBS and resuspended in PBS to standardize the OD_{600} to 1.0.

PBS-suspended cells were inoculated into Nfb broth (without pH indicator) at approximately 4% (v/v) inoculation. Cultures were incubated for seven days at their

original isolation temperatures. After incubation, cultures were centrifuged at 10000 g for 10 minutes at +4 °C, and the supernatant was collected.

For each culture, the supernatant was divided into two 2 mL Eppendorf tubes. To each tube, 25 µL/mL of 0.1 M EDTA was added, followed by vortexing and incubation at room temperature for 2–3 minutes. Then, 100 µL/mL of Nessler's reagent was added, vortexed again, and the samples were incubated in the dark for 15 minutes. Finally, absorbance was measured at 420 nm using two technical replicates for each sample.

Ammonia concentrations were calculated using the regression equation from the standard curve. Results were compared against the positive control (*Klebsiella pneumoniae*), each cultured under their respective optimal conditions.

The 30 isolates that produced the highest levels of ammonia were selected for further characterization.

4.7. Biochemical Characterization of Selected Isolates

Biochemical characterization tests were performed on 30 selected bacterial isolates in order to obtain preliminary taxonomic information. These tests are essential for understanding the physiological and metabolic properties of the isolates and provide insight into their potential species or at least genus-level affiliations. The applied biochemical assays included carbohydrate utilization, hydrogen sulfide (H₂S) production, indole production, methyl red (MR) and Voges-Proskauer (VP) tests, citrate utilization, urease activity, motility, oxidation–fermentation reactions, amino acid decarboxylation (lysine, ornithine, arginine), and the ONPG test for β-galactosidase activity. Collectively, these tests helped differentiate between closely related strains and supported the evaluation of the isolates' potential functional and taxonomic characteristics.

4.7.1. Carbohydrate Utilization Tests

The carbohydrate utilization test is a differential assay used to evaluate the ability of bacterial isolates to ferment specific carbohydrates, leading to acid and/or gas production. This test is commonly applied to characterize bacterial metabolic profiles and support identification through biochemical properties. Acid production is

visualized by a color change in the medium due to the pH indicator phenol red, which turns from red to yellow under acidic conditions [Cappuccino & Welsh, 2019].

In this study, phenol red broth was used as the basal medium with a final carbohydrate concentration of 1% (w/v) for each sugar tested. The sugars included arabinose, fructose, mannitol, cellobiose, galactose, glucose, lactose, mannose, sucrose, trehalose, and xylose. Sugars are syringe filter sterilized before they were added to phenol red broth. All sugars except glucose were tested in 96-well microplates. In these plates, each row was designated for a different bacterial isolate, and each column corresponded to a different carbohydrate. The final column of each row was reserved for negative and positive controls, allowing comparison within the same experimental context. The isolates were initially cultured on LB agar at 35 °C overnight. For inoculation into phenol red broth, bacterial biomass was harvested and resuspended in sterile PBS. A 2% (v/v) inoculum was then transferred into 96-well microplates containing the test medium. The plates were incubated at 35 °C for further observation.

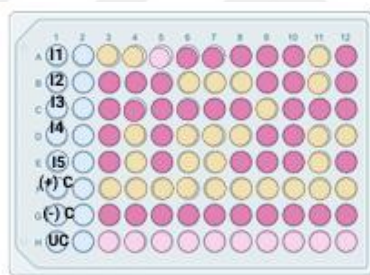


Figure 4.7: Carbohydrate utilization test.

For glucose, the test was performed in phenol red broth tubes containing inverted Durham tubes to detect gas production. Gas formation was interpreted as an additional confirmation of carbohydrate fermentation.

Control strains were selected based on well-established fermentation profiles found in standard microbiology references. *Escherichia coli* ATCC 25922 was used as the primary positive control for all carbohydrates except cellobiose and galactose, due to its broad fermentative capability. *Pseudomonas aeruginosa* ATCC 15692, known as a non-fermentative organism, served as the universal negative control. For cellobiose, *Klebsiella pneumoniae* was selected as the positive control strain, while *Enterococcus faecalis* MCTC 8213 was used for galactose fermentation assessment. Control strains

were included in every experimental set to validate the reliability of results obtained from unknown isolates.

4.7.2. Triple Sugar Iron Test

Triple Sugar Iron (TSI) agar test is a differential biochemical assay used to evaluate bacterial carbohydrate fermentation patterns (glucose, lactose, and sucrose), as well as hydrogen sulfide (H₂S) and gas production. This test is particularly useful for identifying and distinguishing members of the family *Enterobacteriaceae*. The medium contains 0.1% glucose, 1% lactose, and 1% sucrose, along with peptone, sodium thiosulfate, and ferrous sulfate for H₂S detection. Phenol red is included as a pH indicator, turning yellow in acidic conditions and red under alkaline conditions.



Figure 4.8: Triple sugar iron agar test.

Each isolate was inoculated into a TSI slant by stabbing the butt and streaking the slant surface with a sterile inoculating needle. Tubes were incubated at 35°C for 18–24 hours. Color changes in both the slant and the butt were recorded to determine the fermentation of sugars. Gas production was identified by the presence of cracks or bubbles in the medium, while H₂S production was observed as black precipitate (ferrous sulfide) in the butt region [Alexander and Strete, 2001].

Table 4.2: Interpretation of TSI agar test results [Leboffe and Pierce, 2011].

Symbol	Meaning	Interpretation
A/A	Acid/Acid	Glucose and other sugars fermented; yellow slant, yellow butt
K/A	Alkaline/Acid	Only glucose fermented; red slant and yellow butt
K/K	Alkaline/Alkaline	No carbohydrate fermentation; red slant and red butt

Table 4.2 Continued: Interpretation of TSI agar test results [Leboffe and Pierce, 2011].

A/A, H ₂ S	Acid/Acid	Glucose and other sugars fermented with hydrogen sulfite production; yellow slant, black
K/A, H ₂ S	Alkaline/Alkaline	Only glucose fermented with hydrogen sulfite production; red slant and black butt
NC	No Change	No visible growth or metabolic activity; medium remains unchanged
Gas	Gas formation	Gas produced during fermentation; visible as cracks or bubbles in the medium

Escherichia coli ATCC 25922 was used as a positive control for glucose, lactose, and sucrose fermentation without H₂S production, producing a yellow slant and yellow butt (A/A). *Proteus vulgaris* served as a positive control for fermentation with H₂S production (A/A, H₂S). *Pseudomonas aeruginosa* ATCC 27853 was employed as a negative control, exhibiting no sugar fermentation and no H₂S production (K/K or NC).

This test allowed rapid differentiation of enteric isolates based on characteristic acid/alkaline reactions and blackening patterns indicative of sulfur reduction.

4.7.3. IMViC

IMViC tests are a group of biochemical assays used to differentiate enteric bacteria. The acronym IMViC stands for Indole, Methyl Red, Voges-Proskauer, and Citrate tests.

4.7.3.1. Indole, Hydrogen Sulphite, and Motility Tests

The Indole test is the “I” of the IMViC tests. This test is used to identify indole-positive bacteria such as *Escherichia coli* and *Proteus vulgaris*. The amino acid tryptophan can be broken down by the enzyme tryptophanase, resulting in the production of indole, pyruvic acid, and ammonia. To perform this test, SIM (Sulfide-Indole-Motility) medium is used, which contains peptone and meat extract to support bacterial growth.

Peptone provides the tryptophan needed for indole formation. A small amount of agar is added to solidify the medium.

The medium is prepared in test tubes (butt form), and fresh bacterial cultures are inoculated using a sterile needle with a straight stab. Tubes are incubated overnight at 35°C. After incubation, 5 drops of Kovac's reagent are added to the surface of the medium. This reagent contains amyl alcohol, hydrochloric acid, and para-dimethylaminobenzaldehyde, which reacts with any indole present to form a red-colored compound on the surface [Alexander and Strete, 2001].



Figure 4.9: Indole test.

As positive control *P. vulgaris* is used and for negative control *Klebsiella pneumoniae* is used.

4.7.3.2. Methyl-Red Test

The methyl red test is the “M” in the IMViC series and is used to identify enteric bacteria based on how they metabolize glucose. All enteric bacteria first convert glucose into pyruvic acid, but what happens afterward can change depending on species. Some bacteria use the mixed acid pathway, producing stable acids like lactic, acetic, and formic acid. These are known as methyl red-positive organisms and include *Escherichia coli*. Others use the butylene glycol pathway, producing more neutral end products, and are considered methyl red-negative, such as *Serratia marcescens* [Alexander and Strete, 2001].

For this test, MR-VP medium is used. It contains peptone to support bacterial growth and glucose as a fermentable carbohydrate. The medium is inoculated with the selected isolate using a sterile loop and incubated at 35 °C for 2 to 5 days -minimum 2 days. After incubation, about 2.5 mL of the culture is transferred into a clean tube. Then, 5 drops of methyl red solution as an indicator are added, and the tube is gently mixed to homogenise the reagent [Leboffe and Pierce, 2011].

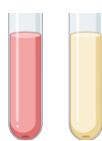


Figure 4.10: Methyl Red test.

A positive result is shown by a red color, indicating stable acid production and a low pH. A negative result appears yellow or orange, meaning the pH did not stay low. As positive control *P. vulgaris* is used and for negative control *Klebsiella pneumoniae* is used.

4.7.3.3. Voges Proskauer Test

The Voges-Proskauer test is the “V” in the IMViC tests. Enteric bacteria use glucose and convert it into pyruvic acid. Those that eventually produce acidic end products are detected by the MR test. Others, which form more neutral end products like acetoin from pyruvic acid, are identified using the VP test [Leboffe and Pierce, 2011].

In our study, we used the MR-VP medium for both tests. After taking 2.5 mL of the culture for the MR test, another 2.5 mL was transferred into a clean tube for the VP test. The reagents used were Barritt’s reagent A (alpha-naphthol solution) and reagent B (KOH solution). First, 6 drops of reagent A were added and gently mixed. Then, 2 drops of reagent B were added, and the contents were mixed again. The tube was left to stand for 15 minutes for the color to develop. The Voges-Proskauer test is the “V” in the IMViC tests. Enteric bacteria use glucose and convert it into pyruvic acid. Those that eventually produce acidic end products are detected by the MR test. Others, which form more neutral end products like acetoin from pyruvic acid, are identified using the VP test [Alexander and Strete, 2001].

In our study, we used the MR-VP medium for both tests. After taking 2.5 mL of the culture for the MR test, another 2.5 mL was transferred into a clean tube for the VP test. The reagents used were Barritt’s reagent A (alpha-naphthol solution) and reagent B (KOH solution). First, 6 drops of reagent A were added and gently mixed. Then, 2 drops of reagent B were added, and the contents were mixed again. The tube was left to stand for 15 minutes for the color to develop.

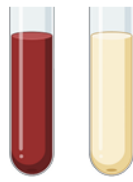


Figure 4.11: Voges Proskauer test.

As positive control *Klebsiella pneumoniae* is used and for negative control *P. vulgaris* is used.

4.7.3.3. Citrate Test

The citrate utilization test is the “C” in the IMViC tests. Citrase is the enzyme that utilizes citrate as a substrate. Bacteria like *Klebsiella pneumoniae* can utilize this organic molecule, bacteria such as *Escherichia coli* are unable to utilize this molecule [Leboffe and Pierce, 2011].

To detect the bacteria that can utilize citrate, Simmon’s citrate agar is used. This media contains citrate as the only carbon source and bromothymol blue as the pH indicator. This media's initial pH is 6.9 and color is green, when citrate is used alkaline end-products are produced and color shifts to blue. Citrase positive bacteria show color changing to blue and growth while citrase negative ones stay green and show no growth [Alexander and Strete, 2001].

Every isolate is inoculated on Simmon’s citrate agar plates (60 mm) with a sterile transfer loop. The plates are incubated at 35 °C for 24 hours, observations for color change is carried out.

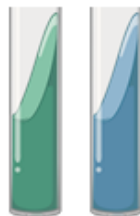


Figure 4.12: Citrate test.

As positive control *Klebsiella pneumoniae* is used and for negative control *E. faecalis* MCTC 8213 is used.

4.7.4. Oxidation-Fermentation (O-F) Test

Organisms can utilize carbohydrates via two ways; aerobically or anaerobically. Aerobically carbon source is oxidized (aerobes), while anaerobically the molecule is fermented (facultative anaerobes). This test is used to detect if the bacteria is aerobes or facultative anaerobes. O-F test separates Gram-negative bacilli, such as *Pseudomonas aeruginosa* and *Escherichia coli*, and Gram-positive cocci, such as *Micrococcus* and *Staphylococcus* aerobes from facultative anaerobes [Leboffe and Pierce, 2011].

For this test, oxidation-fermentation medium is used. This medium contains a low concentration of peptone to decrease the amount of alkaline products that might affect the pH indicator bromothymol blue. High concentration of sugar -glucose is added because it is vastly utilized between bacteria species to force bacteria to utilize the carbon for acidic end-products. Bromothymol blue is green at the initial pH 7.1 and yellow under pH 6.0. A small portion of agar is added to solidify the medium [Alexander and Strete, 2001].



Figure 4.13: Oxidation/fermentation test.

An isolate is inoculated in two O-F medium tubes with sterile transfer needle. After inoculation 2-3 mL of mineral oil is added to one of the tubes for an anaerobic medium and the other one left exposed to air for an aerobic environment. The tubes are incubated at 35 °C for 24 hours, after that observations for color change were carried out.

Table 4.3: Interpretation of O-F test results.

Symbol	Meaning	Interpretation
FA	Facultative Anaerobe	Color change to yellow in both tubes
A	Aerobe	Color change in non-mineral oil tube
I	Inert	No color change in both tube
UC	Uninoculated Control	Tube with no organism

As positive facultative anaerobe *Escherichia coli* ATCC 25922 , positive aerobe *Pseudomonas aeruginosa* ATCC 15592 , and *Pseudomonas putida* KT244 was used as a weak control strain.

4.7.5. Lysine, Ornithine, and Arginine Decarboxylase Test

Amino acids are structured with four groups; a hydrogen, an α - carboxyl group, an α -amine group, and an R-group. Decarboxylase removes carboxyl (-COOH) group from amino acids, which is called decarboxylation. For each amino acid, there is a specific enzyme that removes its carboxyl group. To distinguish enteric bacteria only three decarboxylase is used; lysine, ornithine, and arginine [Leboffe and Pierce, 2011].

In this test, Moeller decarboxylase broth medium is used. To detect each decarboxylase, each amino acid is implemented into the medium separately. The media also contains peptone and meat extract to support bacterial growth. It contains glucose as a carbon source and also, presence of a coenzyme called pyridoxal facilitates the decarboxylation. Bromocresol purple exists as a pH indicator in the medium, which changes color to purple pH above 6.8 and to yellow under pH 5.2.

As a result of the glucose utilization environment turning into more acidic, it plays a role in promoting decarboxylase activity. A positive decarboxylase reaction makes the environment alkaline, leading to a color change from yellow to purple. So, both positive and negative decarboxylase isolate turns the media into yellow, but positive test turns the environment to purple as a result of decarboxylation and negative test remains yellow [Alexander and Strete, 2001].

Test is carried out by inoculating every isolate in the three different decarboxylase media with sterile transfer loop. After that, 2–3 mL of sterile mineral oil is added on top of the medium to make it anaerobic. This helps the bacteria ferment glucose, causing acidification, which promotes decarboxylase activity. The tubes were incubated at 35 °C for 24 hours. After that color changes were observed. The tubes were monitored during incubation to detect the first shift from yellow (acidic) to purple (alkaline), indicating decarboxylase activity [Alexander and Strete, 2001].

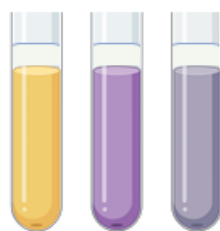


Figure 4.14: Decarboxylase test.

Positive control strains were selected as follows: *K. pneumoniae* was used for lysine and *Escherichia coli* ATCC 25922 ornithine decarboxylase tests, and *Pseudomonas aeruginosa* ATCC 15692 was used for the arginine decarboxylase test. Negative control strains were selected as follows: *P. vulgaris* was used for lysine and *Pseudomonas aeruginosa* ATCC 15692 ornithine decarboxylase tests, and *K. pneumoniae* was used for the arginine decarboxylase test

4.7.6. Urease Test

This test is used to detect urease enzymes produced by bacteria that catalyze urea. Rapid urea catalysis within 24 hours is a known trait of *Proteus* species, and this characteristic is used to help distinguish them from other enteric bacteria.

In this test, urea broth is used, which contains yeast extract, urea, and the pH indicator phenol red. The medium is yellow-orange at its original pH but turns red when the pH rises to 8.4 or above, which indicates urease-positive test [Alexander and Strete, 2001].



Figure 4.15: Urease test.

Each isolate is inoculated into a tube using a sterile transfer loop. Tubes are incubated at 35 °C for 24 hours, and then checked for any color change.

Proteus vulgaris was used as the positive control, while *Escherichia coli* ATCC 25922 was used as the negative control.

4.7.7. Phenylalanine Deaminase Test

In this test, phenylalanine deaminase is the enzyme that catalyzes the conversion of the amino acid phenylalanine into phenylpyruvic acid and ammonia. The purpose of the test is to distinguish enteric bacteria that produce this enzyme, such as *Proteus vulgaris*, from those that do not, like *Escherichia coli* [Leboffe and Pierce, 2011].

To detect the deamination of phenylalanine, phenylalanine agar is used. This medium contains yeast extract, DL-phenylalanine, and agar, and is prepared as a slant.

Each isolate is inoculated using a sterile transfer loop. The tubes are incubated overnight at 35 °C. After incubation, 4–5 drops of 10% ferric chloride are added and spread evenly across the surface of the slant. Color change is then observed [Alexander and Strete, 2001].



Figure 4.16: Phenylalanine deaminase agar test.

Proteus vulgaris was used as the positive control, and *Escherichia coli* as the negative control.

4.7.8. ONPG Test

A bacteria needs two enzymes to ferment lactose; permease and β -galactosidase. To metabolize lactose, permease is needed to bring lactose into the cell and then β -galactosidase processes lactose into glucose and galactose. Absence of permease can cause late lactose fermentation or the bacteria is unable to use lactose. The ONPG test is used to detect real lactose fermenters which produce β -galactosidase.

In this test, ONPG (ortho-nitrophenyl- β -D-galactopyranoside)- an artificial substrate goes into the cell without the help of permease, hydrolysis occurs by β -galactosidase and at the end of this reaction the ortho-nitrophenol turns the media into yellow while negative ones stay the same [MacFaddin, 2000].

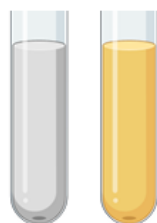


Figure 4.17: ONPG test.

As positive control *Escherichia coli* ATCC 25922, as negative control *Proteus vulgaris* is selected.

4.8. 16S rDNA Analysis

The selected 30 isolates from ammonium detection assay for their nitrogen fixing ability, 16S rDNA analysis is carried out to identify the bacteria at genus and species level. Isolation of genomic DNA is carried out from 24 h culture by a commercial DNA extraction kit (Quick-DNA Miniprep Plus Kit - ZYMO) following the manufacturer's instructions. Quantity, purity and concentration of obtained genomic DNA is assured via agarose gel electrophoresis and spectrophotometric measurements.

4.8.1. PCR for 16S rDNA Gene Region Amplification

PCR master mix for amplification is prepared with isolated genomic DNA using Thermo Scientific Dream Taq Polymerase Green PCR Kit and universal bacterial primers 27F and 1492R .

Tablo 4.4: Primer information of 16S rDNA PCR

Primer	Sequence	PCR Product Size
27F	(5'-AGAGTTTGATCMTGGCTCAG-3')	1500 bp ~
1492R	(5'-TACGGYTACCTTGTTACGACTT-3')	

Tablo 4.5: PCR mix content.

Compotent	1X Reaction (30 µL)
10X Phusion HF Buffer	3 µL
10 mM dNTPs	0,6 µL
25 µM 27F prime	0,6 µL
25 µM 1492R primer	0,6 µL
DNA sample	>150 ng~
Nuclease-free water	to complete 30 µL

Tablo 4.6: PCR Condition.

Cycle Step	Temperature	Time	Cycles
Initial denaturation	98 °C	30 s	1
Denaturation	98 °C	10 s	30
Annealing	58 °C	30 s	
Extension	72 °C	30 s	
Final Extension	72 °C	10 m	1

Amplification is carried out for each isolate with a blank contains apyrogenic water as a replacement for template to detect any contamination. After PCR amplification, 4 µL of PCR products is loaded into wells on agarose gel with blank and also 1Kb DNA ladder s loaded with to compare the size of PCR products. Electrophoresis is performed with 90 V for 30 min. The presence of ~1500 bp 16S rDNA amplicon is confirmed via a UV transilluminator system. External sequencing service (BMLabosis) is used for the bidirectional sequencing of the PCR products. The results of the sequencing are then aligned using BioEdit the software, and consensus sequences were generated. These sequences were compared against the NCBI (National Center for Biotechnology Information) database using BLAST (Basic Local Alignment Search Tool) to determine the closest species level matches

5. RESULTS

5.1. Rhizospheric Soil Collection

To isolate nitrogen fixing bacteria from the potato rhizosphere, five provinces; Niğde, Afyonkarahisar, Konya, Kayseri, and İzmir are selected (Figure 5.1). According to section 4.1 soil samples, total of 15 soil samples were collected (Table 5.1.)

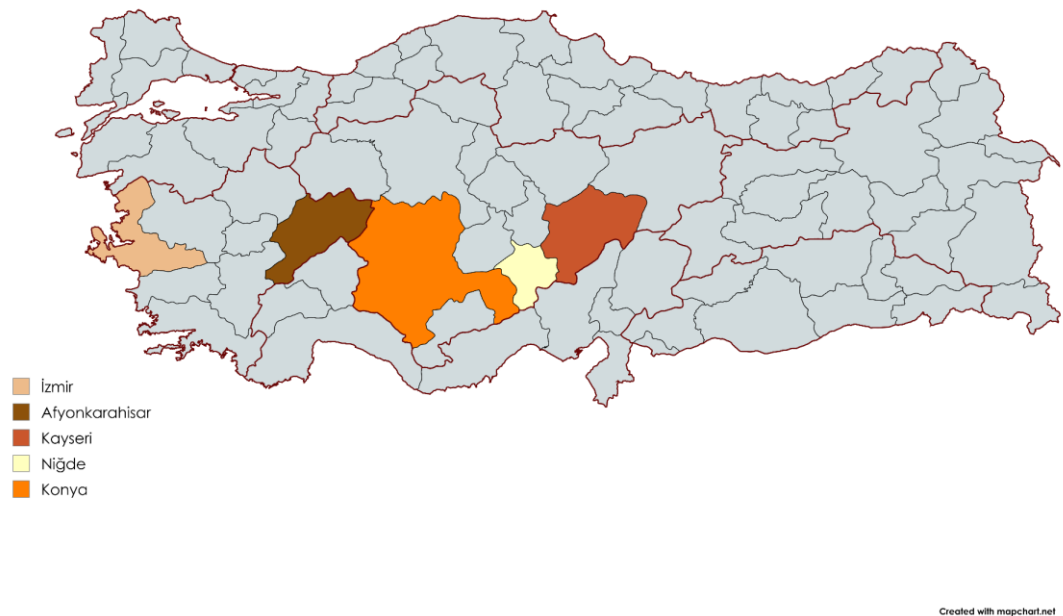


Figure 5.1: Provinces selected for the collection of potato rhizospheric soil.

Tablo 5.1: Information about soil collection sites.

Province	Number of Fields	Location (District-Village)	Coordinates	Codes
Niğde	1/3	Bor - Balcı Köyü (Farmer: Ali Cansın)	37.93023°N, 34.44898°E	ND1
	2/3	Bor - Balcı Köyü (Farmer: Nazım Kurt)	37.92842°N, 34.45139°E	ND2
	3/3	Bor - Balcı Köyü (Farmer: Muharrem Aydın)	37.92951°N, 34.43965°E	ND3
Afyon.	1/3	Dinar - Okçular	38.22454°N, 30.42450°E	AF1
	2/3	Dinar - Okçular	38.23233°N, 30.42524°E	AF2
	3/3	Haydarlı	38.25320°N, 30.39180°E	AF3
Konya	1/3	Seydişehir- Kesecik Köyü	37.39388°N, 31.92712°E	KN1
	2/3	Seydişehir - Gevrekli Köyü	37.55811°N 31.88323°E	KN2
	3/3	Seydişehir - Gevrekli Köyü	37.55937°N 31.88432°E	KN3
Kayseri	1/3	Sarız - Çörekdere Köyü, Erkekoğlu	38.46922°N 36.47450°E	KY1
	2/3	Sarız - Çörekdere Köyü, Kayseri Yolu	38.47372°N 36.47340°E	KY2
	3/3	Sarız - Çörekdere Köyü, Kayseri Yolu	38.47725°N 36.47322°E	KY3
İzmir	1/3	Ödemiş - Gölcük	38.30678°N 28.02625°E	İZ1
	2/3	Ödemiş - Ovacık	38.30504°N 28.05175°E	İZ2
	3/3	Ödemiş - Ödemiş Road	38.31948°N 28.05781°E	İZ3

5.2. Physicochemical Analysis Results of Soil Samples

Table 5.2: Physiochemical analysis results of collected rhizospheric soil samples. (In the scope of 124O159 COST project, this table is prepared by Prof. Dr. Mehmet Parlak and his team).

Location of Samples	Clay (%)	Silt (%)	Sand (%)	Bulk density (g/cm ³)	Aggregate stability (%)	pH
Afyon.	39.37±7.24 b	28.74±7.9 7 a	31.88±5.6 4 c	1.13±0.08 b	88.19±4.7 6	7.43±0.3 5 a
Niğde	22.93±2.82 c	29.89±7.6 7 a	47.50±7.1 4 b	1.25±0.16 a	83.50±12. 62	7.02±0.7 5 a
Kayseri	39.89±5.78 b	32.47±4.6 0 a	27.63±4.2 0 c	1.26±0.07 a	70.21±15. 80	7.60±0.1 6 a
Konya	47.98±7.24 a	22.75±3.1 9 b	29.27±7.0 8 c	1.09±0.12 b	74.78±19. 60	7.10±0.7 2 a
İzmir	11.11±1.07 d	29.47±2.9 0 a	59.41±2.1 5 a	1.29±0.11 a	69.00±23. 34	4.69±0.6 1 b
ANOVA p-value	0.000	0.014	0.000	0.003	0.072	0.000

Table 5.2: Continued.

Location of Samples	EC (dS/m)	Lime (%)	Organic matter (%)	Total N (%)	Available P (mg/kg)	Available K (mg/kg)
Afyon.	0.58±0.13 b	2.14±0.98 bc	1.32±0.20 a	0.06±0.01 bc	31.61±12.68 a	1144.9±32.8 a
Niğde	0.61±0.14 b	0.58±0.21 c	0.99±0.20 b	0.05±0.01 c	22.85±12.26 b	277.8±90.0 bc
Kayseri	0.48±0.04 b	8.32±4.62 a	1.95±0.73 a	0.09±0.03 a	2.51±0.67 c	247.6±126.2 c
Konya	0.87±0.08 a	3.26±2.49 b	1.86±0.54 a	0.08±0.01 ab	1.49±0.97 c	377.3±157.6 b
İzmir	0.84±0.49 a	2.14±0.35 bc	2.00±0.49 a	0.10±0.02 a	4.52±2.26 c	69.5±83.6 d
p-value	0.003	0.000	0.000	0.000	0.000	0.000

Location of Samples	Available Ca (mg/kg)	Available Mg (mg/kg)	Available Cu (mg/kg)	Available Fe (mg/kg)	Available Mn (mg/kg)	Available Zn (mg/kg)
Afyon.	5676±572 a	261.8±69.4 c	2.66±0.13 a	4.31±0.91 b	10.99±6.28 abc	0.71±0.16 b
Niğde	2720±380 b	386.3±104.15 b	2.48±0.34 a	6.63±2.77 b	20.66±19.55 ab	0.42±0.10 bc
Kayseri	6120±1374 a	141.20±25.7 d	1.24±0.16 c	7.15±1.11 b	4.53±1.83 c	0.29±0.11 c
Konya	6686±2846 a	601.90±78.19 a	1.73±0.57 b	9.22±12.32 b	9.12±12.01 bc	0.30±0.18 c
İzmir	1297±804 c	150.59±101.23 d	2.61±0.11 a	162.45±51.61 a	25.04±20.97 a	2.53±0.68 a
p-value	0.000	0.000	0.000	0.000	0.022	0.000

5.3. Rhizobacteria Isolation from Soil

Total 600 isolates are obtained from 15 different potato rhizosphere soil samples. After the evaluation of morphological characterizations total 540 isolates (Table 5.4) are selected to continue with the experiments. Distribution of total isolates according to isolation sites is shown in **Figure 5.2**.

The highest isolation was obtained from Niğde soil samples with a total of 172 isolates, followed by İzmir with 97 isolates, Afyonkarahisar with 92 isolates, Konya with 91 isolates and Kayseri with 90 isolates (Figure 5.2).

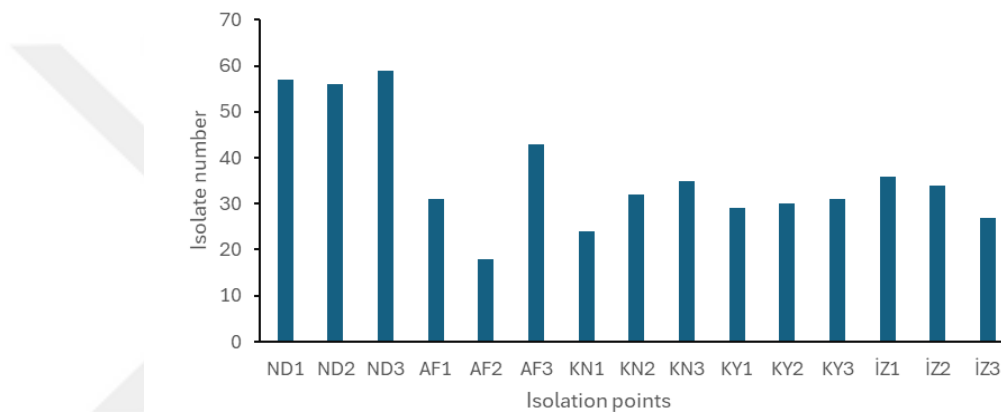


Figure 5.2: Distribution of the isolates by isolation point.

5.3.1. Morphological Characterization Results

The isolates are selected according to how they differ in colony morphological characteristics . Such as pigmentation, shape, margin, etc. Approximately 600 isolates are characterized according to these traits. The characterizations were analyzed in each category as it is shown in the **Figure 5.3**. The pie graph for pigmentation revealed that majority of the colonies were white (243 isolates) or yellowish white (216 isolates) , followed by yellow (51 isolates), with other pigmentation types observed at much lower frequencies. Colony shape was predominantly irregular (266 isolates) or circular (263 isolates), while rhizoid, punctiform, filamentous, and fimbriate were rare. Regarding surface texture, smooth colonies were most common

8378 isolates), with fewer finely granular, arborescent, wavy interlaced, filamentous, coarsely granular, wrinkled surfaces. The majority of margin morphologies were entire

(185 isolates) and wavy (137 isolate) followed by lobate (107 isolates) and creanate, scalloped, fimbriate, and ciliate margins were in less frequent. In the terms of appearance shiny (390 isolates) colonies was dominant to dull (152) colonies. Elevation was mostly raised (383 isolates) followed by effuse (84 isolates) and for colony size colonies were mostly large (195 isolates) and medium 195 isolates). For optical properties opaque colonies are predominant, followed by translucent and transparent colonies. Detailed colony morphologies for each the isolates are presented in **Appendix A**.



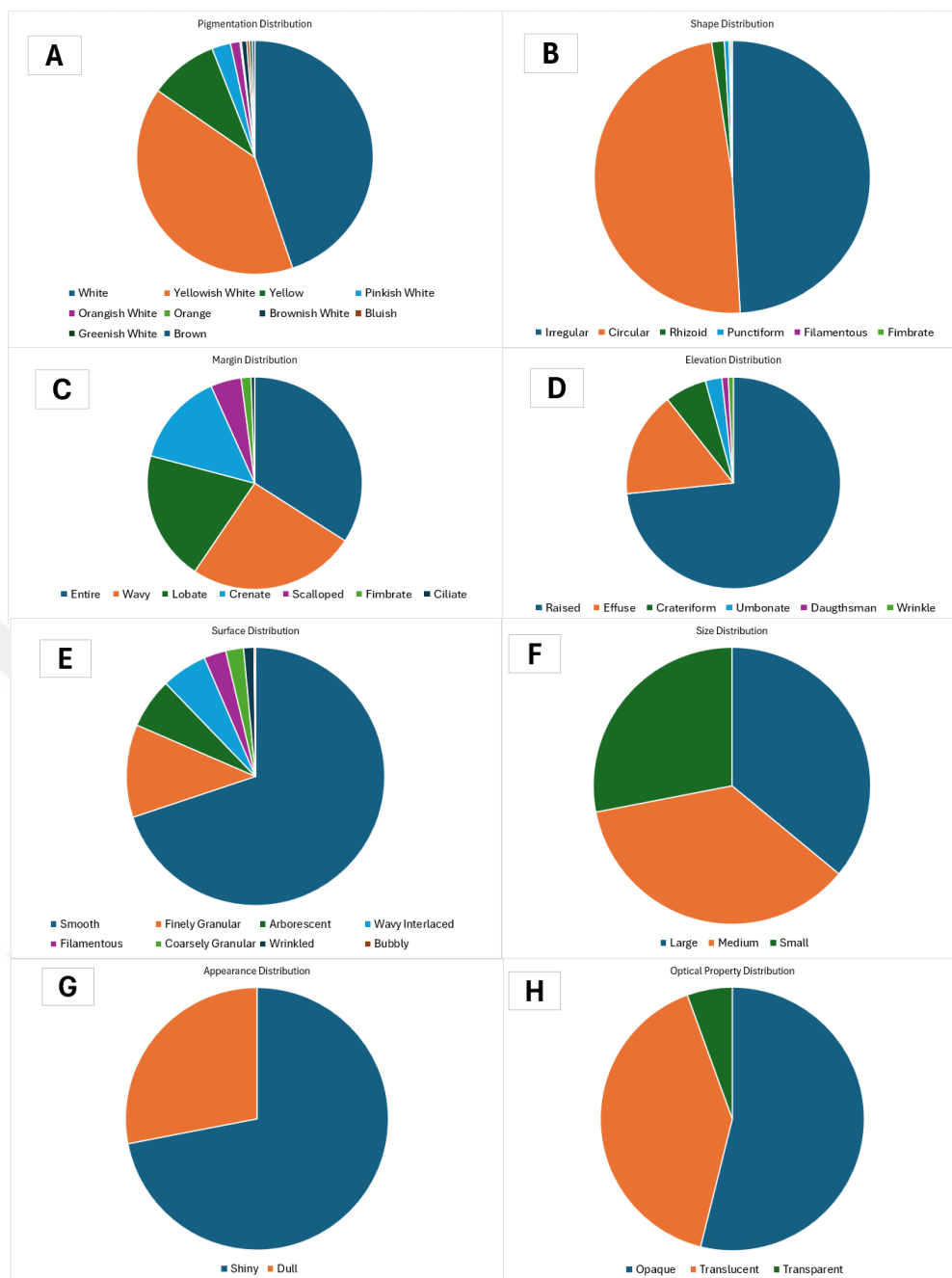


Figure 5.3: Distribution of morphological characteristics observed among bacterial colonies isolated from soil samples. (A) Pigmentation distribution, (B) Colony shape distribution, (C) Margin type distribution, (D) Elevation profile distribution, (E) Surface texture distribution, (F) Colony size distribution, (G) Appearance (Shiny or dull) distribution, (H) Optical property distribution (opaque, translucent, or transparent). Each pie chart represents the relative frequency of the corresponding morphological trait observed during the preliminary characterization of isolates



Figure 5.4: Isolate samples of the Niğde soil with different morphologies on purification plates.

5.4. Preliminary Characterization Test Results

The isolates underwent four preliminary tests for further characterization. Gram staining, catalase test, oxidase test, and endospore staining. Gram staining enabled classification of the isolates as Gram-positive and Gram-negative based on their peptidoglycan cell wall characteristics. Catalase and oxidase tests were used to detect the presence of catalase and cytochrome c oxidase enzymes, respectively. Endospore staining was carried out exceptionally on Gram-positive rod-shaped (bacilli) isolates, to assess their spore-forming capacity. These preliminary tests provided an initial characterization of the isolates. Detailed information for each isolate is presented in **Appendix B**.

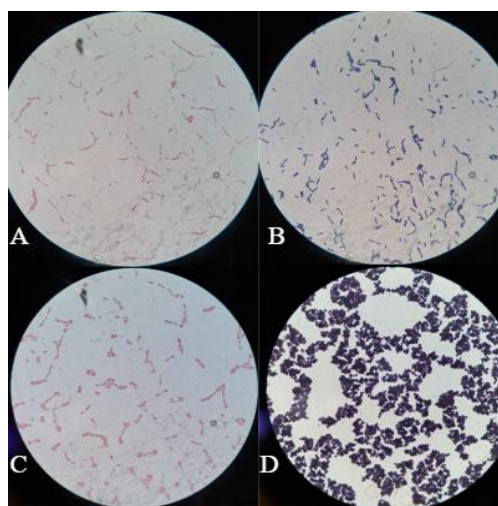


Figure 5.5: Gram staining of some of the isolates. A, AF1B/22-Gram (-) bacillus; B, AF1B/21-Gram (+) bacillus; C, AF3B/29-Gram (-) Coccus; D, İZ3B/8-Gram (+) Coccus.

Evaluation of Gram staining results revealed that Gram-negative rod-shaped bacteria constitute most of the isolates, accounting for 56.5% (306 isolates) of the total 542 isolates. Gram-negative cocci were the second most abundant group, representing 24.9% (135 isolates). Gram-positive rods accounted for 12.2% (66 isolates), ranking third in abundance, while Gram-positive cocci were observed at the lowest proportion 6.5% (35 isolates). These findings indicate that Gram-negative rods were the dominant morphology among the isolates, whereas coccoid forms, in both Gram-negative and Gram-positive groups, were present at respectively lower proportions. This distribution reflects the morphological diversity of the isolates and provides fundamental classification for subsequent identification processes.

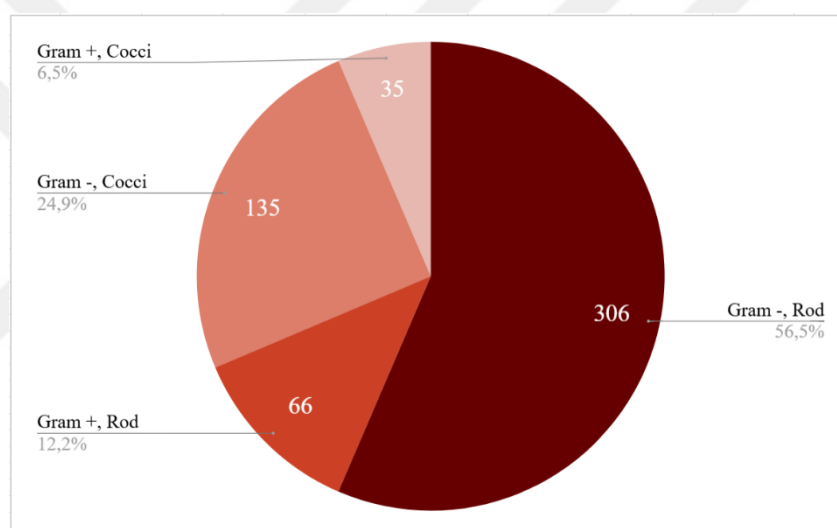


Figure 5.6: Distribution of isolates according to Gram staining and bacterial morphologies.

Other physiological characteristics (catalase activity) of all isolates were screened. Therefore, 158 isolates (28.9%) were determined as strong (+++), 254 isolates (46.4%) were intermediate (++), and 94 isolates (17.2%) were weak (+) against *P. aeruginosa* (+++) control strain. Finally, 41 isolates were evaluated as negative (no activity).

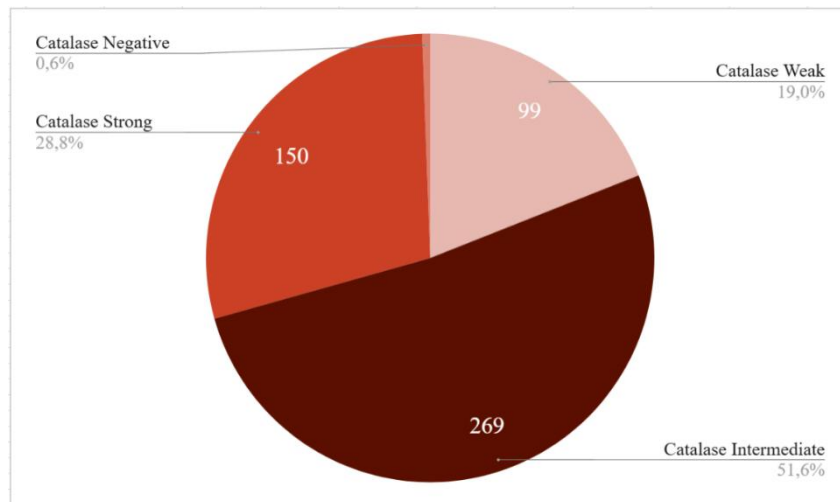


Figure 5.7: Distribution of isolates by catalase activity; strong, (+++); intermediate, (++) weak, (+); no activity, (-).

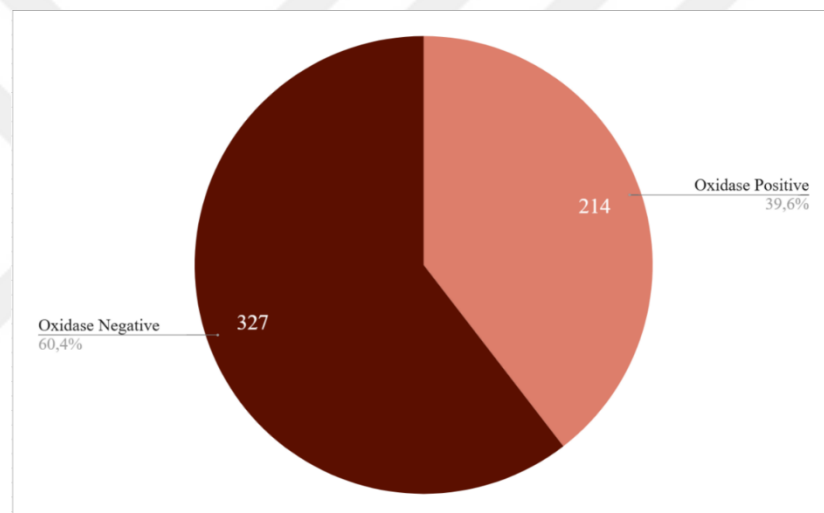


Figure 5.8: Distribution of Isolates by Oxidase Test Results.

The other assay of physiological characterization is the oxidase test. These test findings showed that 212 isolates (39.2%) were oxidase (+) and 329 (60.8%) were oxidase (-).

5.5. Screening Test Results of Isolates in Nitrogen Fixation

Isolates are evaluated according to color change of the Nfb medium according to color scale **Figure 5.9**, respectively, at days 1, 2, 3, and 7. The isolates showing better nitrogen fixation ability are selected for Nessler' reagent assay for quantitative determination of ammonia accumulation in **Appendix C**.

	Level 1
	Level 2
	Level 3
	Level 4
	Level 5

Figure 5.9: Scale of color change on Nfb medium.

CONTROL	Nfb Medium Results			
	Day 1	Day 2	Day 3	Day 7
<i>K. pneumoniae</i> 30 °C				
<i>K. pneumoniae</i> 37 °C				

Figure 5.10: Color change of *K. pneumoniae* on NFB medium over 7 days, used as the positive control

5.6. Ammonium Quantification Using Nessler's Reagent

Isolates that underwent ammonium detection assay were (i) morphologically identified, (ii) grown on nitrogen-free broth (Nfb) medium, and (iii) pre-selected based on the medium's colour change. Ammonium detection is performed via measuring optical density of the colored complex which is occurred as a final product of the assay. After, to calculate the concentration a standard curve is needed and optimization of the protocol was essential. Experiments are carried out for this purpose.

5.6.1. Optimization of the Protocol

Detection of ammonium in bacterial culture media by Nessler's reagent required optimizations. Two main factors for this protocol are tested. Firstly, adding or not adding 0,1 M EDTA as chelating element and secondly, experiments to help decide on concentration of Nessler's reagent to be used in ammonia detection assay.

5.6.1.1. EDTA and Nessler's Reagent Ratio Optimization

Ammonium solutions with known concentrations are prepared and the assay is carried out with and without 0.1 M EDTA. It is observed that in the absence of EDTA accuracy of detected ammonium is decreased while the concentration of ammonium is increased.

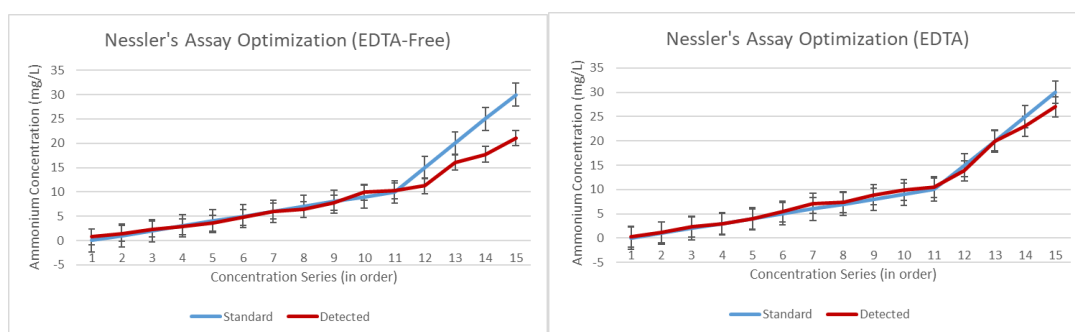


Figure 5.11: Comparison of ammonium detection in standard solutions using Nessler's reagent assay. Left: measurements without EDTA; right: with EDTA added. Blue points represent known ammonium concentrations; red points represent absorbance values obtained via Nessler's reagent assay. Each data point reflects the mean of two independent experimental replicates, with three technical replicates per experiment.

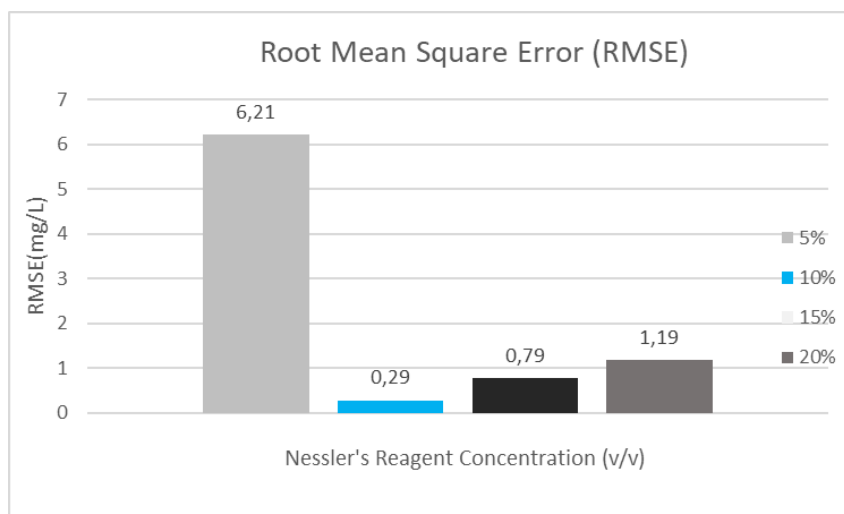


Figure 5.12: Root mean square root (RMSE) comparison between each group.

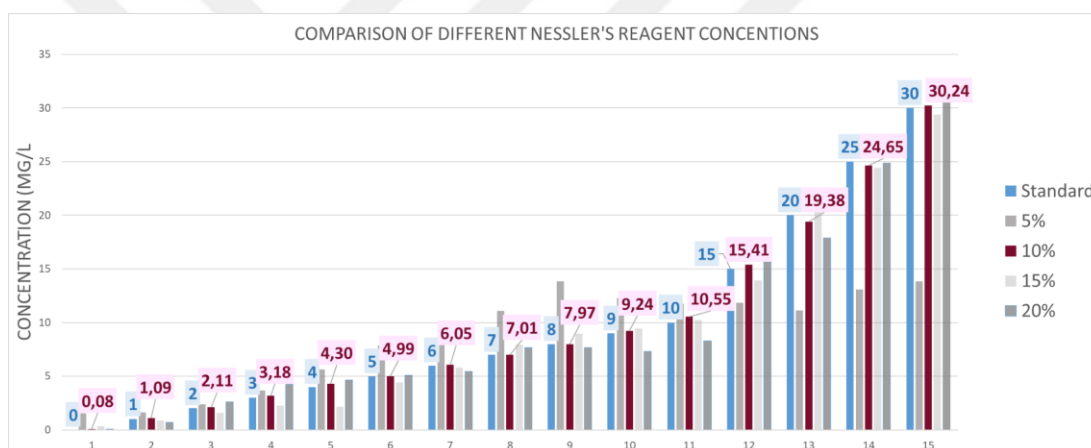


Figure 5.13: Comparison of detected concentrations to known concentrations by applying different concentrations of Nessler's reagent. O, original known concentration; A, 5%; B, 10%; C, 15%; D, 20% Nessler's reagent.

When we combine two figures, **Figure 5.12** and **Figure 5.13** Nessler's reagent concentration on measurement accuracy becomes evident. The Root Mean Square Error (RMSE) value (Figure 5.12) indicate that the lowest deviation from the standard concentrations was obtained with 10% Nessler's reagent, yielding an RMSE of 0.29 mg/L. This concentration demonstrates the highest measurement accuracy among all tested conditions. In contrast, the 5% concentration produced a markedly higher RMSE (6.21 mg/L), indicating substantial measurement error. The 15% and 20 % concentrations showed moderate deviations, with RMSE values of 0.79 mg/L and 1.19 mg/L, respectively.

The comparison of measured concentration of measured concentrations with the standard values (Figure 5.13) further supports these findings the results obtained with 10% Nesller's reagent (red bars) closely matched the standard concentrations (blue bars) across nearly all measurement points. In comparison, the 5% concentration generally underestimated the standard values, while the 15% and 20% concentrations tended to produce slightly higher readings at certain points.

Overall, both RMSE analysis and direct comparison with standard concentrations indicate that 10% Nessler's reagent provides the most accurate and reliable measurements for the intended applicaiton.

5.6.2. Ammonium Sulfate Standard Curve

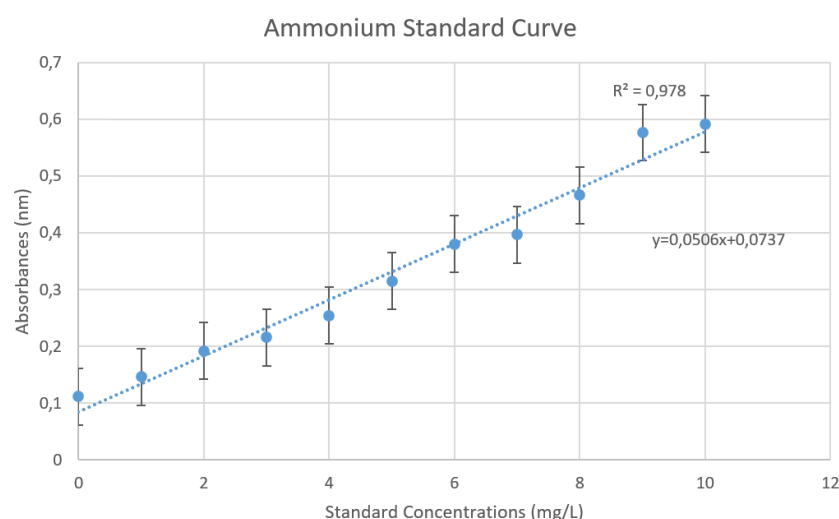


Figure 5.14: Standard curve for ammonia quantification. Standard concentrations ranged from 1 to 10 mg/L. The assay was performed using 10% Nessler's reagent and 0.1 M EDTA, and absorbance was measured at 420 nm. The standard curve was constructed using X–Y data points with two experimental replicates, each comprising three technical replicates, as indicated by the error bars.

The R^2 value of 0.978 demonstrates a strong linear correlation, confirming the reliability of the assay within the tested concentration range. Error bars represent the variability across measurements, which remained low and consistent throughout the dataset.

5.6.3. Ammonium Detection in Isolate Cultures

Cultivation. Each isolate was inoculated, in duplicate, into Nfb medium without bromothymol blue. Optical density at 600 nm (OD₆₀₀) was measured before and after incubation. Cultures were then incubated for 7 days at the temperature at which the isolate had originally been obtained.

Preparation for the Nessler assay. After incubation, supernatants were harvested from each duplicate culture and split in half, yielding two independent Nessler reactions per culture. Each Nessler reaction was measured in duplicate in 96-well microplates, and absorbance was read at 420 nm (OD₄₂₀). Mean values were calculated and tabulated.

Ammonium concentrations (mg /L) were computed from the OD₄₂₀ data using a previously generated ammonium-sulfate standard curve; means and standard deviations were recorded.

Positive (*Klebsiella pneumoniae*) and negative control (*Staphylococcus aureus*) were run at both 30 °C and 37 °C. Isolates were evaluated against the control grown at the same temperature:

At 30 °C, isolates that produced more than 2 mg/L of NH₄⁺ were retained, considering that the *Klebsiella pneumoniae* control reached 1.2 mg/L. At 37 °C, a threshold of 2.5 mg/L NH₄⁺ was applied, although the *K. pneumoniae* control reached 3.7 mg/L, a lower cut-off was intentionally selected to allow the inclusion of more candidate isolates. The 30 isolates which match this criteria are as in **Table 5.5**.

Table 5.5: Detected ammonium concentration produced by 30 isolates selected by Nessler' reagent assay.

Isolate Name	Concentration (mg/L)	Isolate Name	Concentration (mg/L)
<i>K. pneumoniae</i> (30°C)	1,25 ± 0,20	ND3B/30	2,83 ± 0,03
<i>K. pneumoniae</i> (37°C)	3,68 ± 0,03	ND3B/36	3,91 ± 0,17
ND3A/18	2,27 ± 0,19	AF1B/5	4,50 ± 0,06
AF1A/22	2,03 ± 0,22	AF2B/2	2,61 ± 0,04
AF1A/23	3,35 ± 0,10	AF2B/6	3,19 ± 0,16
AF2A/29	2,42 ± 0,28	AF2B/19	2,58 ± 0,20
AF3A/4	3,28 ± 0,35	AF2B/20	2,68 ± 0,06
AF3A/9	2,06 ± 0,13	AF3B/1	2,67 ± 0,09
AF3A/16	2,55 ± 0,19	AF3B/22	2,71 ± 0,36
AF3A/17	2,29 ± 0,17	AF3B/25	3,01 ± 0,38
AF3A/25	2,41 ± 0,20	KN1B/3	3,43 ± 0,46
AF3A/26	2,92 ± 0,19	KN1B/13	3,34 ± 0,13
ND2B/20	2,46 ± 0,11	KN2B/2	2,92 ± 0,16
ND3B/11	4,46 ± 0,07	KY1B/5	2,65 ± 0,13
ND3B/23	5,96 ± 0,28	KY2B/10	3,43 ± 0,06
ND3B/29	4,07 ± 0,07	KY2B/11	3,12 ± 0,04

Values sharing the same letter are not significantly different from each other ($p < 0.05$).

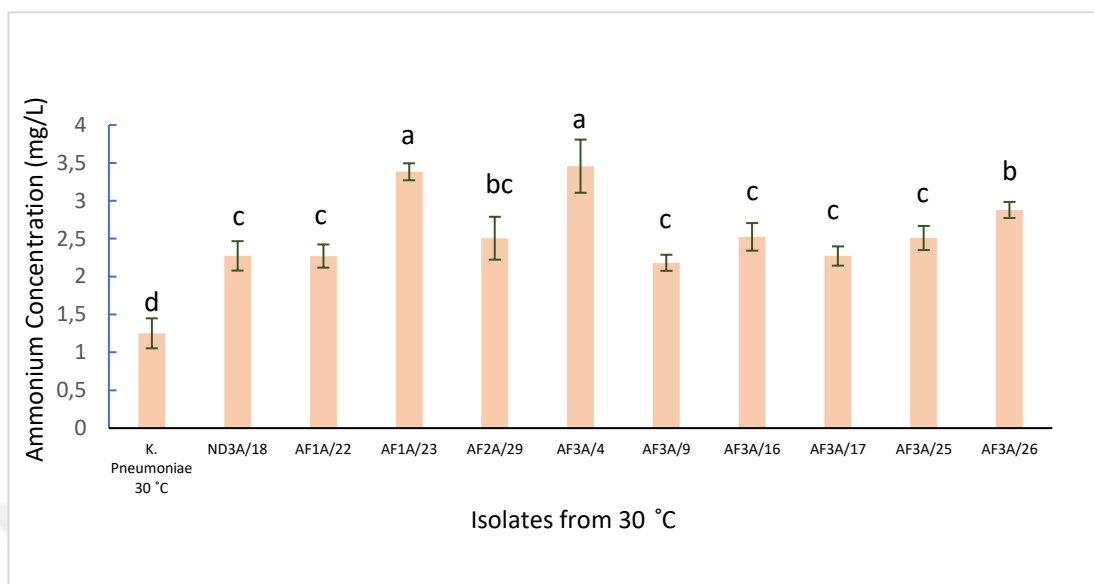


Figure 5.15: Ammonium concentrations of the nitrogen-fixing bacteria isolated at 30° C compared with positive control.

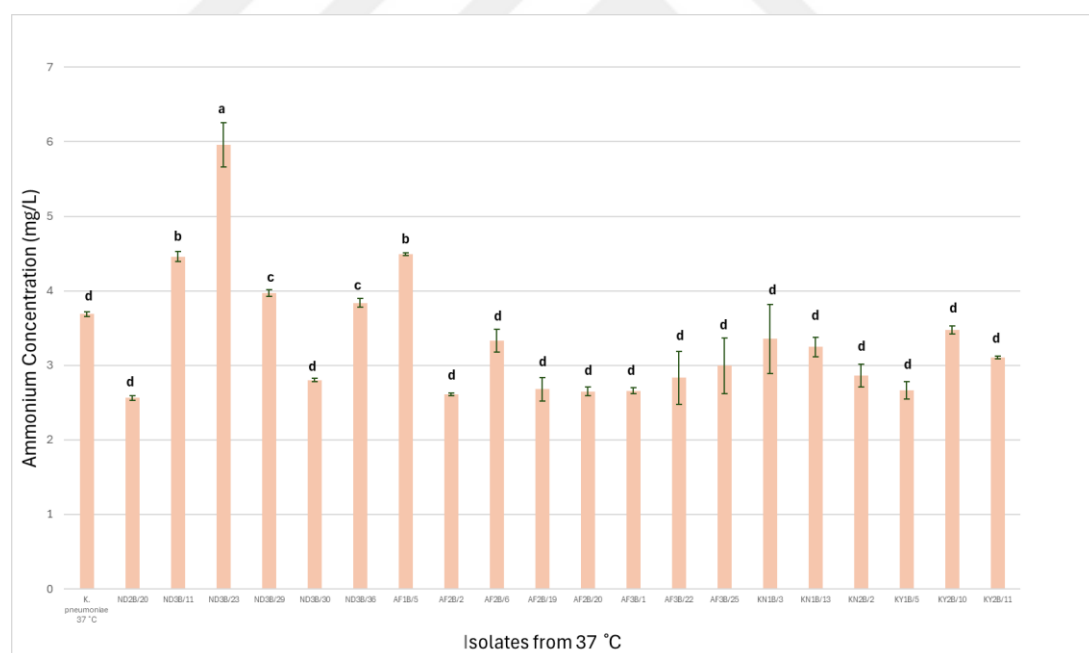


Figure 5.16: Ammonium contractions of the nitrogen-ficing bacteria isolated at 37° C compared with positive control

This workflow ensures that only the most promising nitrogen-fixing candidates advance to detailed characterisation.

5.7. Biochemical Characterization Test Results

Biochemical characterizations of nitrogen fixing bacteria selected by ammonia detection assay are performed for each 30 isolates.

5.7.1. Carbohydrate Utilization Test Results

Phenol red broth is used to detect carbohydrate utilization of the isolates for eleven sugars (glucose, fructose, sucrose, mannitol, mannose, xylose, trehalose, galactose, lactose, cellobiose, and arabinose). For glucose utilization, gas production is also observed via Durham tubes. Other sugar tests are performed in 96-well plates.

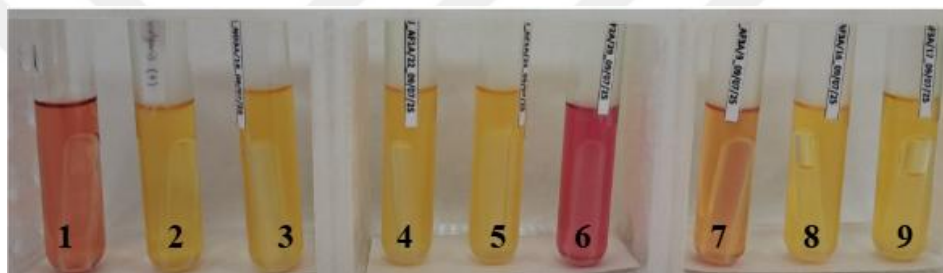


Figure 5.17: Sample of glucose utilization test tubes with Durham tubes. In the order of uninoculated control, *P. vulgaris* (positive control), ND3A/18 (+), AF1A/22 (+), AF1A/23 (+), AF2A/29 (-), AF3A/9 (-), AF3A/16 (+), AF3A/17(+).

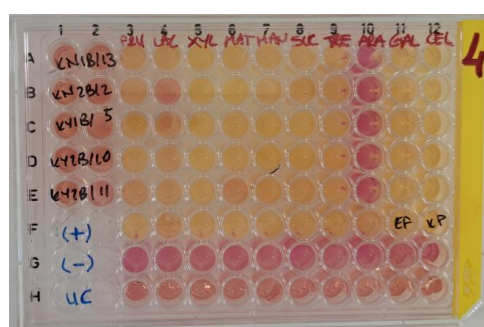


Figure 5.18: Sample of the other ten carbohydrate utilization tests on 96-well plate. In the order of fructose, lactose, xylose, mannitol, mannose, sucrose, trehalose, arabinose, galactose, cellobiose from the third to twelfth column. Bacteria are placed in the rows in the order of KN1B/13, KN2B/2, KY1B/5, KY2B/10, KY2B/11, *E. coli* ATCC 25922 (positive control), *P. aeruginosa* ATCC 15692, uninoculated control.

5.7.2. Triple Sugar Iron Agar Test Results

This media contains three sugars (glucose, sucrose, and lactose) and it is used to observe sugar utilization, gas formation, hydrogen sulphite production.

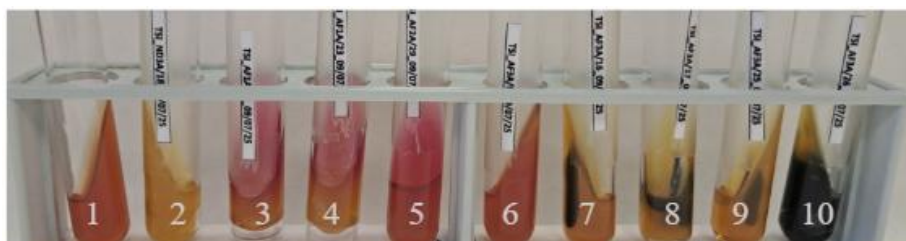


Figure 5.19: Sample of TSI slant agar test tubes. In the order of uninoculated control, ND3A/18, AF1A/22, AF1A/23, AF2A/29, AF3A/9, AF3A/16, AF3A/17, AF3A/25, AF3A/26.

5.7.3. IMViC Tests Results

Indole production, methyl red for pyruvic acid end product, Voges Proskauer, citrate utilization tests.

5.7.3.1. Indole, Hydrogen Sulphite, and Motility Test Results

For indole production tests with motility and hydrogen sulphite production SIM media is used.

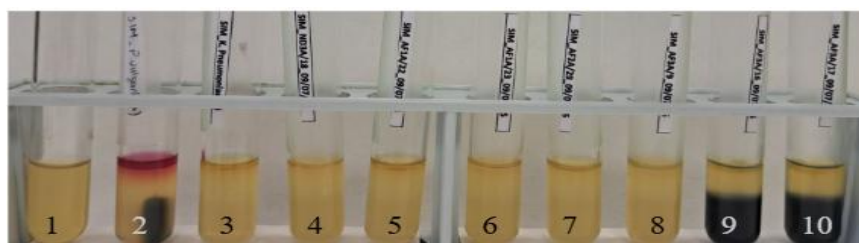


Figure 5.20: Sample of SIM media tubes. In the order of uninoculated control, *P. vulgaris* (positive control), *K. pneumoniae* (negative control), ND3A/18 (-), AF1A/22 (-), AF1A/23 (-), AF2A/29 (-), AF3A/9 (-), AF3A/16 (H₂S, +), AF3A/17 (H₂S, +).

5.7.3.2. Methyl Red Test Results

Pyruvic acid can be broken down into other acids or neutral products. Color change indicates which pathway is utilized to catalyze the sugar.

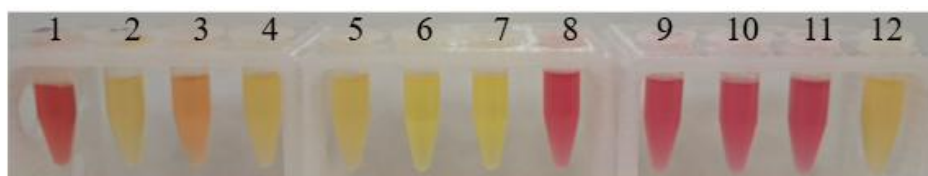


Figure 5.21: Samples of MR test tubes. *P. vulgaris* (positive control), *K. pneumoniae* (negative control), ND3A/18 (-), AF1A/22 (-), AF1A/23 (-), AF2A/29 (-), AF3A/9 (-), AF3A/16 (+), AF3A/17 (+), AF3A/25 (+), AF3A/26 (+), ND2B/20 (-).

5.7.3.3. Voges Proskauer Test Results

Acetoin is a neutral end product of glucose fermentation. VP test is used to detect the production of acetoin.

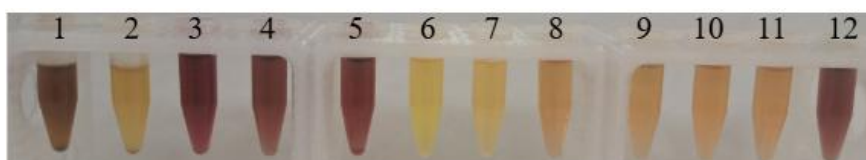


Figure 5.22: Samples of VP test tubes. *K. pneumoniae* (positive control), *P. vulgaris* (negative control), ND3A/18 (+), AF1A/22(+), AF1A/23(+), AF2A/29 (-), AF3A/9 (-), AF3A/16 (-), AF3A/17 (-), AF3A/25 (-), AF3A/26 (-), ND2B/20 (+).

5.7.3.4. Citrate Test Results

Citratase enzyme catalyzes citrate resulting in change of color in the media for this test.

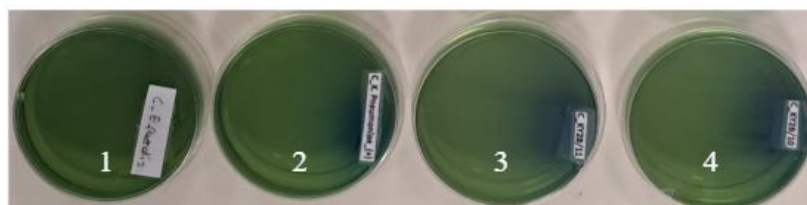


Figure 5.23: Sample of citrate utilization test. In the order of *E. faecalis* MCTC 8213 (negative control), *K. pneumoniae* (positive control), KY2A/11 (+), KY2A/10 (+).

5.7.4. Oxidation-Fermentation Test Results

Some bacteria are facultative anaerobe and some of them are aerobe. OF medium is used to detect how bacteria utilizes sugar.

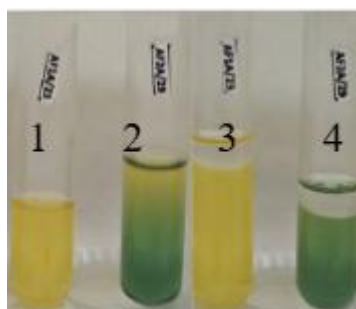


Figure 5.24: Samples of oxidation / fermentation tests. Aerobic (1, 2) and anaerobic (3, 4) environment. In the order of AF1A/ 23 (aerobic test), AF2A/29 (aerobic test), AF1A/23 (anaerobic test), AF2A/29 (aerobic test).

5.7.5. Lysine, Ornithine, and Arginine Test Results

Decarboxylation of lysine, ornithine, and arginine is detected in this test.

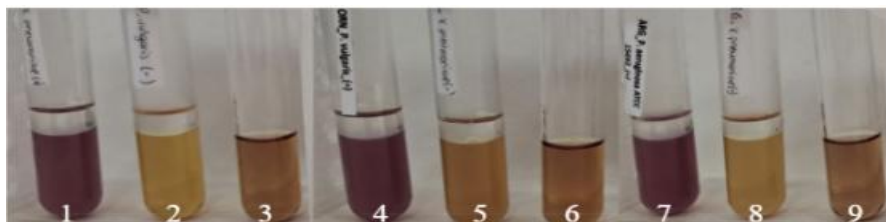


Figure 5.25: Sample of decarboxylase test tubes. From left to right; lysine (1, 2, 3), ornithine (4, 5, 6), and arginine (7, 8, 9). *K. pneumoniae* (lysine, +), *P. vulgaris* (lysine, -), uninoculated control (lysine), *P. vulgaris* (ornithine, +), *K. pneumoniae* (ornithine, -), uninoculated control (ornithine), *P. aeruginosa* ATCC 15962 (arginine, +), *K. pneumoniae* (arginine, -), uninoculated control (arginine).

5.7.6. Urease Test Results

Detection of urease positive isolates via color change in urea broth.



Figure 5.26: Sample of urease test tubes. In the order of uninoculated control, *P. vulgaris* (positive control), ND3A/18 (-), AF1A/22 (-), AF1A/23 (-), AF2A/29 (-), AF3A/9 (-), AF3A/16 (-), AF3A/17 (-), AF3A/25 (-).

5.7.7. Phenylalanine Deaminase Test Results

Detection of deamination phenylalanine from bacterial isolates are tested.

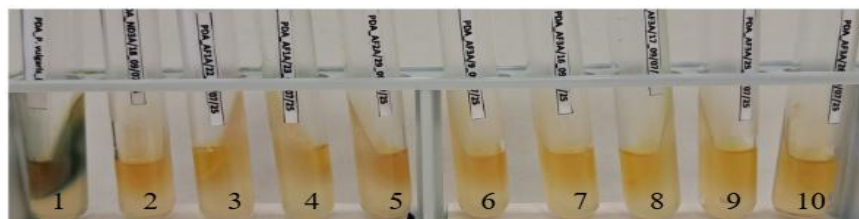


Figure 5.27: Samples of phenylalanine deaminase slant agar test tubes. In the order of *P. vulgaris* (positive control) ND3A/18 (-), AF1A/22(-), AF1A/23(-), AF2A/29 (-), AF3A/9 (-), AF3A/16 (-), AF3A/17 (-), AF3A/25 (-), AF3A/26 (-).

5.7.8. ONPG Test Results

β -galactosidase enzyme existence is detected via artificial ONPG resulting in colored media when catalyzed.

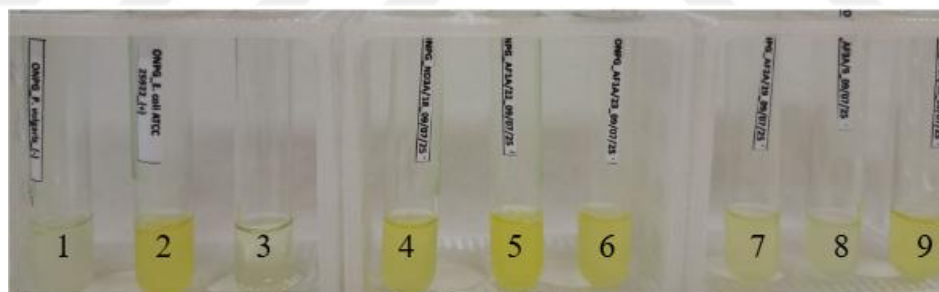


Figure 5.28: Samples of ONPG test tubes. In the order of *P. vulgaris* (negative control), *E. coli* ATCC 25922 (positive control), ND3A/18 (-), AF1A/22 (-), AF1A/23 (-), AF2A/29 (-), AF3A/9 (-), AF3A/16 (-), AF3A/17 (-), AF3A/25 (-).

In total, 30 isolates were selected and prepared for acetylene reduction assay and biochemical characterization tests. The biochemical tests are carried out to characterize isolates closer to their identities. For the indole production test, all 30 isolates were found to be indole-negative. In the methyl red test, 5 isolates were positive and 25 were negative. In the Voges–Proskauer test, 21 isolates were positive and 9 were negative. For the citrate utilization test, 28 isolates were positive and 2 was

negative. Regarding hydrogen sulfide production and motility, 4 isolates were positive and 26 were negative.

In the carbohydrate utilization tests, the results were as follows: glucose — 25 positive and 4 negative; fructose — 24 positive and 6 negative; lactose — 18 positive and 12 negative; xylose — 26 positive and 4 negative; mannitol — 24 positive and 6 negative; mannose — 28 positive and 2 negative; sucrose — 20 positive and 10 negative; trehalose — 26 positive and 4 negative; galactose — 25 positive and 5 negative; cellobiose — 26 positive and 4 negative.

In the phenylalanine deaminase agar test, all 30 isolates were negative. For the decarboxylase tests, the results were as follows: lysine — 2 positive, 27 negative, and 1 inert; ornithine — 28 positive, 1 negative and 1 inert; arginine — 28 positive, 1 negative and 1 inert. For the ONPG test 27 isolates positive and 3 isolates negative had results. Finally, in the urease test, all isolates were negative. Collective results of biochemical characterization tests are presented in **Appendix E**.

These results collectively highlight the biochemical diversity among the selected isolates and provide essential information for their further taxonomic identification.

5.8. 16S rDNA Analysis

The 30 selected nitrogen-fixing bacteria are first morphologically analyzed, screened for nitrogen fixation, characterized biochemically and for their taxonomic identification 16S rDNA analysis is performed.

In the scope of this purpose each isolate underwent DNA isolation to be used in further PCR amplifications. DNA is isolated from 24 h cell cultures of the isolates via Quick DNA Miniprep Plus Kit (ZYMO). The quality and integrity of the extracted genomic DNA were primarily assessed by agarose gel electrophoresis, which confirmed the presence of high-molecular-weight DNA suitable for downstream amplification. DNA concentration and purity were measured using a NanoDrop spectrophotometer, providing A260/A280 and A260/A230 absorbance ratios as indicators of protein and polysaccharide/phenolic compound contamination, respectively. All samples yielded concentrations and purity ratios within acceptable ranges for PCR amplification. Representative gel images are presented in the main text, while detailed NanoDrop measurements for all isolates are provided in **Table 5.6**.

AF2B/20	1.74	44.3
AF3A/9	1.78	84.6
AF3A/16	1.83	119.5
AF3A/17	1.77	82.7
AF3A/25	1.96	145.7
AF3A/26	1.79	194.1
AF3B/1	1.79	36.3
AF3B/22	1.88	19.3
AF3B/25	1.75	51.6
KN1B/3	1.76	62.2
ND3B/26	1.85	88.5

AF2B/20	1.74	44.3
AF3A/9	1.78	84.6
AF3A/16	1.83	119.5
AF3A/17	1.77	82.7
AF3A/25	1.96	145.7
AF3A/26	1.79	194.1
AF3B/1	1.79	36.3
AF3B/22	1.88	19.3
AF3B/25	1.75	51.6
KN1B/3	1.76	62.2
ND3B/26	1.85	88.5

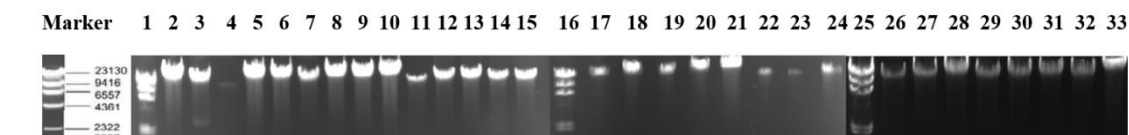


Figure 5.29: The DNA samples assessed on agarose gel (1%) electrophoresis. In the order from left to right; Marker, Lambda Hind III DNA ladder; 1) Marker, 2-15) isolates, 16) marker, 17-24) isolates; 25) marker; 26-33, isolates. Isolate names are presented in **Table 5.6**.

5.8.1. PCR for 16S rDNA Gene Region Amplification

The nearly full-length bacterial 16S rRNA gene was amplified using universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1491R (5'-GGTTACCTTGTTACGACTT-3'), which target conserved regions at the 5' and 3' ends of the gene, respectively. These primers are widely used for bacterial phylogenetic and taxonomic studies due to their broad coverage across diverse bacterial taxa. Thermo Scientific Dream Taq Polymerase Green Kit is used following the protocol mentioned in the **Section 4.8.1**. Obtained PCR products are then assessed on agarose gel electrophoresis as it is shown in the **Figure 5.30**. Expected band is around 1500 bp and when we compare the PCR products with 1Kb DNA ladder they showed band around the expected size.

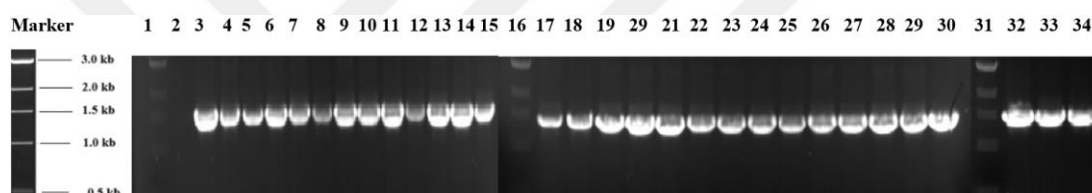


Figure 5.30: The samples from PCR products on agarose gel (0.8%) electrophoresis. In the order from left to right In the order from left to right; Marker, Lambda Hind III DNA ladder; 1) marker, 2) blank, 3-15) isolates, 16) marker, 17-30) isolates; 31) marker; 32-34, isolates. Isolate names are presented in **Table 5.6**.

The PCR products of the 30 selected isolates were sequenced via BM LAB's service. The final results were screened using the Basic Local Alignment Search Tool (BLASTN) to determine sequence similarity. **Table 5.7** shows the top three matches with the highest similarity percentage.

Table 5.7: Identification of isolates based on 16S rDNA sequence analysis results.

Isolate Number	Isolate Name	BLASTn Results	% Identity	Estimated Taxonomy
109	ND2B/20	<i>Enterobacter cloacae</i> strain AZ3	100%	<i>Enterobacter</i> sp.
		<i>Enterobacter ludwigii</i> strain A30606	100%	
		<i>Pantoea agglomerans</i> strain 21-a blue	100%	

Table 5.8: Continued.

Isolate Number	Isolate Name	BLASTn Results	% Identity	Estimated Taxonomy
126	ND3A/18	<i>Lelliottia amnigena</i> strain B29-6	100%	<i>Enterobacter</i> sp.
		<i>Enterobacter</i> sp. 638	100%	
		<i>Klebsiella aerogenes</i> strain g24	100%	
148	ND3B/11	<i>Curtobacterium</i> sp	99.72%	<i>Curtobacterium</i> sp.
159	ND3B/23	<i>Enterobacter ludwigii</i> strain A30606	100%	<i>Enterobacter</i> sp.
		<i>Enterobacter cloacae</i> strain Ec022	100%	
165	ND3B/29	<i>Enterobacter cloacae</i> strain MS-27	98.13%	<i>Enterobacter</i> sp.
		<i>Enterobacter ludwigii</i> strain DP5	98.13%	
166	ND3B/30	<i>Enterobacter roggenkampii</i> strain Colony143	100%	<i>Enterobacter</i> sp.
		<i>Enterobacter chengduensis</i> strain SKC/As-6	100%	
		<i>Enterobacter asburiae</i> strain 42A:	100%	
172	ND3B/36	<i>Enterobacter ludwigii</i> strain A30606	100%	<i>Enterobacter</i> sp.
		<i>Enterobacter cloacae</i> subsp. <i>dissolvens</i> strain 22STR470	100%	
186	AF1A/22	<i>Enterobacter cancerogenus</i> JCM 3947	99.86%	<i>Enterobacter</i> sp.
		<i>Enterobacter asburiae</i> TA61661	99.86%	
187	AF1A/23	<i>Enterobacter cancerogenus</i> strain 3.2.17	99.86%	<i>Enterobacter</i> sp.
		<i>Enterobacter cloacae</i>	99.86%	
192	AF1B/5	<i>Pseudomonas stutzeri</i> strain 2Aben1	98.04%	<i>Pseudomonas</i> sp.
		<i>Pseudomonas</i> sp. CF14-10	98.04%	
212	AF2A/29	<i>Enterobacter hormaechei</i> strain DP29	100%	<i>Enterobacter</i> sp.
		<i>Enterobacter hormaechei</i> FUJ80022	100%	
213	AF2B/2	<i>Enterobacter hormaechei</i> FUJ80022	100%	<i>Enterobacter</i> sp.
		<i>Enterobacter cloacae</i> complex sp. ECL411	100%	

Table 5.8: Continued.

Isolate Number	Isolate Name	BLASTn Results	% Identity	Estimated Taxonomy
214	AF2B/6	<i>Pseudomonas putida</i> strain AFUM4	100%	<i>Pseudomonas</i> sp.
		<i>Pseudomonas</i> sp. P1B16	100%	
220	AF2B/19	<i>Enterobacter</i> sp. BR1	98.33%	<i>Enterobacter</i> sp.
		<i>Enterobacter cloacae</i> strain 309-2	98.25%	
		<i>Enterobacter hormaechei</i> strain 21STR103	98.25%	
221	AF2B/20	<i>Enterobacter hormaechei</i> FUJ80022	100%	<i>Enterobacter</i> sp.
		<i>Enterobacter cloacae</i> complex sp. ECL411	100%	
227	AF3A/9	<i>Pseudomonas</i> sp. strain CEMTC_3966	100%	<i>Pseudomonas</i> sp.
		<i>Pseudomonas putida</i> strain XT911	100%	
		<i>Pseudomonas qingdaonensis</i> strain AS279	100%	
234	AF3A/16	<i>Citrobacter braakii</i> strain RHB44-SE-C08	98~%	<i>Citrobacter</i> sp.
		<i>Citrobacter freundii</i> strain CH-GX-LA13-2022	98~%	
235	AF3A/17	<i>Citrobacter braakii</i> strain Colony409	98~%	<i>Citrobacter</i> sp.
		<i>Citrobacter braakii</i> strain RHB09-SO-C07	98~%	
241	AF3A/25	<i>Citrobacter braakii</i> strain Colony409	98~%	<i>Citrobacter</i> sp.
		<i>Citrobacter freundii</i> strain CH-GX-LA13-2022	98~%	
242	AF3A/26	<i>Yokenella</i> sp. strain FP3GSM01a	97~%	<i>Citrobacter</i> sp.
		<i>Enterobacter</i> sp. strain YUSE7	97~%	
		<i>Citrobacter freundii</i> strain AtetA	97~%	

Table 5.8: Continued

Isolate Number	Isolate Name	BLASTn Results	% Identity	Estimated Taxonomy
247	AF3B/1	<i>Enterobacter ludwigii</i> strain A30606	100%	<i>Enterobacter</i> sp.
		<i>Enterobacter cloacae</i> subsp. <i>dissolvens</i> strain 22STR470	100%	
		<i>Pantoea agglomerans</i> strain 16SrDNA-55	100%	
261	AF3B/22	<i>Enterobacter ludwigii</i> strain A30606	100%	<i>Enterobacter</i> sp.
		<i>Enterobacter cloacae</i> subsp. <i>dissolvens</i> strain 22STR470	100%	
263	AF3B/24	<i>Pseudomonas stutzeri</i> JCM 20400	100%	<i>Pseudomonas</i> sp.
		<i>Pseudomonas</i> sp. strain BS-2	100%	
264	AF3B/25	<i>Citrobacter braakii</i> strain Colony409	98~%	<i>Citrobacter</i> sp.
		<i>Citrobacter</i> sp. MGH105	98~%	
281	KN1B/3	<i>Enterobacter mori</i> strain ECC-007	98~%	<i>Enterobacter</i> sp.
		<i>Pantoea agglomerans</i> strain PVM	98~%	
288	KN1B/13	<i>Enterobacter mori</i> strain 08-091	98~%	<i>Enterobacter</i> sp.
		<i>Enterobacter roggenkampii</i> strain WL111	98~%	
306	KN2B/2	<i>Enterobacter roggenkampii</i> strain Colony143	98~%	<i>Enterobacter</i> sp.
		<i>Enterobacter asburiae</i> strain 42A:C	98~%	
		<i>Enterobacter</i> sp. strain NF14	98~%	
372	KY1B/5	<i>Enterobacter ludwigii</i> strain A30606	98~%	<i>Enterobacter</i> sp.
		<i>Enterobacter</i> sp. BNK-18	98~%	
412	KY2B/10	<i>Acinetobacter</i> sp. D009	100%	<i>Acinetobacter</i> sp.
		<i>Acinetobacter pittii</i> strain BJ6	100%	

		<i>Acinetobacter calcoaceticus</i> strain YL014	100%	
413	KY2B/11	<i>Enterobacter ludwigii</i> strain A30606	98~%	<i>Enterobacter</i> sp.

Analysis for each of the 30 isolates are evaluated according to both 16S rRNA BLASTn results and biochemical test results.



6. DISCUSSION

This study aims to isolate rhizospheric nitrogen-fixing bacteria from the soil that is collected from major potato producing regions in Türkiye, including Niğde, Afyonkarahisar, Konya, Kayseri and İzmir. The locations are selected considering not only their economic importance in potato production but they were selected to capture potential microbial diversity associated with different physiochemical characteristics. Parameters such as pH, organic matter content, electrical conductivity, and nutrient availability are known to influence the composition and activity of soil microbial communities, including nitrogen-fixing bacteria. In general, soils with neutral to slightly alkaline pH and moderate-to-high organic matter are considered favorable for diazotrophs, as they support both microbial growth and enzymatic activity [Sylvia et al., 2005]. In the present study, the highest number of nitrogen-fixing isolates was obtained from Niğde soils, despite İzmir and Kayseri exhibiting the highest organic matter contents. This finding suggests that while organic matter provides essential carbon and energy sources for microbial activity, it does not solely determine the abundance of nitrogen-fixing bacteria. Other factors, such as pH, EC, and soil structure, likely play significant roles.

High EC, which can indicate salinity stress, has been reported to negatively affect microbial diversity and nitrogen-fixing capacity [Rietz & Haynes, 2003]. If İzmir or Kayseri soils had elevated EC levels despite their higher organic matter, this could have limited microbial proliferation. Unfortunately, the complex interactions among soil properties often make it difficult to attribute microbial abundance to a single factor.

Moreover, soil texture influences microbial habitats by affecting porosity, aeration, and moisture retention. Loamy or sandy-loam soils, for example, tend to provide better aeration and microenvironments for aerobic and facultatively anaerobic diazotrophs compared to heavy clay soils [Ikeda et al., 2003]. The variety in the sampling of rhizospheric soil provided a ground for isolation of rhizobacteria in the scope of designing a biofertilizer specific to the plant and the region. So, while pH and organic matter are important factors for microbial activity, the results of this study demonstrate that a combination of soil characteristics, including EC and texture, likely influenced the observed distribution of nitrogen-fixing isolates. Further investigation into the

relative contributions of these factors could provide deeper insight into the ecology of diazotrophs in potato rhizospheres.

The pH values across the soils were within a range generally favorable for microbial activity — close to neutral or slightly alkaline — which aligns with optimal conditions reported for nitrogen-fixing bacteria like *Bacillus*, *Pseudomonas*, and *Enterobacter* [Chen et al., 2007]. However, the Niğde soil, from which the highest number of isolates was obtained, did not have the most neutral pH nor the highest organic matter. This suggests that additional factors, such as lower EC, suitable aeration due to soil texture, or historical cultivation practices, might have contributed to the higher isolate recovery. The variety in the sampling of rhizospheric soil provided a ground for isolation of rhizobacteria in the scope of designing a biofertilizer specific to the plant and the region.

The soil samples underwent pretreatments and experiments for bacterial isolation; around 600 isolates are selected by observing colonies that differ in morphology (pigmentation, elevation, margin, etc.) Preliminary tests such as Gram staining, catalase test, etc. are performed on each and every one of the isolates. These isolates are initially screened to detect the ones with high resemblance in colony characterization. Isolates with similar colony morphological traits and preliminary test results are clustered and one of them is left as a representation. Total of 542 distinct isolates subjected to further tests to investigate the nitrogen fixing bacteria.

When the final results of preliminary tests were evaluated (Appendix B), they were consistent with literature findings. Our results showed that 82,8% of the isolates are catalase (+) and 39.2% of the isolates are oxidase (+). Literature provided that soil bacteria were predominantly aerobic or facultative anaerobic which enables growth in oxygen-rich environments. Since they are exposed to reactive oxygen species as a result of aerobic respiration, 70 % of catalase activity is commonly detected among the culture-based isolates. Similarly, in aerobic culture, 20-40% of isolates are oxidase (+) [Torsvik and Øvreås, 2002; Schneegurt, 2013].

After obtaining 542 isolates, they were screened to detect nitrogen fixing bacteria. In the sense of this purpose, initial screening of potential nitrogen-fixing bacteria was carried out using N-free bromothymol blue (NFB) medium, which allows for a quick

and effective preliminary selection. Nitrogen fixation is carried out by diazotrophs. These bacteria fixate atmospheric nitrogen into ammonium which is a molecule that plants can directly use in the form of ammonia. Ammonia accumulation in the media causes increase in the pH levels. The utilization of NFB medium is a crucial step in the sense of enhancing nitrogen fixation and detecting pH shift by color change of pH indicator bromothymol blue. NFB medium contains malic acid as only carbon source along with multiple salt compounds, with some of them supplying essential micronutrients such as, iron, magnesium, molybdate. Nitrogen fixing bacteria, generally, can metabolize malic acid, this media provides key elements required for activation of nitrogenase enzyme. To conserve space and facilitate simultaneous observation and comparison of multiple bacterial isolates, the medium is dispensed into 24 well-plates. Observations are performed on day 1, 2, 3, and 7 to detect the better nitrogen fixing bacteria according to color change. Positive control *K. pneumoniae* and negative control *S. aureus*, are also inoculated on the plates alongside with the isolates for comparison with each other. Some of the isolates were slow but consistent, some of them changed color on day 1, some of them didn't change at all and some of them changed color closer to the end of incubation. They were evaluated according to **Figure 5.9** individually and the faster and better nitrogen fixers, relatively, are selected. Since this evaluation is dependent on color change of media which is influenced from medium pH, NFB medium alone is not definitive. Many non-diazotrophic bacteria can tolerate nitrogen-free conditions temporarily or generate alkalinity through other metabolic processes, leading to false positives. Therefore, while useful for narrowing down candidates, NFB results must be confirmed by more quantitative methods to avoid overestimating the nitrogen-fixing potential. For this reason, an ammonium detection assay (Nessler's reagent assay) is carried out. Approximately 150 isolates are selected in NFB medium to be assayed on Nessler's reagent assay.

To address the limitations of NFB medium, Nessler's reagent was employed to quantify the ammonia released by the isolates. The reagent is generally used to detect ammonia contamination in water sources, aqueous environments. The Nessler's reagent possesses potassium tetraiodomercurate (II) a compound that forms a colored complex when come into reaction with ammonia. The logic behind this assay is to detect ammonia concentration via measuring color density of bacteria culture substrate

after being treated with Nessler's reagent. Quantitative measurements and calculation of ammonia concentration is carried out via a standard curve made with known ammonia concentrations and Nessler's reagent. In the literature, Nessler's reagent is vastly used to detect ammonia in water sources, so a protocol for bacterial isolates was not available. Therefore, optimizations for this assay's protocol is performed on known concentrations of ammonia in NFB medium, before starting with the isolates.

Giovanni (2011) shared a protocol on how to prepare a standard curve with ammonium sulphate for Nessler's reagent. In the original protocol, Rochelle salt was used as a chelating agent. However, since it was not available in our laboratory, 0.1 M EDTA was chosen as an alternative chelating agent. Therefore, the first optimization was performed to evaluate whether the inclusion of 0.1 M EDTA was necessary or not. Known concentrations are prepared (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30) mg/L. One set of experiments was treated with 0.1 M EDTA and the other set was not. 10% of Nessler reagent and for the measurement 420 nm wavelength is applied as it was recommended in the literature. Results showed that 0.1 M EDTA is needed as a chelating factor. When Nessler's assay results of known concentration are compared with their true concentration, EDTA-free ones showed a decrease in ammonia detection. For each experiment their own standard curve is created and calculations were performed according to equations of each curve.

In the literature, the recommended amount of Nessler's reagent is reported to be 10–20% (v/v) of the sample volume. To determine the least but most efficient amount of Nessler's reagent, experiments were conducted using 5%, 10%, 15%, and 20% (v/v) concentrations; a standard curve was prepared for each concentration to calculate the ammonia concentrations accurately. The comparison of the groups revealed no statistically significant differences ($p > 0.05$). However, when the RMSE values were considered, the 10% group showed the smallest deviation from the original concentration, suggesting it was the most accurate. Also, since Nessler's reagent contains mercury, working with lower concentrations is safer for both the user and the environment. Therefore, 10% Nessler's reagent was chosen as the most appropriate concentration among the four tested. The final protocol was thus performed using 0.1 M EDTA, 10% Nessler's reagent, and absorbance measured at 420 nm.

After the optimization of the protocol, isolate assays were carried out. The isolates were cultured in LB broth, and to remove any residual carbon and nitrogen, they were

washed multiple times with PBS. To start with a known bacterial density, OD₆₀₀ measurements were performed. Inoculations into the NFB broth medium were carried out as equally as possible, in duplicates. NFB broth (without bromothymol blue) was used as a nitrogen-free medium to promote and detect ammonia fixed from atmospheric nitrogen. After 7 days of incubation, the supernatant was collected for the Nessler's assay and divided into two portions for testing. Replications were performed as much as possible to verify the results. Measurements were conducted using a microplate reader. The obtained absorbance values were applied to the standard curve equation to calculate the ammonia concentration in the medium.

Isolates that surpassed the positive control were selected, resulting in approximately 30 isolates. The assay provided valuable quantitative data, supporting the selection of the most promising isolates based on actual ammonia production rather than growth alone. Thus, the Nessler's assay complemented the NFB medium by verifying and ranking the isolates' ability to fix nitrogen.

Since the isolates were inoculated at their original isolation temperatures, the positive control *K. pneumoniae* was also incubated at both 30 °C and 37 °C. Each group was evaluated according to the concentration value presented by the positive control at the corresponding temperature. At 30 °C, *K. pneumoniae* produced an ammonia concentration of approximately 1.2 mg/L; therefore, isolates that yielded ≥ 2.0 mg/L were selected. At 37 °C, where the positive control showed approximately 3.7 mg/L, isolates that yielded ≥ 2.5 mg/L were selected.

The biochemical characterization of the 29 nitrogen-fixing isolates revealed a diverse set of metabolic traits, which are largely consistent with the reported characteristics of diazotrophic bacteria in the literature. Indole production was negative in all isolates, which aligns with the typical profile of genera such as *Bacillus*, *Pseudomonas*, and *Azospirillum*, which generally lack tryptophanase activity. The methyl red test was positive in 4 isolates, suggesting mixed-acid fermentation, a trait sometimes associated with *Enterobacter* species. Conversely, 21 isolates were Voges-Proskauer positive, indicating acetoin production through the butylene glycol pathway, a hallmark of *Enterobacter* and *Klebsiella*. Nearly all isolates utilized citrate (28/29), which is expected of many rhizobacteria, including *Enterobacter*, *Klebsiella*, *Azospirillum*, and *Pseudomonas*, as it reflects their ability to metabolize alternative carbon sources.

Hydrogen sulfide production and motility were observed in only 4 isolates, which may correspond to motile diazotrophs such as *Pseudomonas* or certain *Enterobacter* strains. Carbohydrate utilization patterns showed that most isolates could metabolize glucose, fructose, mannitol, mannose, galactose, and cellobiose — pathways commonly active in rhizospheric diazotrophs. Sucrose and lactose utilization were less prevalent, consistent with the variability of these pathways among *Klebsiella* and *Pseudomonas* species. High utilization of trehalose (26/29) is noteworthy, as this sugar is often metabolized by *Bacillus* and *Pseudomonas*.

Decarboxylase activities further supported this diversity: the majority of isolates were positive for ornithine and arginine decarboxylases, while only two isolates were positive for lysine decarboxylase. Such patterns are in agreement with *Enterobacter*, *Pseudomonas*, and *Azospirillum*, which commonly exhibit ornithine and arginine decarboxylase activity but variable lysine activity. Notably, none of the isolates exhibited phenylalanine deaminase or urease activity, helping to rule out non-diazotrophic genera such as *Proteus*.

These findings indicate that the selected isolates likely include members of several diazotrophic genera, most notably *Enterobacter*, *Klebsiella*, *Pseudomonas*, *Bacillus*, and possibly *Azospirillum*. Their biochemical profiles not only align with those reported in previous studies of nitrogen-fixing bacteria from various crops [Chen et al., 2007; Hafeez et al., 2006; Baldani & Baldani, 2005] but also highlight their metabolic versatility and ecological potential.

While biochemical and physiological tests suggested that the isolates likely belonged to known diazotrophic genera such as *Enterobacter*, *Pseudomonas*, and *Klebsiella* these conventional methods do not offer sufficient resolution to distinguish closely related taxa reliably. The 16S rDNA gene, being highly conserved yet containing variable regions, enables precise identification of bacteria at the genus and sometimes species level. Moreover, sequence alignment and phylogenetic tree construction provide insights into the evolutionary relationships of the isolates with known diazotrophs. This molecular approach, therefore, validates and strengthens the biochemical characterization, ensuring accurate classification of the isolates and supporting their potential as biofertilizer candidates.

To confirm the taxonomic identity of the best isolates, sequencing of the 16S rDNA gene was performed. 16S rRNA BLASTn analysis and the biochemical test results, each isolate was assigned to a specific genus. Based on these data, isolate 109 appears to be more closely related to the genus *Enterobacter* rather than *Pantoea*. This conclusion was mainly supported by results of the decarboxylase test. *Enterobacter* species that matched the isolate are known not to utilize lysine but do utilize arginine and ornithine, whereas *Pantoea* species typically do not show decarboxylase activity for any of these three amino acids [BacDive, 2025]. Therefore, the estimated taxonomy for isolate 109 is *Enterobacter* sp.

Isolate 126 is also assigned to the genus *Enterobacter*. When compared with *Klebsiella aerogenes*, the isolate was found to be lysine-negative, similar to *K. aerogenes*. Based on this characteristic, the isolate appears to be more closely related to *Enterobacter* [BacDive, 2025].

Isolate 148 is thought to be a *Curtobacterium* sp. The biochemical test results and morphological characterization of this isolate were consistent with the literature, such as being Gram-positive, rod-shaped and with negative results for all IMViC tests, optimized for Gram-negative enteric bacteria [Collins & Jones, 1983; BacDive, 2025].

Isolates 159, 165, 166, 172, 186, and 187 are thought to be *Enterobacter* spp. They had largely the same biochemical test profile: all were indole and MR negative, and all were VP and citrate positive [BacDive, 2025]. Sugar utilization patterns were largely consistent except for lactose, sucrose, and arabinose. In particular, isolate 172 utilized lactose whereas the others did not. Isolates 186 and 187 were able to utilize arabinose but not sucrose, while the others showed the opposite pattern. For arabinose, the difference could be due to the configuration of the sugar used in our tests: We used D-arabinose are usually reported in literature, with *Enterobacter* species showing positive utilization [Brady et al., 2013; BacDive, 2025]. This could explain why other isolates showed negative utilization of arabinose. When comparing the matched species, isolates 186 and 187 correlated more with *Enterobacter cancerogenus*, as the literature reports that *E. cancerogenus* cannot utilize sucrose, whereas *E. asburiae* and *E. cloacae* can [BacDive, 2025].

Isolates 247 and 261 also showed very similar 16S rRNA and biochemical test results, so they probably belong to *Enterobacter* sp.

Isolates 192 and 263 are probably *Pseudomonas* sp., in particular *Pseudomonas stutzeri*, which is known to be a strong nitrogen fixer. Their biochemical results were consistent with literature reports, including the lack of sugar utilization and decarboxylase activity [BacDive, 2025]. Importantly, isolate 263 produced the highest ammonium concentration in the Nessler's reagent test.

Isolate 212 is estimated to be *Enterobacter* sp. and is consistent with several *E. hormaechei* strains, and its biochemical profile showed no major differences from the expected results. Similarly, isolate 213 was also identified as *Enterobacter* sp.

Isolates 214 and 227 are presumed to be *Pseudomonas* spp., as their O/F test results and sugar utilization patterns consistent with descriptions in the literature [Palleroni, 2010; BacDive, 2025].

Isolates 220 and 221 are presumably *Enterobacter* spp. and their biochemical test results were almost identical. Isolate 220 showed ~98% similarity to *Enterobacter* sp., while isolate 221 was closer to species such as *E. cloacae* or *E. hormaechei*.

Isolates 281, 288, 306, 372, and 413 all showed ~98% similarity to *Enterobacter* sp., and their biochemical test results consistent with this assignment. However, isolates 306 and 372 showed no utilization of fructose, making species-level identification difficult.

Isolates 234, 235 and 241 showed ~98% similarity to *Citrobacter* sp. and their biochemical test results were consistent with this genus, including the important characteristic of H₂S production [BacDive, 2025]. Isolate 264 was also judged to be *Citrobacter*, but did not produce H₂S, suggesting that it may be *C. braakii*, a species known not to produce H₂S [Brenner et al., 2015]. Isolate 242 also produced H₂S and was consistent with *Citrobacter* spp., further supporting this classification at the genus-level.

Finally, isolate 412 matched 100% with several *Acinetobacter* strains, with a 1430 bp alignment in BLAST analysis, and its biochemical characteristics were consistent with the genus [Bouvet & Grimont, 1986; BacDive, 2025].

Although 16S rRNA sequencing in combination with biochemical tests has provided valuable insights at the genus level, the identification of bacteria at the species level remains a challenge. In closely related genera such as *Enterobacter*, *Klebsiella*, and *Pantoea*, overlapping phenotypic characteristics often limit the discriminatory power of biochemical tests [Hoffmann & Roggenkamp, 2003; Brady et al., 2013]. In addition, 16S rRNA gene sequences often show >98% similarity between species within the Enterobacteriaceae, making assignment to species-level uncertain [Brady & Cleenwerck, 2014]. Additional molecular markers such as *hsp60* (heat shock protein 60 gene) and *rpoB* (RNA polymerase β -subunit gene) or multilocus sequence typing (MLST), are therefore recommended for higher resolution.

It should also be noted that despite compliance with aseptic conditions, possible undetected contamination may lead to deviations in the biochemical test results. Moreover, variations of the sugar configuration (e.g., D- vs. L-arabinose) or the medium composition may explain discrepancies between our results and those reported in the literature.

Nevertheless, the isolates characterized in this study represent valuable microbial resources. Their potential for nitrogen fixation, especially the isolates identified as *Pseudomonas stutzeri* and *Enterobacter* spp., underlines their importance for agricultural applications, including the development of biofertilizer and sustainable crop production.

7. CONCLUSION

In this study, initial 600 isolates are collected in the purpose of finding nitrogen-fixing bacteria. The isolate were first characterized morphologically and biochemically and screened for their nitrogen-fixing ability in NFB medium and selected isolates then further quantitatively analysed by ammonium detection assay. The thirty isolates which had the highest ammonium accumulation in their surrounding are selected and biochemical tests are performed to charecterize them. For further taxonomic identification 16S rRNA analysis is carried out and finally they are identified as such genera as *Pseudomonas*, *Enterobacter*, *Klebsiella*, and etc. These results highlight the considerable diversity of native diazotrophs inhabiting potato rhizospheres in Türkiye and emphasize their potential role as biofertilizer candidates. By identifying and characterizing such strains, gives information about the possibility of their contribution in reducing reliance on chemical fertilizers Future studies should focus on validating the plant growth-promoting effects of these isolates under greenhouse and field conditions, as well as whole-genome analyses to better understand the molecular basis of nitrogen fixation and other plant-beenficial traits.to improve sustainability, ecological cost, soil health and product quality.

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BIOGRAPHY

Sena USTA, she completed her secondary education at Türk Kızılayı Kartal Anadolu High School in İstanbul and she enrolled in the Department of Molecular Biology and Genetics at Gebze Technical University, where she received her Bachelor of Science degree in 2023. During her undergraduate studies, she developed a strong interest in microbiology, biotechnology and plant-microbe interactions.

Following her graduation, she was admitted to the Master's program in Molecular Biology and Genetics at Gebze Technical University where she focused on microbial ecology, nitrogen-fixing bacteria, and plant-microbe interactions. Her thesis project, supported by TÜBİTAK 2210-A and COST Action Grant No. 1240159, involves the isolation, characterization, and molecular identification of plant growth-promoting rhizobacteria (PGPR) from potato rhizosphere soils collected from various regions of Türkiye.



PUBLICATIONS AND PRESENTATIONS FROM THE THESIS

Usta S. (2025), “Isolation, Purification and Characterization of Nitrogen-fixing Bacteria from Potato Rhizosphere Soils in Türkiye”, 9th GTU Graduate Research Symposium, 41400, Kocaeli, Türkiye, 26-27 May.



APPENDICES

APPENDIX-A: Colony morphologies of the 542 Isolates

Isolate Number	Isolate Name	Colony Morphology							
		Pigmentation	Shape	Margin	Elevation	Texture	Size	Appearance	Optical Property
1	ND1A/2	Yellowish White	Circular	Entire	Raised	Smooth	Small	Shiny	Opaque
2	ND1A/3	White	Circular	Entire	Raised	Smooth	Medium	Shiny	Opaque
3	ND1A/4	Yellowish	Circular	Entire	Raised	Smooth	Medium	Shiny	Opaque
4	ND1A/5	Yellow	Circular	Entire	Effuse	Smooth	Small	Dull	Opaque
5	ND1A/6	White	Circular	Entire	Raised	Smooth	Small	Shiny	Opaque
6	ND1A/7	White	Irregular	Wavy	Effuse	Smooth	Medium	Dull	Translucent
7	ND1A/8	Yellow	Circular	Entire	Effuse	Smooth	Small	Dull	Opaque
8	ND1A/10	White	Irregular	Wavy	Effuse	Smooth	Large	Dull	Translucent
9	ND1A/11	Yellowish White	Circular	Entire	Raised	Finely Granular	Medium	Shiny	Opaque
10	ND1A/12	Yellow	Irregular	Lobate	Raised	Smooth	Medium	Shiny	Opaque
11	ND1A/13	White	Irregular	Lobate	Raised	Smooth	Medium	Shiny	Opaque
12	ND1A/15	Yellow	Circular	Entire	Raised	Smooth	Medium	Dull	Opaque
13	ND1A/16	Yellowish White	Irregular	Wavy	Effuse	Smooth	Large	Dull	Translucent
14	ND1A/17	White	Circular	Lobate	Effuse	Smooth	Small	Dull	Transparent
15	ND1A/18	Yellow	Circular	Entire	Raised	Smooth	Medium	Shiny	Opaque
16	ND1A/19	White	Circular	Entire	Raised	Finely Granular	Medium	Shiny	Opaque
17	ND1A/20	Yellowish White	Irregular	Wavy	Raised	Finely Granular	Medium	Shiny	Opaque
18	ND1A/21	Yellow	Irregular	Wavy	Raised	Smooth	Small	Shiny	Opaque
19	ND1A/22	White	Circular	Entire	Raised	Coarsely Granular	Small	Shiny	Translucent
20	ND1A/23	White	Irregular	Wavy	Effuse	Finely Granular	Large	Shiny	Translucent
21	ND1A/24	Yellowish White	Circular	Entire	Raised	Smooth	Medium	Shiny	Opaque
22	ND1A/25	Yellow	Circular	Entire	Raised	Smooth	Medium	Dull	Opaque
23	ND1A/26	White	Irregular	Wavy	Raised	Smooth	Medium	Shiny	Translucent
24	ND1A/27	White	Irregular	Wavy	Raised	Arborescent	Large	Shiny	Translucent
25	ND1A/28	White	Irregular	Lobate	Raised	Smooth	Large	Shiny	Opaque
26	ND1A/29	Yellowish White	Irregular	Wavy	Raised	Smooth	Large	Shiny	Opaque
27	ND1A/31	White	Irregular	Crenate	Raised	Finely Granular	Large	Shiny	Opaque
28	ND1A/32	White	Irregular	Lobate	Raised	Smooth	Large	Shiny	Opaque
29	ND1A/33	Yellow	Irregular	Wavy	Raised	Smooth	Small	Shiny	Translucent
30	ND1A/34	Yellow	Circular	Entire	Effuse	Smooth	Small	Shiny	Opaque
31	ND1A/36	White	Irregular	Entire	Effuse	Arborescent	Medium	Shiny	Translucent
32	ND1B/1	Yellow	Circular	Entire	Raised	Smooth	Small	Shiny	Opaque
33	ND1B/3	Yellow	Circular	Entire	Raised	Smooth	Medium	Shiny	Opaque
34	ND1B/6	White	Circular	crenate	Raised	Arborescent	Large	Shiny	Opaque
35	ND1B/7	White	Circular	crenate	Raised	Arborescent	Medium	Shiny	Opaque
36	ND1B/8	White	Irregular	Entire	Raised	Wavy Interlaced	Medium	Shiny	Transparent
37	ND1B/9	White	Irregular	Lobate	Raised	Smooth	Medium	Shiny	Opaque
38	ND1B/10	White	Irregular	Wavy	Effuse	Smooth	Large	Dull	Translucent
39	ND1B/11	Yellowish White	Irregular	Lobate	Raised	Smooth	Small	Shiny	Opaque
40	ND1B/12	White	Irregular	crenate	Raised	Smooth	Large	Shiny	Opaque
41	ND1B/13	Yellowish White	Irregular	Lobate	Effuse	Smooth	Large	Dull	Translucent
42	ND1B/14	Yellowish White	Irregular	Wavy	Umbonate	Arborescent	Large	Shiny	Translucent
43	ND1B/16	Yellowish White	Irregular	crenate	Umbonate	Arborescent	Medium	Shiny	Translucent
44	ND1B/17	Yellowish White	Punctiform	Entire	Raised	Smooth	Small	Shiny	Opaque
45	ND1B/18	Yellowish White	Irregular	Wavy	Umbonate	Arborescent	Small	Shiny	Translucent
46	ND1B/19	Yellowish White	Irregular	Ciliate	Raised	Arborescent	Medium	Shiny	Translucent
47	ND1B/20	Yellow	Circular	crenate	Umbonate	Arborescent	Large	Shiny	Translucent
48	ND1B/21	White	Circular	crenate	Umbonate	Arborescent	Large	Shiny	Translucent
49	ND1B/22	Yellowish White	Circular	Entire	Raised	Smooth	Small	Shiny	Opaque
50	ND1B/23	Yellow	Circular	Entire	Raised	Smooth	Medium	Shiny	Opaque
51	ND1B/24	White	Irregular	crenate	Umbonate	Arborescent	Large	Shiny	Translucent
52	ND1B/25	Yellowish White	Irregular	crenate	Umbonate	Wavy Interlaced	Large	Shiny	Translucent
53	ND1B/26	Yellowish White	Irregular	Entire	Effuse	Wavy Interlaced	Large	Shiny	Translucent
54	ND1B/28	Yellowish White	Irregular	Wavy	Effuse	Wavy Interlaced	Medium	Shiny	Translucent
55	ND1B/30	Yellowish White	Irregular	crenate	Umbonate	Arborescent	Large	Shiny	Translucent
56	ND1B/32	White	Irregular	Wavy	Effuse	Finely Granular	Large	Dull	Opaque
57	ND1B/34	White	Irregular	crenate	Raised	Smooth	Large	Dull	Opaque
58	ND2A/4	White	Irregular	Wavy	Raised	Smooth	Medium	Shiny	Translucent
59	ND2A/5	White	Circular	Entire	Effuse	Smooth	Small	Dull	Translucent
60	ND2A/6	White	Circular	Entire	Raised	Smooth	Medium	Shiny	Translucent
61	ND2A/7	Yellowish White	Circular	Entire	Raised	Smooth	Small	Shiny	Translucent
62	ND2A/8	Yellowish White	Circular	Entire	Raised	Smooth	Small	Shiny	Opaque
63	ND2A/9	Yellowish White	Irregular	Wavy	Raised	Smooth	Medium	Shiny	Translucent
64	ND2A/10	White	Circular	Entire	Raised	Smooth	Medium	Dull	Translucent
65	ND2A/11	Yellowish White	Circular	Entire	Raised	Smooth	Medium	Shiny	Opaque
66	ND2A/12	White	Irregular	Wavy	Effuse	Smooth	Medium	Dull	Translucent
67	ND2A/13	White	Circular	Entire	Raised	Smooth	Small	Shiny	Opaque
68	ND2A/14	Yellow	Irregular	Entire	Raised	Smooth	Small	Shiny	Opaque
69	ND2A/15	White	Circular	Wavy	Effuse	Finely Granular	Small	Dull	Opaque
70	ND2A/16	Yellowish White	Circular	Entire	Raised	Smooth	Small	Shiny	Transparent

APPENDIX-A: Continued.

Isolate Number	Isolate Name	Colony Morphology							
		Pigmentation	Shape	Margin	Elevation	Texture	Size	Appearance	Optical Property
71	ND2A/17	Yellowish White	Circular	Entire	Effuse	Smooth	Small	dull	Opaque
72	ND2A/34	Yellowish White	Irregular	Lobate	Raised	Smooth	Medium	Shiny	Opaque
73	ND2A/36	Yellowish White	Irregular	Wavy	Effuse	Smooth	Small	Dull	Translucent
74	ND2A/37	White	Circular	Crenate	Effuse	Coarsely Granular	Large	Shiny	Opaque
75	ND2A/38	Yellowish White	Circular	Wavy	Raised	Smooth	Small	Shiny	Opaque
76	ND2A/39	Yellowish White	Irregular	Ciliate	Effuse	Filamentous	Large	Dull	Translucent
77	ND2A/40	Yellowish White	Irregular	Wavy	Effuse	Smooth	Large	Dull	Opaque
78	ND2A/41	White	Irregular	Lobate	Raised	Smooth	Large	Shiny	Opaque
79	ND2A/42	White	Irregular	Wavy	Raised	Coarsely Granular	Small	Shiny	Transparent
80	ND2A/43	Yellowish White	Circular	Entire	Effuse	Smooth	Small	Shiny	Opaque
81	ND2A/44	White	Irregular	Fimbriate	Effuse	Smooth	Medium	Dull	Translucent
82	ND2A/45	Yellowish White	Filamentous	Wavy	Raised	Smooth	Medium	Shiny	Opaque
83	ND2A/46	White	Circular	Entire	Raised	Smooth	Large	Dull	Opaque
84	ND2A/47	Yellowish White	Circular	Entire	Raised	Smooth	Medium	Dull	Opaque
85	ND2A/48	Yellow	Circular	Entire	Effuse	Smooth	Small	Dull	Opaque
86	ND2A/49	White	Circular	Wavy	Raised	Smooth	Small	Dull	Translucent
87	ND2A/61	Yellowish White	Circular	Entire	Raised	Smooth	Small	Shiny	Opaque
88	ND2A/62	Yellow	Circular	Crenate	Raised	Arborescent	Small	Dull	Translucent
89	ND2A/63	Yellowish White	Circular	Crenate	Raised	Arborescent	Medium	Shiny	Translucent
90	ND2A/65	Yellowish White	Circular	Crenate	Effuse	Finely Granular	Small	Dull	Transparent
91	ND2B/1	White	Irregular	Wavy	Effuse	Smooth	Medium	Dull	Translucent
92	ND2B/2	Yellowish White	Circular	Entire	Raised	Smooth	Small	Shiny	Opaque
93	ND2B/3	White	Circular	Crenate	Raised	Finely Granular	Large	Shiny	Opaque
94	ND2B/4	White	Circular	Entire	Raised	Smooth	Small	Shiny	Opaque
95	ND2B/5	Yellowish White	Circular	Entire	Raised	Smooth	Small	Shiny	Opaque
96	ND2B/6	White	Circular	Entire	Raised	Smooth	Medium	Shiny	Opaque
97	ND2B/7	White	Circular	Entire	Raised	Smooth	Small	Shiny	Opaque
98	ND2B/8	Yellowish White	Irregular	Crenate	Effuse	Wavy Interlaced	Large	Shiny	Translucent
99	ND2B/9	Yellowish White	Circular	Crenate	Raised	Finely Granular	Small	Shiny	Translucent
100	ND2B/11	White	Circular	Entire	Raised	Smooth	Small	Shiny	Opaque
101	ND2B/12	Sari	Irregular	Entire	Raised	Smooth	Small	Dull	Opaque
102	ND2B/13	White	Circular	Wavy	Raised	Coarsely Granular	Medium	Dull	Translucent
103	ND2B/14	Yellowish White	Irregular	Wavy	Effuse	Finely Granular	Medium	Dull	Opaque
104	ND2B/15	White	Circular	Entire	Raised	Smooth	Small	Shiny	Opaque
105	ND2B/16	Yellowish White	Circular	Entire	Effuse	Finely Granular	Medium	Shiny	Opaque
106	ND2B/17	White	Irregular	Crenate	Effuse	Arborescent	Large	Shiny	Translucent
107	ND2B/18	Yellow	Circular	Entire	Raised	Smooth	Small	Shiny	Opaque
108	ND2B/19	Yellowish White	Circular	Entire	Raised	Finely Granular	Medium	Dull	Opaque
109	ND2B/20	White	Circular	Entire	Raised	Coarsely Granular	Large	Shiny	Translucent
110	ND2B/21	White	Circular	Entire	Raised	Smooth	Small	Shiny	Opaque
111	ND2B/22	White	Circular	Entire	Raised	Smooth	Large	Shiny	Opaque
112	ND2B/23	Yellowish White	Irregular	Crenate	Effuse	Arborescent	Large	Dull	Translucent
113	ND2B/24	Yellowish White	Irregular	Entire	Raised	Finely Granular	Large	Dull	translucent
114	ND3A/2	Orange	Circular	Crenate	Raised	Smooth	Small	Shiny	Opaque
115	ND3A/4	White	Irregular	Lobate	Effuse	Smooth	Large	Shiny	Opaque
116	ND3A/5	White	Circular	Crenate	Raised	Smooth	Small	Shiny	Opaque
117	ND3A/8	White	Circular	Crenate	Raised	Finely Granular	Small	Dull	Opaque
118	ND3A/9	White	Circular	Entire	Raised	Finely Granular	Medium	Shiny	Opaque
119	ND3A/11	Yellowish White	Circular	Wavy	Raised	Smooth	Medium	Shiny	Opaque
120	ND3A/12	Yellowish White	Circular	Wavy	Raised	Smooth	Small	Shiny	Opaque
121	ND3A/13	Yellowish White	Irregular	Lobate	Raised	Smooth	Small	Shiny	Opaque
122	ND3A/14	White	Circular	Entire	Raised	Smooth	Medium	Shiny	Translucent
123	ND3A/15	White	Circular	Entire	Raised	Smooth	Small	Shiny	Opaque
124	ND3A/16	White	Circular	Entire	Raised	Smooth	Small	Shiny	Opaque
125	ND3A/17	Yellow	Irregular	Lobate	Raised	Smooth	Small	Shiny	Opaque
126	ND3A/18	White	Irregular	Lobate	Raised	Finely Granular	Small	Shiny	Opaque
127	ND3A/19	White	Irregular	Lobate	Effuse	Smooth	Large	Shiny	Opaque
128	ND3A/20	Yellow	Circular	Entire	Raised	Smooth	Small	Shiny	Opaque
129	ND3A/21	White	Irregular	Lobate	Raised	Smooth	Medium	Dull	Translucent
130	ND3A/22	Yellowish White	Circular	Entire	Raised	Smooth	Medium	Shiny	Opaque
131	ND3A/23	White	Circular	Entire	Raised	Smooth	Medium	Shiny	Translucent
132	ND3A/24	Yellow	Irregular	Lobate	Raised	Smooth	Medium	Shiny	Opaque
133	ND3A/25	White	Irregular	Lobate	Raised	Smooth	Medium	Shiny	Opaque
134	ND3A/26	White	Irregular	Lobate	Raised	Smooth	Large	Shiny	Opaque
135	ND3A/27	White	Circular	Entire	Raised	Smooth	Small	Shiny	Opaque
136	ND3A/29	White	Circular	Crenate	Raised	Smooth	Medium	Shiny	Opaque
137	ND3A/30	White	Irregular	Crenate	Raised	Smooth	Medium	Shiny	Translucent
138	ND3A/31	White	Irregular	Wavy	Effuse	Smooth	Large	Shiny	Translucent
139	ND3A/32	White	Circular	Entire	Raised	Smooth	Large	Dull	Translucent
140	ND3A/33	White	Circular	Wavy	Raised	Smooth	Large	Dull	Opaque

APPENDIX-A: Continued.

Isolate Number	Isolate Name	Colony Morphology							
		Pigmentation	Shape	Margin	Elevation	Texture	Size	Appearance	Optical Property
141	ND3B/2	Yellowish White	Irregular	Lobate	Raised	Smooth	Medium	Shiny	Opaque
142	ND3B/4	Yellowish White	Circular	Crenate	Effuse	Smooth	Small	Shiny	Translucent
143	ND3B/5	White	Circular	Wavy	Raised	Smooth	Medium	Shiny	Opaque
144	ND3B/7	White	Circular	Entire	Raised	Smooth	Medium	Shiny	Opaque
145	ND3B/8	Yellow	Circular	Entire	Raised	Smooth	Small	Shiny	Opaque
146	ND3B/9	Yellow	Circular	Crenate	Raised	Smooth	Medium	Shiny	Opaque
147	ND3B/10	White	Irregular	Fimbriate	Effuse	Arborescent	small	dull	Translucent
148	ND3B/11	Yellowish White	Irregular	Fimbriate	Raised	Arborescent	Large	Dull	Translucent
149	ND3B/12	Yellowish White	Irregular	Lobate	Raised	Finely Granular	Medium	Dull	Opaque
150	ND3B/13	Yellowish White	Circular	Entire	Raised	Smooth	Small	Shiny	Opaque
151	ND3B/14	White	Circular	Entire	Raised	Smooth	Medium	Shiny	Opaque
152	ND3B/15	White	Irregular	Lobate	Effuse	Wavy Interlaced	Large	Shiny	Translucent
153	ND3B/16	White	Circular	Entire	Raised	Smooth	Small	Shiny	Translucent
154	ND3B/17	White	Circular	Crenate	Raised	Finely Granular	Medium	Dull	Opaque
155	ND3B/18	Yellowish White	Circular	Entire	Raised	Smooth	Small	Shiny	Opaque
156	ND3B/19	Yellowish White	Irregular	Ciliate	Raised	Finely Granular	Large	Dull	Opaque
157	ND3B/20	Yellowish White	Circular	Entire	Raised	Finely Granular	Large	Shiny	Opaque
158	ND3B/22	Yellowish White	Irregular	Lobate	Raised	Wavy Interlaced	Large	Shiny	Opaque
159	ND3B/23	White	Irregular	Wavy	Raised	Wavy Interlaced	Large	Shiny	Opaque
160	ND3B/24	White	Irregular	Wavy	Raised	Finely Granular	Large	Shiny	Opaque
161	ND3B/25	White	Irregular	Lobate	Raised	Wavy Interlaced	Large	Shiny	Opaque
162	ND3B/26	White	Circular	Wavy	Effuse	Smooth	Large	Shiny	Translucent
163	ND3B/27	White	Irregular	Wavy	Raised	Smooth	Medium	Shiny	Translucent
164	ND3B/28	White	Irregular	Wavy	Raised	Wavy Interlaced	Large	Shiny	Opaque
165	ND3B/29	Yellowish White	Irregular	Wavy	Raised	Arborescent	Medium	Shiny	Translucent
166	ND3B/30	White	Circular	Entire	Effuse	Smooth	Large	Shiny	Translucent
167	ND3B/31	Pinkish White	Irregular	Wavy	Raised	Smooth	Large	Dull	Opaque
168	ND3B/32	Yellowish White	Circular	Entire	Raised	Smooth	Large	Dull	Opaque
169	ND3B/33	White	Circular	Wavy	Raised	Smooth	Large	Shiny	Opaque
170	ND3B/34	White	Irregular	Wavy	Raised	Smooth	Large	Shiny	Translucent
171	ND3B/35	Yellowish White	Irregular	Entire	Raised	Smooth	Medium	Shiny	Opaque
172	ND3B/36	White	Irregular	Lobate	Effuse	Smooth	Large	Shiny	Opaque
173	AF1A/1	White	Irregular	Entire	Raised	Smooth	Large	Dull	Translucent
174	AF1A/2	Yellow	Irregular	Wavy	Raised	Arborescent	Large	Shiny	Opaque
175	AF1A/3	White	Irregular	Scalloped	Raised	Wavy Interlaced	Large	Shiny	Transparent
176	AF1A/4	White	Irregular	Lobate	Raised	Arborescent	Large	Shiny	Opaque
177	AF1A/9	White	Irregular	Wavy	Raised	Smooth	Medium	Shiny	Translucent
178	AF1A/12	White	Circular	Wavy	Raised	Finely Granular	Large	Dull	Opaque
179	AF1A/13	Yellowish White	Circular	Entire	Raised	Smooth	Small	Shiny	Translucent
180	AF1A/14	Pinkish White	Irregular	Wavy	Effuse	Smooth	Large	Dull	Opaque
181	AF1A/15	White	Circular	Crenate	Raised	Wavy Interlaced	Large	Shiny	Translucent
182	AF1A/17	White	Circular	Entire	Raised	Smooth	Medium	Shiny	Translucent
183	AF1A/18	White	Irregular	Lobate	Raised	Smooth	Large	Shiny	Opaque
184	AF1A/20	Yellowish White	Irregular	Lobate	Effuse	Smooth	Large	Shiny	Translucent
185	AF1A/21	White	Circular	Lobate	Effuse	Smooth	Large	Shiny	Opaque
186	AF1A/22	White	Circular	Wavy	Raised	Smooth	Large	Shiny	Opaque
187	AF1A/23	White	Circular	Entire	Effuse	Smooth	Medium	Shiny	Translucent
188	AF1B/1	Yellow	Irregular	Wavy	Raised	Arborescent	Medium	Shiny	Translucent
189	AF1B/2	Yellow	Irregular	Crenate	Raised	Coarseley Granular	Large	Shiny	Translucent
190	AF1B/3	Yellowish White	Circular	Entire	Raised	Smooth	Small	Dull	Translucent
191	AF1B/4	White	Irregular	Crenate	Effuse	Finely Granular	Large	Dull	Translucent
192	AF1B/5	Yellow	Irregular	Wavy	Raised	Arborescent	Small	Shiny	Translucent
193	AF1B/7	Orange	Irregular	Lobate	Raised	Arborescent	Large	Shiny	Translucent
194	AF1B/8	Yellow	Irregular	Fimbriate	Effuse	Filamentous	Large	Dull	Translucent
195	AF1B/11	Orange	Circular	Entire	Raised	Smooth	Medium	Shiny	Opaque
196	AF1B/13	Yellow	Irregular	Fimbriate	Raised	Filamentous	Large	Shiny	Translucent
197	AF1B/15	White	Irregular	Wavy	Raised	Smooth	Large	Shiny	Translucent
198	AF1B/16	White	Irregular	Crenate	Effuse	Coarseley Granular	Large	Dull	Translucent
199	AF1B/17	White	Irregular	Lobate	Raised	Smooth	Large	Shiny	Translucent
200	AF1B/18	Orange	Circular	Wavy	Raised	Smooth	Small	Shiny	Opaque
201	AF1B/21	White	Irregular	Crenate	Raised	Finely Granular	Medium	Dull	Opaque
202	AF1B/25	Yellowish White	Circular	Lobate	Raised	Arborescent	Large	Shiny	Translucent
203	AF1B/26	Yellowish White	Circular	Wavy	Raised	Smooth	Large	Shiny	Translucent
204	AF2A/1	White	Circular	Wavy	Raised	Smooth	Small	Shiny	Translucent
205	AF2A/2	Yellowish White	Circular	Entire	Raised	Smooth	Medium	Shiny	Opaque
206	AF2A/3	Yellowish White	Circular	Lobate	Raised	Smooth	Medium	Shiny	Opaque
207	AF2A/13	Yellowish White	Circular	Wavy	Raised	Smooth	Small	Shiny	Translucent
208	AF2A/16	Pinkish White	Irregular	Lobate	Raised	Smooth	Medium	Shiny	Opaque
209	AF2A/19	Yellowish White	Irregular	Wavy	Effuse	Smooth	Large	Dull	Translucent
210	AF2A/23	White	Irregular	Wavy	Raised	Smooth	Large	Shiny	Transparent

APPENDIX-A: Continued.

Isolate Number	Isolate Name	Colony Morphology							
		Pigmentation	Shape	Margin	Elevation	Texture	Size	Appearance	Optical Property
211	AF2A/28	Brownish White	Circular	Entire	Raised	Smooth	Small	Shiny	Opaque
212	AF2A/29	White	Irregular	Lobate	Raised	Smooth	Large	Shiny	Opaque
213	AF2B/2	Yellowish White	Circular	Entire	Raised	Smooth	Small	Shiny	Translucent
214	AF2B/6	Yellowish White	Irregular	Wavy	Effuse	Smooth	Large	Shiny	Translucent
215	AF2B/9	White	Irregular	Scalloped	Raised	Arborescent	Medium	Dull	Opaque
216	AF2B/12	White	Irregular	Crenate	Raised	Filamentous	Large	Dull	Opaque
217	AF2B/13	Brownish White	Irregular	Lobate	Raised	Smooth	Medium	Shiny	Opaque
218	AF2B/15	Yellowish White	Irregular	Lobate	Raised	Smooth	Large	Shiny	Translucent
219	AF2B/16	White	Circular	Entire	Raised	Smooth	Medium	Shiny	Translucent
220	AF2B/19	White	Circular	Entire	Raised	Smooth	Small	Shiny	Translucent
221	AF2B/20	White	Irregular	Lobate	Raised	Smooth	Medium	Shiny	Translucent
222	AF3A/1	White	Circular	Wavy	Raised	Smooth	Large	Shiny	Opaque
223	AF3A/2	Yellowish White	Circular	Wavy	Raised	Smooth	Large	Dull	Opaque
224	AF3A/3	Yellow	Circular	Lobate	Raised	Smooth	Medium	Shiny	Translucent
225	AF3A/4	White	Circular	Lobate	Raised	Smooth	Large	Shiny	Opaque
226	AF3A/5	White	Circular	Entire	Raised	Smooth	Large	Shiny	Opaque
227	AF3A/9	Brownish White	Irregular	Wavy	Raised	Smooth	Medium	Shiny	Opaque
228	AF3A/10	Yellow	Circular	Lobate	Effuse	Smooth	Medium	Dull	Translucent
229	AF3A/11	Yellowish White	Irregular	Lobate	Raised	Smooth	Medium	Shiny	Translucent
230	AF3A/12	Yellowish White	Irregular	Wavy	Effuse	Smooth	Medium	Shiny	Opaque
231	AF3A/13	Yellowish White	Irregular	Lobate	Umbonate	Finely Granular	Large	Dull	Opaque
232	AF3A/14	Yellowish White	Irregular	Wavy	Raised	Smooth	Medium	Shiny	Opaque
233	AF3A/15	Pinkish White	Irregular	Lobate	Raised	Smooth	Large	Dull	Opaque
234	AF3A/16	White	Irregular	Lobate	Effuse	Smooth	Large	Dull	Translucent
235	AF3A/17	Pinkish White	Circular	Entire	Raised	Smooth	Medium	Shiny	Opaque
236	AF3A/19	White	Irregular	Fimbriate	Effuse	Filamentous	Medium	Shiny	Opaque
237	AF3A/20	Bluish	Irregular	Wavy	Raised	Smooth	Large	Shiny	Opaque
238	AF3A/22	White	Circular	Wavy	Crateriform	Smooth	Small	Dull	Translucent
239	AF3A/23	Yellowish White	Irregular	Wavy	Raised	Smooth	Medium	Shiny	Opaque
240	AF3A/24	Yellowish White	Circular	Entire	Raised	Smooth	Large	Shiny	Opaque
241	AF3A/25	Yellowish White	Circular	Wavy	Effuse	Smooth	Large	Shiny	Opaque
242	AF3A/26	Yellowish White	Irregular	Wavy	Raised	Smooth	Medium	Shiny	Opaque
243	AF3A/29	Yellowish White	Circular	Entire	Raised	Smooth	Large	Shiny	Opaque
244	AF3A/31	Yellowish White	Irregular	Crenate	Raised	Wavy/Interlaced	Large	Shiny	Translucent
245	AF3A/32	White	Irregular	Crenate	Effuse	Smooth	Large	Dull	Transparent
246	AF3A/33	Bluish	Irregular	Entire	Raised	Smooth	Medium	Shiny	Opaque
247	AF3B/1	White	Irregular	Wavy	Raised	Smooth	Large	Shiny	Opaque
248	AF3B/3	Yellowish White	Irregular	Lobate	Effuse	Wavy/Interlaced	Large	Shiny	Translucent
249	AF3B/4	Yellowish White	Irregular	Lobate	Effuse	Smooth	Large	Dull	Translucent
250	AF3B/5	Yellowish White	Irregular	Wavy	Raised	Smooth	Large	Shiny	Opaque
251	AF3B/7	Pinkish White	Circular	Wavy	Raised	Wavy/Interlaced	Small	Shiny	Opaque
252	AF3B/10	White	Circular	Wavy	Raised	Smooth	Large	Dull	Opaque
253	AF3B/11	White	Irregular	Wavy	Raised	Wrinkled	Medium	Shiny	Opaque
254	AF3B/14	Yellowish White	Circular	Entire	Raised	Smooth	Small	Shiny	Opaque
255	AF3B/15	White	Irregular	Wavy	Raised	Wavy/Interlaced	Large	Shiny	Translucent
256	AF3B/17	Pinkish White	Irregular	Lobate	Raised	coarsely granular	Large	Shiny	Translucent
257	AF3B/18	Yellowish White	Irregular	Lobate	Raised	Wrinkled	Large	Shiny	Translucent
258	AF3B/19	Yellowish White	Irregular	Wavy	Raised	Wavy/Interlaced	Small	Shiny	Translucent
259	AF3B/20	Yellow	Circular	Entire	Raised	Smooth	Medium	Dull	Opaque
260	AF3B/21	White	Circular	Wavy	Raised	Smooth	Medium	Shiny	Opaque
261	AF3B/22	White	Circular	Wavy	Raised	Smooth	Medium	Shiny	Translucent
262	AF3B/23	Yellowish White	Irregular	Crenate	Effuse	Filamentous	Large	Shiny	Transparent
263	AF3B/24	Yellowish White	Irregular	Crenate	Raised	Wrinkled	Medium	Shiny	Translucent
264	AF3B/25	Yellowish White	Irregular	Entire	Raised	Wavy/Interlaced	Small	Shiny	Translucent
265	KN1A/1	White	Irregular	Entire	Crateriform	Smooth	Medium	Dull	Opaque
266	KN1A/2	White	Irregular	Wavy	Crateriform	Finely Granular	Medium	Shiny	Opaque
267	KN1A/3	White	Irregular	Lobate	Crenate	Smooth	Small	Shiny	Opaque
268	KN1A/4	White	Circular	Entire	Raised	Smooth	Medium	Shiny	Translucent
269	KN1A/5	White	Circular	Entire	Raised	Smooth	Medium	Shiny	Opaque
270	KN1A/6	Pinkish White	Circular	Lobate	Raised	Smooth	Small	Shiny	Opaque
271	KN1A/7	White	Circular	Entire	Convex	Coarsely Granular	Small	Shiny	Opaque
272	KN1A/9	Yellowish White	Circular	Lobate	Raised	Smooth	Small	Shiny	Opaque
273	KN1A/10	White	Irregular	Crenate	Effuse	Finely Granular	Large	Shiny	Opaque
274	KN1A/13	Yellowish White	Circular	Crenate	Raised	Finely Granular	Medium	Dull	Opaque
275	KN1A/14	Yellowish White	Circular	Entire	Raised	Smooth	Small	Shiny	Opaque
276	KN1A/16	White	Irregular	Crenate	Crateriform	Finely Granular	Small	Shiny	Translucent
277	KN1A/22	Yellowish White	Irregular	Entire	Convex	Smooth	Small	Shiny	Opaque
278	KN1A/23	Yellowish White	Irregular	Crenate	Raised	Finely Granular	Large	Dull	Opaque
279	KN1B/1	Yellowish White	Circular	Entire	Raised	Smooth	Medium	Shiny	Opaque
280	KN1B/2	Pinkish White	Circular	Wavy	Raised	Smooth	Medium	Shiny	Opaque

APPENDIX-A: Continued.

Isolate Number	Isolate Name	Colony Morphology							
		Pigmentation	Shape	Margin	Elevation	Texture	Size	Appearance	Optical Property
281	KN1B/3	White	Irregular	Lobate	Raised	Smooth	Large	Shiny	Translucent
282	KN1B/5	Yellowish White	Irregular	Lobate	Effuse	Finely Granular	Large	Dull	Opaque
283	KN1B/6	White	Irregular	Wavy	Braughtsma	Finely Granular	Large	Dull	Opaque
284	KN1B/7	White	Irregular	Lobate	Raised	Smooth	Small	Shiny	Opaque
285	KN1B/9	Pinkish White	Circular	Entire	Raised	Smooth	Medium	Shiny	Opaque
286	KN1B/10	Yellowish White	Irregular	Wavy	Raised	Finely Granular	Medium	Dull	Opaque
287	KN1B/11	White	Circular	Entire	Raised	Smooth	Small	Shiny	Opaque
288	KN1B/13	White	Circular	Entire	Raised	Smooth	Small	Shiny	Translucent
289	KN2A/1	Yellowish White	Irregular	Entire	Raised	Smooth	Medium	Shiny	Translucent
290	KN2A/2	White	Circular	Entire	Raised	Smooth	Medium	Shiny	Opaque
291	KN2A/4	Yellowish White	Irregular	Lobate	Raised	Smooth	Medium	Shiny	Opaque
292	KN2A/5	Yellowish White	Irregular	Lobate	Raised	Arborescent	Small	Shiny	Translucent
293	KN2A/6	Yellowish White	Irregular	Lobate	Raised	Arborescent	Medium	Shiny	Translucent
294	KN2A/7	Yellow	Irregular	Lobate	Effuse	Smooth	Large	Dull	Transparent
295	KN2A/8	White	Irregular	Crenate	Raised	Finely Granular	Large	Shiny	Opaque
296	KN2A/9	Yellowish White	Circular	Entire	Crateriform	Smooth	Small	Shiny	Translucent
297	KN2A/10	Yellowish White	Irregular	Lobate	Raised	Arborescent	Large	Shiny	Translucent
298	KN2A/11	White	Irregular	Entire	Raised	Smooth	Medium	Dull	Opaque
299	KN2A/12	White	Irregular	Crenate	Wrinkled	Arborescent	Medium	Shiny	Opaque
300	KN2A/13	Pinkish White	Circular	Entire	Raised	Smooth	Small	Shiny	Opaque
301	KN2A/14	White	Irregular	Crenate	Raised	Arborescent	Large	Shiny	Opaque
302	KN2A/15	Pinkish White	Irregular	Wavy	Raised	Smooth	Medium	Shiny	Opaque
303	KN2A/16	White	Irregular	Wavy	Effuse	Filamentous	Medium	Shiny	Translucent
304	KN2A/18	White	Irregular	Lobate	Effuse	Smooth	Large	Dull	Translucent
305	KN2B/1	White	Circular	Wavy	Raised	Smooth	Medium	Shiny	Opaque
306	KN2B/2	White	Irregular	Wavy	Raised	Smooth	Medium	Dull	Opaque
307	KN2B/3	Yellowish White	Circular	Entire	Raised	Smooth	Medium	Shiny	Opaque
308	KN2B/4	Yellowish White	Circular	Entire	Raised	Smooth	Medium	Shiny	Opaque
309	KN2B/5	Yellow	Circular	Wavy	Effuse	Smooth	Large	Dull	Opaque
310	KN2B/6	White	Circular	Entire	Raised	Smooth	Small	Shiny	Opaque
311	KN2B/7	Yellowish White	Irregular	Lobate	Effuse	Smooth	Large	Shiny	Translucent
312	KN2B/8	White	Circular	Crenate	Raised	Smooth	Small	Shiny	Opaque
313	KN2B/9	Yellowish White	Circular	Wavy	Raised	Smooth	Medium	Shiny	Opaque
314	KN2B/10	White	Irregular	Wavy	Raised	Finely Granular	Large	Shiny	Opaque
315	KN2B/11	Yellowish White	Irregular	Lobate	Effuse	Smooth	Large	Dull	Translucent
316	KN2B/12	Yellowish White	Irregular	Wavy	Raised	Smooth	Large	Shiny	Opaque
317	KN2B/13	Yellowish White	Irregular	Lobate	Raised	Smooth	Medium	Dull	Translucent
318	KN2B/14	White	Irregular	Entire	Raised	Smooth	Small	Shiny	Opaque
319	KN2B/15	Yellow	Irregular	Crenate	Effuse	Finely Granular	Small	Dull	Translucent
320	KN2B/16	Yellow	Irregular	Wavy	Raised	Smooth	Small	Shiny	Opaque
321	KN3A/1	Orange	Circular	Entire	Raised	Smooth	Medium	Shiny	Opaque
322	KN3A/2	Greenish White	Circular	Entire	Raised	Smooth	Medium	Shiny	Opaque
323	KN3A/3	Yellowish White	Circular	Entire	Raised	Smooth	Medium	Shiny	Opaque
324	KN3A/4	White	Circular	Entire	Raised	Smooth	Large	Shiny	Opaque
325	KN3A/5	White	Irregular	Lobate	Raised	Smooth	Large	Shiny	Opaque
326	KN3A/6	Yellow	Circular	Wavy	Raised	Smooth	Large	Shiny	Opaque
327	KN3A/7	White	Circular	Entire	Raised	Smooth	Medium	Shiny	Opaque
328	KN3A/8	Yellowish White	Irregular	Lobate	Raised	Smooth	Large	Shiny	Opaque
329	KN3A/9	White	Irregular	Lobate	Raised	Smooth	Large	Shiny	Opaque
330	KN3A/10	White	Irregular	Wavy	Effuse	Smooth	Medium	Dull	Translucent
331	KN3A/11	White	Irregular	Wavy	Crateriform	Filamentous	Large	Shiny	Opaque
332	KN3A/12	White	Irregular	Entire	Raised	Smooth	Medium	Shiny	Opaque
333	KN3A/13	White	Irregular	Lobate	Effuse	Smooth	Medium	Dull	Transparent
334	KN3A/14	Greenish White	Circular	Crenate	Raised	Smooth	Medium	Shiny	Opaque
335	KN3A/15	Pinkish White	Circular	Entire	Raised	Smooth	Medium	Shiny	Opaque
336	KN3A/16	Yellow	Circular	Wavy	Raised	Smooth	Large	Shiny	Opaque
337	KN3A/17	Yellowish White	Circular	Entire	Raised	Smooth	Small	Shiny	Opaque
338	KN3A/21	White	Circular	Crenate	Raised	Finely Granular	Large	Shiny	Translucent
339	KN3A/22	White	Irregular	Crenate	Raised	Finely Granular	Large	Shiny	Opaque
340	KN3A/23	Yellowish White	Irregular	Lobate	Raised	Smooth	Small	Shiny	Opaque
341	KN3A/24	White	Circular	Entire	Crateriform	Smooth	Small	Dull	Translucent
342	KN3B/1	Yellow	Circular	Entire	Raised	Smooth	Medium	Shiny	Opaque
343	KN3B/2	White	Circular	Entire	Raised	Smooth	Medium	Shiny	Translucent
344	KN3B/3	Yellowish White	Circular	Entire	Raised	Smooth	Large	Shiny	Opaque
345	KN3B/4	White	Circular	Entire	Raised	Smooth	Medium	Shiny	Opaque
346	KN3B/5	White	Irregular	Fimbriate	Raised	Filamentous	Large	Shiny	Translucent
347	KN3B/6	Yellowish White	Circular	Entire	Raised	Smooth	Large	Shiny	Opaque
348	KN3B/7	Yellowish White	Irregular	Wavy	Effuse	Filamentous	Large	Shiny	Translucent
349	KN3B/8	White	Irregular	Lobate	Raised	Finely Granular	Large	Shiny	Opaque
350	KN3B/10	White	Irregular	Lobate	Raised	Finely Granular	Large	Shiny	Translucent

APPENDIX-A: Continued.

Isolate Number	Isolate Name	Colony Morphology							
		Pigmentation	Shape	Margin	Elevation	Texture	Size	Appearance	Optical Property
351	KN3B/11	Yellowish White	Circular	Wavy	Raised	Smooth	Small	Shiny	Translucent
352	KN3B/12	White	Rhizoid	Crenate	Raised	Filamentous	Large	Shiny	Opaque
353	KN3B/13	White	Rhizoid	Crenate	Effuse	Filamentous	Large	Shiny	Translucent
354	KN3B/14	White	Irregular	Crenate	Effuse	Smooth	Medium	Dull	Opaque
355	KN3B/15	Yellowish White	Irregular	Lobate	Effuse	Filamentous	Large	Shiny	Translucent
356	KY1A/2	White	Circular	Crenate	Raised	Smooth	Medium	Shiny	Translucent
357	KY1A/3	Yellow	Circular	Entire	Raised	Smooth	Medium	Shiny	Opaque
358	KY1A/4	Orange	Circular	Entire	Raised	Smooth	Small	Shiny	Opaque
359	KY1A/6	Yellowish White	Circular	Wavy	Raised	Wavy Interlaced	Medium	Shiny	Opaque
360	KY1A/7	Yellowish White	Circular	Scalloped	Umbonate	Wavy Interlaced	Small	Shiny	Translucent
361	KY1A/8	Yellowish White	Circular	Wavy	Raised	Smooth	Medium	Shiny	Translucent
362	KY1A/9	Yellowish White	Circular	Entire	Raised	Smooth	Medium	Shiny	Opaque
363	KY1A/10	White	Irregular	Lobate	Effuse	Smooth	Medium	Dull	Translucent
364	KY1A/11	White	Punctiform	Entire	Raised	Smooth	Small	Shiny	Opaque
365	KY1A/12	White	Circular	Crenate	Raised	Smooth	Small	Shiny	Translucent
366	KY1A/13	Yellowish White	Irregular	Scalloped	Raised	Smooth	Medium	Shiny	Opaque
367	KY1A/14	White	Irregular	Scalloped	Effuse	Wavy Interlaced	Large	Dull	Transparent
368	KY1B/1	White	Circular	Entire	Crateriform	Smooth	Large	Shiny	Translucent
369	KY1B/2	White	Irregular	Wavy	Crateriform	Smooth	Large	Shiny	Translucent
370	KY1B/3	Yellowish White	Circular	Entire	Raised	Smooth	Small	Shiny	Opaque
371	KY1B/4	White	Irregular	Lobate	Raised	Finely Granular	Large	Shiny	Translucent
372	KY1B/5	White	Circular	Entire	Crateriform	Smooth	Small	Shiny	Translucent
373	KY1B/6	Yellowish White	Irregular	Lobate	Crateriform	Smooth	Large	Dull	Translucent
374	KY1B/7	White	Irregular	Entire	Raised	Smooth	Small	Shiny	Opaque
375	KY1B/8	Yellowish White	Irregular	Scalloped	Raised	Finely Granular	Medium	Shiny	Opaque
376	KY1B/9	Yellowish White	Irregular	Lobate	Effuse	Smooth	Large	Dull	Transparent
377	KY1B/10	White	Irregular	Wavy	Raised	Smooth	Large	Dull	Opaque
378	KY1B/11	Yellowish White	Rhizoid	Lobate	Effuse	Smooth	Large	Dull	Transparent
379	KY1B/12	Yellowish White	Circular	Entire	Crateriform	Smooth	Medium	Shiny	Opaque
380	KY1B/13	White	Circular	Scalloped	Raised	Smooth	Medium	Shiny	Translucent
381	KY1B/14	Yellowish White	Circular	Scalloped	Raised	Smooth	Small	Shiny	Translucent
382	KY1B/16	White	Rhizoid	Lobate	Raised	Smooth	Large	Shiny	Opaque
383	KY1B/17	Yellowish White	Irregular	Crenate	Crateriform	Smooth	Medium	Shiny	Translucent
384	KY1B/18	White	Circular	Entire	Raised	Smooth	Medium	Shiny	Opaque
385	KY2A/1	Yellowish White	Circular	Wavy	Crateriform	Smooth	Small	Shiny	Translucent
386	KY2A/2	Yellow	Circular	Entire	Raised	Smooth	Medium	Shiny	Translucent
387	KY2A/3	White	Circular	Entire	Crateriform	Smooth	Medium	Shiny	Translucent
388	KY2A/4	Yellowish White	Circular	Entire	Raised	Smooth	Small	Shiny	Translucent
389	KY2A/5	White	Circular	Lobate	Raised	Smooth	Small	Shiny	Translucent
390	KY2A/6	Yellow	Circular	Wavy	Crateriform	Smooth	Large	Shiny	Translucent
391	KY2A/7	Yellowish White	Irregular	Lobate	Raised	Smooth	Large	Shiny	Translucent
392	KY2A/9	White	Circular	Entire	Raised	Smooth	Medium	Shiny	Opaque
393	KY2A/10	Yellowish White	Irregular	Lobate	Raised	Smooth	Large	Shiny	Translucent
394	KY2A/11	White	Circular	Scalloped	Raised	Finely Granular	Large	Shiny	Opaque
395	KY2A/12	White	Irregular	Lobate	Effuse	Smooth	Medium	Dull	Translucent
396	KY2A/13	White	Circular	Crenate	Raised	Finely Granular	Medium	Shiny	Opaque
397	KY2A/14	Yellowish White	Circular	Entire	Raised	Smooth	Small	Shiny	Transparent
398	KY2A/15	White	Irregular	Wavy	Raised	Smooth	Small	Shiny	Translucent
399	KY2A/16	Yellowish White	Irregular	Scalloped	Raised	Finely Granular	Medium	Dull	Transparent
400	KY2A/17	Yellowish White	Irregular	Crenate	Effuse	Wavy Interlaced	Large	Dull	Transparent
401	KY2A/18	Yellowish White	Circular	Wavy	Raised	Smooth	Small	Shiny	Translucent
402	KY2A/20	White	Circular	Entire	Raised	Smooth	Small	Shiny	Translucent
403	KY2B/1	White	Irregular	Scalloped	Raised	Finely Granular	Large	Shiny	Opaque
404	KY2B/2	Yellowish White	Irregular	Wavy	raughtsma	Smooth	Large	Shiny	Translucent
405	KY2B/3	Yellowish White	Irregular	Lobate	Crateriform	Smooth	Large	Shiny	Translucent
406	KY2B/4	White	Irregular	Scalloped	raughtsma	Finely Granular	Large	Shiny	Opaque
407	KY2B/5	Yellowish White	Punctiform	Entire	Raised	Smooth	Small	Shiny	Translucent
408	KY2B/6	Yellowish White	Irregular	Scalloped	Raised	Wrinkled	Medium	Shiny	Transparent
409	KY2B/7	Yellowish White	Circular	Entire	Raised	Smooth	Small	Shiny	Translucent
410	KY2B/8	Yellowish White	Irregular	Crenate	Raised	Arborescent	Medium	Shiny	Translucent
411	KY2B/9	Yellowish White	Circular	Scalloped	Crateriform	Wrinkled	Small	Shiny	Translucent
412	KY2B/10	Yellowish White	Circular	Entire	Raised	Smooth	Small	Shiny	Translucent
413	KY2B/11	White	Circular	Wavy	Raised	Smooth	Small	Shiny	Translucent
414	KY2B/12	Yellowish White	Irregular	Crenate	Raised	Wrinkled	Medium	Shiny	Transparent
415	KY3A/1	White	Circular	Wavy	Crateriform	Smooth	Large	Shiny	Opaque
416	KY3A/2	Orange	Circular	Entire	Crateriform	Smooth	Medium	Shiny	Opaque
417	KY3A/4	Yellow	Circular	Entire	Raised	Smooth	Medium	Shiny	Opaque
418	KY3A/5	White	Irregular	Crenate	Effuse	Wavy Interlaced	Large	Dull	Transparent
419	KY3A/7	Yellowish White	Circular	Crenate	Raised	Smooth	Medium	Shiny	Opaque
420	KY3A/8	Yellowish White	Circular	Scalloped	Raised	Smooth	Large	Shiny	Translucent

APPENDIX-A: Continued.

Isolate Number	Isolate Name	Colony Morphology							
		Pigmentation	Shape	Margin	Elevation	Texture	Size	Appearance	Optical Property
421	KY3A/9	Orangish White	Circular	Scalloped	Raised	Smooth	Medium	Shiny	Translucent
422	KY3A/10	White	Irregular	Lobate	Raised	Wrinkled	Large	Shiny	Opaque
423	KY3A/11	Yellowish White	Irregular	Lobate	Raised	Smooth	Large	Dull	Translucent
424	KY3A/12	Yellowish White	Circular	Entire	Raised	Smooth	Medium	Shiny	Translucent
425	KY3A/13	White	Circular	Entire	Raised	Smooth	Small	Shiny	Translucent
426	KY3A/14	White	Irregular	Crenate	Crateriform	Smooth	Large	Dull	Transparent
427	KY3A/15	White	Irregular	Crenate	Effuse	Wavy Interlaced	Large	Dull	Transparent
428	KY3A/16	Yellowish White	Irregular	Entire	Effuse	Smooth	Small	Dull	Transparent
429	KY3A/17	White	Circular	Wavy	Raised	Smooth	Medium	Shiny	Translucent
430	KY3A/18	White	Irregular	Lobate	Raised	Smooth	Medium	Shiny	Translucent
431	KY3A/19	Yellowish White	Circular	Entire	Raised	Smooth	Large	Shiny	Opaque
432	KY3A/20	Yellow	Circular	Lobate	Raised	Finely Granular	Small	Shiny	Translucent
433	KY3A/23	White	Circular	Entire	Crateriform	Smooth	Small	Shiny	Translucent
434	KY3A/24	Yellowish White	Circular	Entire	Raised	Smooth	Small	Shiny	Translucent
435	KY3B/1	White	Irregular	Wavy	Raised	Smooth	Medium	Shiny	Opaque
436	KY3B/2	Yellow	Circular	Entire	Crateriform	Smooth	Large	Shiny	Translucent
437	KY3B/3	Yellowish White	Circular	Entire	Raised	Smooth	Medium	Shiny	Translucent
438	KY3B/4	Yellowish White	Irregular	Wavy	raughtsma	Smooth	Large	Dull	Translucent
439	KY3B/5	White	Circular	Entire	Raised	Smooth	Small	Shiny	Opaque
440	KY3B/6	White	Circular	Wavy	Raised	Smooth	Small	Shiny	Translucent
441	KY3B/7	Yellowish White	Circular	Entire	Crateriform	Smooth	Small	Shiny	Translucent
442	KY3B/9	Yellowish White	Irregular	Entire	Raised	Smooth	Small	Shiny	Translucent
443	KY3B/10	Yellowish White	Circular	Wavy	Raised	Smooth	Small	Shiny	Opaque
444	KY3B/13	Yellowish White	Irregular	Scalloped	Raised	Finely Granular	Large	Shiny	Opaque
445	KY3B/14	Brownish White	Circular	Wavy	Raised	Smooth	Small	Shiny	Opaque
446	I21A/1	Yellowish White	Irregular	Lobate	Raised	Wavy Interlaced	Medium	Shiny	Opaque
447	I21A/2	White	Irregular	Wavy	Raised	Smooth	Medium	Dull	Opaque
448	I21A/4	Yellow	Circular	Entire	Raised	Smooth	Small	Shiny	Opaque
449	I21A/5	Yellowish White	Circular	Entire	Raised	Smooth	Small	Shiny	Opaque
450	I21A/6	Yellowish White	Circular	Entire	Raised	Finely Granular	Medium	Shiny	Opaque
451	I21A/7	Yellowish White	Circular	Entire	Raised	Smooth	Medium	Shiny	Opaque
452	I21A/8	White	Circular	Crenate	Raised	Smooth	Medium	Shiny	Opaque
453	I21A/9	Yellowish White	Irregular	Wavy	Raised	Smooth	Medium	Shiny	Opaque
454	I21A/10	Yellowish White	Circular	Entire	Raised	Smooth	Medium	Shiny	Opaque
455	I21A/12	White	Irregular	Crenate	Effuse	Coarsely Granular	Medium	Dull	Transparent
456	I21A/13	White	Irregular	Wavy	Raised	Smooth	Medium	Shiny	Opaque
457	I21A/14	Yellowish White	Irregular	Wavy	Effuse	Smooth	Medium	Shiny	Translucent
458	I21A/15	Yellow	Irregular	Lobate	Raised	Smooth	Large	Shiny	Translucent
459	I21A/16	White	Irregular	Lobate	Raised	Smooth	Small	Shiny	Translucent
460	I21A/17	White	Circular	Crenate	Raised	Coarsely Granular	Large	Dull	Translucent
461	I21A/18	Yellowish White	Circular	Entire	Raised	Smooth	Medium	Shiny	Opaque
462	I21A/19	White	Irregular	Wavy	Raised	Smooth	Medium	Shiny	Opaque
463	I21A/20	Yellowish White	Circular	Lobate	Crateriform	Smooth	Medium	Shiny	Translucent
464	I21A/21	White	Irregular	Crenate	Raised	Finely Granular	Large	Shiny	Opaque
465	I21A/23	Yellowish White	Circular	Entire	Crateriform	Smooth	Small	dull	Translucent
466	I21A/24	Yellowish White	Irregular	Crenate	Raised	Wavy Interlaced	Large	Dull	Opaque
467	I21B/1	Yellowish White	Irregular	Crenate	Raised	Wavy Interlaced	Large	Dull	Opaque
468	I21B/2	Yellowish White	Circular	Entire	Raised	Smooth	Large	Shiny	Opaque
469	I21B/3	White	Irregular	Wavy	Raised	Smooth	Medium	Shiny	Opaque
470	I21B/4	White	Circular	Entire	Raised	Smooth	Small	Shiny	Opaque
471	I21B/5	White	Irregular	Lobate	Raised	Wavy Interlaced	Large	Dull	Opaque
472	I21B/6	Yellowish White	Irregular	Wavy	Raised	Smooth	Large	Shiny	Translucent
473	I21B/7	Yellowish White	Irregular	Lobate	Raised	Smooth	Large	Shiny	Opaque
474	I21B/8	Yellowish White	Circular	Entire	Raised	Smooth	Small	Shiny	Opaque
475	I21B/9	Yellowish White	Irregular	Lobate	Raised	Finely Granular	Medium	Shiny	Opaque
476	I21B/10	Yellowish White	Circular	Wavy	Crateriform	Finely Granular	Small	Shiny	Opaque
477	I21B/11	Yellowish White	Circular	Entire	Raised	Smooth	Medium	Shiny	Opaque
478	I21B/13	Yellowish White	Circular	Entire	Raised	Smooth	Small	Shiny	Opaque
479	I21B/14	Yellowish White	Irregular	Lobate	Raised	Smooth	Medium	Shiny	Translucent
480	I21B/15	Yellowish White	Circular	Wavy	Crateriform	Smooth	Large	Dull	Opaque
481	I21B/16	White	Circular	Wavy	Crateriform	Smooth	Small	Shiny	Opaque
482	I22A/1	Yellow	Irregular	Wavy	Raised	Smooth	Medium	Shiny	Translucent
483	I22A/2	Yellowish White	Circular	Entire	Raised	Smooth	Medium	Shiny	Opaque
484	I22A/3	White	Irregular	Lobate	Raised	Smooth	Medium	Shiny	Translucent
485	I22A/4	White	Irregular	Wavy	Raised	Smooth	Medium	Shiny	Translucent
486	I22A/5	Yellowish White	Circular	Entire	Raised	Finely Granular	Medium	Shiny	Opaque
487	I22A/6	Yellow	Circular	Wavy	Crateriform	Smooth	Small	Shiny	Translucent
488	I22A/7	Yellowish White	Circular	Entire	Umbonate	smooth	small	Shiny	Translucent
489	I22A/8	Yellowish White	Irregular	Wavy	Crateriform	Finely Granular	Large	Shiny	Translucent
490	I22A/9	White	Circular	Entire	Umbonate	Smooth	Small	Shiny	Translucent

APPENDIX-A: Continued.

Isolate Number	Isolate Name	Colony Morphology							
		Pigmentation	Shape	Margin	Elevation	Texture	Size	Appearance	Optical Property
491	I22A/10	White	Circular	Wavy	Raised	Smooth	Small	Shiny	Translucent
492	I22A/11	White	Irregular	Lobate	Raised	Finely Granular	Large	Dull	Translucent
493	I22A/12	Yellowish White	Circular	Entire	Raised	Smooth	Small	Dull	Translucent
494	I22A/13	Yellowish White	Irregular	Crenate	Raised	Finely Granular	Small	Dull	Translucent
495	I22A/14	White	Irregular	Crenate	Raised	Finely Granular	Large	Shiny	Opaque
496	I22A/15	White	Circular	Wavy	Raised	Smooth	Medium	Shiny	Translucent
497	I22A/16	White	Circular	Scalloped	Raised	Finely Granular	Large	Shiny	Opaque
498	I22A/17	Yellowish White	Irregular	Entire	Crateriform	Smooth	Medium	Shiny	Translucent
499	I22A/18	White	Circular	Wavy	Raised	Smooth	Small	Shiny	Translucent
500	I22A/19	White	Circular	Entire	Raised	Smooth	Medium	Shiny	Opaque
501	I22A/20	White	Irregular	Crenate	Effuse	Coarsely Granular	Large	Dull	Transparent
502	I22B/1	White	Irregular	Crenate	Raised	Wavy Interlaced	Large	Dull	Opaque
503	I22B/2	Yellowish White	Irregular	Fimbriate	Raised	Arborescent	Large	Shiny	Translucent
504	I22B/3	Yellowish White	Irregular	Crenate	Raised	Finely Granular	Large	Shiny	Translucent
505	I22B/4	White	Circular	Wavy	Raised	Smooth	Medium	Shiny	Opaque
506	I22B/5	Yellowish White	Circular	Entire	Raised	Smooth	Medium	Shiny	Opaque
507	I22B/6	Yellowish White	Irregular	Scalloped	Wrinkled	Smooth	Large	Shiny	Translucent
508	I22B/7	Yellowish White	Rhizoid	Lobate	Raised	Smooth	Medium	Shiny	Translucent
509	I22B/8	Yellowish White	Fimbriate	Lobate	Raised	Filamentous	Large	Shiny	Translucent
510	I22B/9	Yellowish White	Circular	Entire	Raised	Smooth	Small	Shiny	Opaque
511	I22B/10	White	Irregular	Entire	Raised	Smooth	Medium	Shiny	Opaque
512	I22B/11	Yellowish White	Rhizoid	Scalloped	Wrinkled	Filamentous	Medium	Shiny	Translucent
513	I22B/12	Yellowish White	Irregular	Scalloped	Wrinkled	Arborescent	Large	Shiny	Translucent
514	I22B/13	Yellowish White	Rhizoid	Crenate	Raised	Bubbled	Medium	Shiny	Translucent
515	I22B/14	Pinkish White	Irregular	Wavy	Umbonate	Smooth	Medium	Dull	Opaque
516	I23A/1	White	Irregular	Scalloped	Raised	Smooth	Large	Dull	Translucent
517	I23A/2	White	Irregular	Scalloped	Raised	Smooth	Large	Dull	Translucent
518	I23A/3	Yellowish White	Irregular	Scalloped	Raised	Wavy Interlaced	Large	Dull	Translucent
519	I23A/4	Yellowish White	Irregular	Lobate	Raised	Smooth	Large	Shiny	Opaque
520	I23A/5	Yellowish White	Irregular	Scalloped	Raised	Wavy Interlaced	Large	Shiny	Opaque
521	I23A/6	White	Irregular	Wavy	Raised	Smooth	Small	Shiny	Translucent
522	I23A/7	Yellowish White	Circular	Entire	Raised	Smooth	Small	Shiny	Opaque
523	I23A/8	White	Irregular	Crenate	Raised	Finely Granular	Large	Shiny	Opaque
524	I23A/9	White	Circular	Lobate	Raised	Smooth	Medium	Shiny	Opaque
525	I23A/11	Yellowish White	Circular	Entire	Raised	Smooth	Small	Shiny	Translucent
526	I23A/12	Brown	Irregular	Lobate	Effuse	Smooth	Medium	Dull	Translucent
527	I23A/13	White	Circular	Entire	Crateriform	Smooth	Small	Dull	Translucent
528	I23A/15	Brown	Irregular	Wavy	Raised	Smooth	Small	Shiny	Translucent
529	I23A/17	White	Irregular	Lobate	Raised	Smooth	Small	Shiny	Translucent
530	I23A/18	Yellow	Circular	Entire	Crateriform	Smooth	Small	Shiny	Opaque
531	I23A/19	Yellowish White	Circular	Entire	Raised	Smooth	Medium	Shiny	Opaque
532	I23A/20	Yellowish White	Circular	Wavy	Raised	Smooth	Medium	Shiny	Translucent
533	I23B/1	Yellowish White	Circular	Wavy	Raised	Smooth	Small	Dull	Opaque
534	I23B/2	White	Irregular	Lobate	Raised	Smooth	Medium	Shiny	Opaque
535	I23B/3	Yellow	Circular	Entire	Raised	Smooth	Small	Shiny	Translucent
536	I23B/4	White	Rhizoid	Wavy	Raised	Smooth	Large	Shiny	Translucent
537	I23B/5	Yellowish White	Irregular	Lobate	Raised	Smooth	Large	Shiny	Translucent
538	I23B/6	White	Circular	Entire	Raised	Smooth	Medium	Shiny	Opaque
539	I23B/7	Yellowish White	Irregular	Lobate	Raised	Smooth	Medium	Shiny	Opaque
540	I23B/8	White	Circular	Entire	Raised	Finely Granular	Small	Shiny	Opaque
541	I23B/9	Yellowish White	Irregular	Lobate	Raised	Smooth	Medium	Shiny	Opaque
542	I23B/10	White	Circular	Wavy	Raised	Smooth	Medium	Shiny	Opaque

APPENDIX-B: Preliminary Test Results of 542 Isolates

Isolate Number	Isolate Name	Preliminary Identification Tests				
		Gram Staining	Bacterial Morphology	Catalase Test	Oxidase Test	Spore Staining
1	ND1A/2	(-)	Coccus	(+++)	(-)	NA
2	ND1A/3	(+)	Cocobacillus	(++)	(-)	
3	ND1A/4	(-)	Coccus	(++)	(-)	NA
4	ND1A/5	(-)	Coccus	(++)	(-)	NA
5	ND1A/6	(-)	Cocobacillus	(+++)	(+)	NA
6	ND1A/7	(-)	Bacillus	(++)	(+)	NA
7	ND1A/8	(-)	Bacillus	(+)	(+)	NA
8	ND1A/10	(-)	Bacillus	(+++)	(+)	NA
9	ND1A/11	(-)	Bacillus	(+++)	(+)	NA
10	ND1A/12	(+)	Coccus	(++)	(-)	NA
11	ND1A/13	(-)	Bacillus	(+)	(-)	NA
12	ND1A/15	(-)	Coccus	(++)	(-)	NA
13	ND1A/16	(-)	Bacillus	(++)	(-)	NA
14	ND1A/17	(-)	Bacillus	(+)	(-)	NA
15	ND1A/18	(+)	Coccus	(+)	(-)	NA
16	ND1A/19	(-)	Bacillus	(+++)	(+)	NA
17	ND1A/20	(-)	Bacillus	(+++)	(+)	NA
18	ND1A/21	(-)	Bacillus	(++)	(+)	NA
19	ND1A/22	(-)	Bacillus	(+)	(-)	NA
20	ND1A/23	(-)	Bacillus	(-)	(-)	NA
21	ND1A/24	(+)	Cocobacillus	(+)	(-)	
22	ND1A/25	(-)	Bacillus	(++)	(-)	NA
23	ND1A/26	(-)	Bacillus	(+)	(-)	NA
24	ND1A/27	(-)	Bacillus	(++)	(-)	NA
25	ND1A/28	(-)	Bacillus	(+++)	(+)	NA
26	ND1A/29	(-)	Bacillus	(++)	(+)	NA
27	ND1A/31	(+)	Bacillus	(+)	(-)	
28	ND1A/32	(-)	Cocobacillus	(+++)	(+)	NA
29	ND1A/33	(-)	Bacillus	(++)	(+)	NA
30	ND1A/34	(-)	Cocobacillus	(++)	(-)	NA
31	ND1A/36	(-)	Bacillus	(++)	(+)	NA
32	ND1B/1	(+)	Coccus	(+)	(-)	NA
33	ND1B/3	(+)	Diplococcus	(++)	(-)	NA
34	ND1B/6	(-)	Bacillus	(+)	(+)	NA
35	ND1B/7	(-)	Bacillus	(++)	(+)	NA
36	ND1B/8	(-)	Bacillus	(++)	(+)	NA
37	ND1B/9	(+)	Coccus	(+)	(-)	NA
38	ND1B/10	(-)	Coccus	(++)	(-)	NA
39	ND1B/11	(+)	Diplococcus	(+)	(-)	
40	ND1B/12	(-)	Bacillus	(+)	(+)	NA
41	ND1B/13	(-)	Bacillus	(++)	(-)	NA
42	ND1B/14	(-)	Bacillus	(+)	(+)	NA
43	ND1B/16	(+)	Cocobacillus	(+++)	(+)	
44	ND1B/17	(-)	Cocobacillus	(±)	(-)	NA
45	ND1B/18	(-)	Bacillus	(+)	(-)	NA
46	ND1B/19	(-)	Bacillus	(++)	(+)	NA
47	ND1B/20	(-)	Cocobacillus	(+++)	(-)	NA
48	ND1B/21	(-)	Coccus	(++)	(-)	NA
49	ND1B/22	(-)	Coccus	(++)	(-)	NA
50	ND1B/23	(-)	Cocobacillus	(+)	(+)	NA
51	ND1B/24	(-)	Bacillus	(++)	(+)	NA
52	ND1B/25	(-)	Diplococcus	(+++)	(+)	NA
53	ND1B/26	(-)	Bacillus	(+)	(+)	NA
54	ND1B/28	(-)	Cocobacillus	(++)	(+)	NA
55	ND1B/30	(-)	Diplococcus	(++)	(+)	NA
56	ND1B/32	(-)	Coccus	(++)	(-)	NA
57	ND1B/34	(-)	Bacillus	(++)	(-)	
58	ND2A/4	(-)	Cocobacillus	(+)	(-)	NA
59	ND2A/5	(+)	Coccus	(+++)	(+)	NA
60	ND2A/6	(+)	Cocobacillus	(+)	(-)	
61	ND2A/7	(-)	Bacillus	(++)	(+)	NA
62	ND2A/8	(-)	Bacillus	(++)	(-)	NA
63	ND2A/9	(-)	Cocobacillus	(+++)	(-)	NA
64	ND2A/10	(-)	Coccus	(+)	(-)	NA
65	ND2A/11	(-)	Cocobacillus	(+++)	(-)	NA
66	ND2A/12	(-)	Coccus	(+)	(-)	NA
67	ND2A/13	(+)	Coccus	(++)	(-)	NA
68	ND2A/14	(+)	Bacillus	(++)	(+)	
69	ND2A/15	(-)	Bacillus	(++)	(-)	NA
70	ND2A/16	(-)	Coccus	(-)	(-)	NA

APPENDIX-B: Continued.

Isolate Number	Isolate Name	Preliminary Identification Tests				
		Gram Staining	Bacterial Morphology	Catalase Test	Oxidase Test	Spore Staining
71	ND2A/17	(-)	Diplococcus	(++)	(-)	NA
72	ND2A/34	(-)	Bacillus	(+++)	(+)	NA
73	ND2A/36	(-)	Coccus	(+++)	(-)	NA
74	ND2A/37	(-)	Cocobacillus	(+)	(-)	NA
75	ND2A/38	(-)	Coccus	(+++)	(+)	NA
76	ND2A/39	(-)	Cocobacillus	(++)	(-)	NA
77	ND2A/40	(-)	Cocobacillus	(+)	(-)	NA
78	ND2A/41	(-)	Diplococcus	(+)	(-)	NA
79	ND2A/42	(-)	Bacillus	(+++)	(+)	NA
80	ND2A/43	(+)	Bacillus	(++)	(-)	(-)
81	ND2A/44	(+)	Coccus	(+)	(-)	NA
82	ND2A/45	(+)	Bacillus	(+)	(-)	(-)
83	ND2A/46	(-)	Coccus	(++)	(-)	NA
84	ND2A/47	(+)	Coccus	(+++)	(-)	NA
85	ND2A/48	(-)	Cocobacillus	(+++)	(-)	NA
86	ND2A/49	(-)	Coccus	(+)	(-)	NA
87	ND2A/61	(+)	Coccus	(-)	(-)	NA
88	ND2A/62	(-)	Cocobacillus	(+++)	(+)	NA
89	ND2A/63	(-)	Bacillus	(+)	(-)	NA
90	ND2A/65	(-)	Cocobacillus	(+++)	(-)	NA
91	ND2B/1	(-)	Cocobacillus	(+)	(-)	NA
92	ND2B/2	(+)	Coccus	(++)	(-)	NA
93	ND2B/3	(+)	Bacillus	(++)	(-)	(+)
94	ND2B/4	(-)	Cocobacillus	(-)	(-)	NA
95	ND2B/5	(-)	Coccus	(-)	(-)	NA
96	ND2B/6	(-)	Coccus	(++)	(-)	NA
97	ND2B/7	(-)	Coccus	(+)	(-)	NA
98	ND2B/8	(+)	Bacillus	(+++)	(-)	NA
99	ND2B/9	(-)	Bacillus	(++)	(-)	NA
100	ND2B/11	(-)	Coccus	(+)	(-)	NA
101	ND2B/12	(-)	Bacillus	(+++)	(-)	NA
102	ND2B/13	(-)	Cocobacillus	(++)	(-)	NA
103	ND2B/14	(+)	Bacillus	(+++)	(-)	NA
104	ND2B/15	(+)	Coccus	(+++)	(-)	NA
105	ND2B/16	(-)	Bacillus	(+++)	(-)	NA
106	ND2B/17	(-)	Coccus	(++)	(-)	NA
107	ND2B/18	(-)	Bacillus	(++)	(-)	NA
108	ND2B/19	(-)	Bacillus	(+)	(-)	NA
109	ND2B/20	(-)	Cocobacillus	(++)	(-)	NA
110	ND2B/21	(+)	Coccus	(-)	(-)	NA
111	ND2B/22	(-)	Coccus	(++)	(-)	NA
112	ND2B/23	(-)	Bacillus	(+++)	(-)	NA
113	ND2B/24	(-)	Coccus	(+++)	(-)	NA
114	ND3A/2	(-)	Diplococcus	(++)	(-)	NA
115	ND3A/4	(+)	Bacillus	(++)	(-)	(-)
116	ND3A/5	(-)	Bacillus	(+++)	(-)	NA
117	ND3A/8	(-)	Bacillus	(++)	(-)	NA
118	ND3A/9	(+)	Cocobacillus	(+++)	(+)	(-)
119	ND3A/11	(-)	Coccus	(++)	(-)	NA
120	ND3A/12	(-)	Cocobacillus	(+++)	(+)	NA
121	ND3A/13	(-)	Cocobacillus	(++)	(-)	NA
122	ND3A/14	(-)	Cocobacillus	(++)	(-)	NA
123	ND3A/15	(-)	Bacillus	(++)	(-)	NA
124	ND3A/16	(-)	Cocobacillus	(+++)	(-)	NA
125	ND3A/17	(-)	Cocobacillus	(++)	(-)	NA
126	ND3A/18	(-)	Cocobacillus	(++)	(-)	NA
127	ND3A/19	(-)	Bacillus	(++)	(-)	NA
128	ND3A/20	(+)	Cocobacillus	(+++)	(-)	(-)
129	ND3A/21	(-)	Cocobacillus	(+)	(-)	NA
130	ND3A/22	(+)	Coccus	(++)	(-)	NA
131	ND3A/23	(-)	Coccus	(++)	(-)	NA
132	ND3A/24	(+)	Bacillus	(+++)	(-)	(-)
133	ND3A/25	(-)	Bacillus	(+++)	(+)	NA
134	ND3A/26	(-)	Bacillus	(++)	(-)	NA
135	ND3A/27	(-)	Cocobacillus	(++)	(-)	NA
136	ND3A/29	(-)	Cocobacillus	(+++)	(-)	NA
137	ND3A/30	(-)	Cocobacillus	(++)	(-)	NA
138	ND3A/31	(-)	Cocobacillus	(++)	(-)	NA
139	ND3A/32	(-)	Bacillus	(++)	(-)	NA
140	ND3A/33	(+)	Coccus	(++)	(-)	NA

APPENDIX-B: Continued.

Isolate Number	Isolate Name	Preliminary Identification Tests				
		Gram Staining	Bacterial Morphology	Catalase Test	Oxidase Test	Spore Staining
141	ND3B/2	(-)	Coccus	(++)	(-)	NA
142	ND3B/4	(+)	Coccus	(+)	(-)	NA
143	ND3B/5	(-)	Cocobacillus	(+)	(-)	NA
144	ND3B/7	(-)	Coccus	(+)	(-)	NA
145	ND3B/8	(-)	Coccus	(++)	(-)	NA
146	ND3B/9	(-)	Bacillus	(+)	(-)	NA
147	ND3B/10	(-)	Coccus	(+)	(-)	NA
148	ND3B/11	(+)	Cocobacillus	(+)	(-)	NA
149	ND3B/12	(+)	Bacillus	(+)	(-)	(-)
150	ND3B/13	(+)	Cocobacillus	(+)	(-)	(-)
151	ND3B/14	(+)	Bacillus	(+)	(-)	(-)
152	ND3B/15	(+)	Bacillus	(+)	(+)	(-)
153	ND3B/16	(+)	Coccus	(++)	(-)	NA
154	ND3B/17	(+)	Coccus	(+)	(-)	NA
155	ND3B/18	(+)	Coccus	(+++)	(-)	NA
156	ND3B/19	(-)	Bacillus	(+++)	(-)	NA
157	ND3B/20	(-)	Bacillus	(+++)	(+)	NA
158	ND3B/22	(-)	Cocobacillus	(++)	(-)	NA
159	ND3B/23	(-)	Bacillus	(+)	(-)	NA
160	ND3B/24	(-)	Cocobacillus	(++)	(-)	NA
161	ND3B/25	(+)	Diplococcus	(+++)	(-)	NA
162	ND3B/26	(+)	Coccus	(+++)	(-)	NA
163	ND3B/27	(-)	Coccus	(++)	(-)	NA
164	ND3B/28	(-)	Bacillus	(+++)	(-)	NA
165	ND3B/29	(-)	Bacillus	(+++)	(-)	NA
166	ND3B/30	(-)	Bacillus	(++)	(-)	NA
167	ND3B/31	(+)	Bacillus	(+++)	(-)	(+)
168	ND3B/32	(-)	Bacillus	(++)	(-)	NA
169	ND3B/33	(-)	Coccus	(++)	(-)	NA
170	ND3B/34	(-)	Cocobacillus	(++)	(-)	NA
171	ND3B/35	(-)	Coccus	(++)	(-)	NA
172	ND3B/36	(-)	Cocobacillus	(++)	(-)	(-)
173	AF1A/1	(-)	Coccus	(++)	(-)	NA
174	AF1A/2	(-)	Bacillus	(+++)	(+)	NA
175	AF1A/3	(-)	Cocobacillus	(++)	(+)	NA
176	AF1A/4	(-)	Cocobacillus	(+++)	(-)	NA
177	AF1A/9	(-)	Cocobacillus	(++)	(-)	NA
178	AF1A/12	(+)	Bacillus	(++)	(-)	(+)
179	AF1A/13	(+)	Coccus	(±)	(-)	NA
180	AF1A/14	(+)	Bacillus	(+++)	(-)	(-)
181	AF1A/15	(+)	Bacillus	(+++)	(-)	(-)
182	AF1A/17	(-)	Coccus	(++)	(-)	NA
183	AF1A/18	(-)	Coccus	(+++)	(-)	NA
184	AF1A/20	(-)	Coccus	(++)	(-)	NA
185	AF1A/21	(-)	Cocobacillus	(+++)	(-)	NA
186	AF1A/22	(-)	Cocobacillus	(+++)	(-)	NA
187	AF1A/23	(-)	Cocobacillus	(+++)	(-)	NA
188	AF1B/1	(-)	Bacillus	(+++)	(-)	NA
189	AF1B/2	(-)	Bacillus	(++)	(+)	NA
190	AF1B/3	(-)	Bacillus	(++)	(-)	NA
191	AF1B/4	(-)	Bacillus	(+++)	(-)	NA
192	AF1B/5	(-)	Bacillus	(++)	(-)	NA
193	AF1B/7	(-)	Cocobacillus	(++)	(-)	NA
194	AF1B/8	(-)	Bacillus	(+++)	(-)	NA
195	AF1B/11	(-)	Bacillus	(++)	(-)	NA
196	AF1B/13	(-)	Coccus	(++)	(-)	NA
197	AF1B/15	(-)	Coccus	(+++)	(-)	NA
198	AF1B/16	(+)	Bacillus	(++)	(-)	(-)
199	AF1B/17	(-)	Cocobacillus	(++)	(-)	NA
200	AF1B/18	(-)	Bacillus	(+++)	(-)	NA
201	AF1B/21	(+)	Bacillus	(++)	(-)	(-)
202	AF1B/25	(-)	Cocobacillus	(+++)	(-)	NA
203	AF1B/26	(+)	Bacillus	(++)	(-)	(-)
204	AF2A/1	(-)	Cocobacillus	(++)	(-)	NA
205	AF2A/2	(-)	Bacillus	(++)	(-)	NA
206	AF2A/3	(-)	Bacillus	(+++)	(+)	NA
207	AF2A/13	(-)	Coccus	(+)	(-)	NA
208	AF2A/16	(-)	Cocobacillus	(+++)	(-)	NA
209	AF2A/19	(-)	Cocobacillus	(++)	(-)	NA
210	AF2A/23	(-)	Coccus	(+)	(-)	NA

APPENDIX-B: Continued.

Isolate Number	Isolate Name	Preliminary Identification Tests				
		Gram Staining	Bacterial Morphology	Catalase Test	Oxidase Test	Spore Staining
211	AF2A/28	(-)	Bacillus	(++)	(-)	NA
212	AF2A/29	(-)	Bacillus	(++)	(-)	NA
213	AF2B/2	(-)	Cocobacillus	(++)	(-)	NA
214	AF2B/6	(-)	Cocobacillus	(++)	(-)	NA
215	AF2B/9	(+)	Bacillus	(+)	(-)	
216	AF2B/12	(+)	Bacillus	(+)	(-)	
217	AF2B/13	(+)	Bacillus	(+)	(-)	
218	AF2B/15	(-)	Coccus	(++)	(-)	NA
219	AF2B/16	(+)	Cocobacillus	(++)	(-)	
220	AF2B/19	(-)	Cocobacillus	(++)	(-)	NA
221	AF2B/20	(-)	Coccus	(++)	(-)	NA
222	AF3A/1	(-)	Bacillus	(+++)	(-)	NA
223	AF3A/2	(-)	Cocobacillus	(+++)	(-)	NA
224	AF3A/3	(-)	Bacillus	(+)	(-)	NA
225	AF3A/4	(-)	Coccus	(++)	(-)	NA
226	AF3A/5	(-)	Coccus	(++)	(-)	NA
227	AF3A/9	(-)	Cocobacillus	(+++)	(-)	NA
228	AF3A/10	(-)	Cocobacillus	(+++)	(-)	NA
229	AF3A/11	(-)	Cocobacillus	(++)	(-)	NA
230	AF3A/12	(+)	Coccus	(++)	(-)	NA
231	AF3A/13	(-)	Coccus	(+++)	(+)	NA
232	AF3A/14	(-)	Bacillus	(++)	(-)	NA
233	AF3A/15	(-)	Coccus	(+++)	(-)	NA
234	AF3A/16	(-)	Coccus	(++)	(-)	NA
235	AF3A/17	(-)	Bacillus	(+++)	(-)	NA
236	AF3A/19	(-)	Cocobacillus	(+++)	(-)	NA
237	AF3A/20	(-)	Cocobacillus	(++)	(+)	NA
238	AF3A/22	(+)	Coccus	(±)	(-)	NA
239	AF3A/23	(-)	Bacillus	(++)	(-)	NA
240	AF3A/24	(+)	Coccus	(++)	(-)	NA
241	AF3A/25	(-)	Coccus	(++)	(+)	NA
242	AF3A/26	(-)	Bacillus	(+)	(-)	NA
243	AF3A/29	(+)	Bacillus	(+)	(-)	
244	AF3A/31	(-)	Cocobacillus	(+++)	(-)	NA
245	AF3A/32	(-)	Cocobacillus	(+)	(+)	NA
246	AF3A/33	(-)	Cocobacillus	(+++)	(-)	NA
247	AF3B/1	(-)	Cocobacillus	(+++)	(-)	NA
248	AF3B/3	(-)	Cocobacillus	(+++)	(-)	NA
249	AF3B/4	(-)	Coccus	(++)	(-)	NA
250	AF3B/5	(-)	Cocobacillus	(+++)	(-)	NA
251	AF3B/7	(-)	Bacillus	(++)	(+)	NA
252	AF3B/10	(-)	Coccus	(++)	(-)	NA
253	AF3B/11	(-)	Coccus	(+++)	(+)	NA
254	AF3B/14	(-)	Coccus	(+++)	(-)	NA
255	AF3B/15	(-)	Cocobacillus	(+++)	(+)	NA
256	AF3B/17	(-)	Coccus	(++)	(+)	NA
257	AF3B/18	(-)	Coccus	(++)	(-)	NA
258	AF3B/19	(-)	Cocobacillus	(+++)	(-)	NA
259	AF3B/20	(-)	Coccus	(++)	(-)	NA
260	AF3B/21	(-)	Coccus	(+)	(-)	NA
261	AF3B/22	(-)	Cocobacillus	(++)	(-)	NA
262	AF3B/23	(-)	Coccus	(+++)	(-)	NA
263	AF3B/24	(-)	Cocobacillus	(+++)	(-)	NA
264	AF3B/25	(-)	Coccus	(+++)	(-)	NA
265	KN1A/1	(+)	Bacillus	(++)	(-)	
266	KN1A/2	(-)	Cocobacillus	(++)	(+)	NA
267	KN1A/3	(-)	Cocobacillus	(++)	(+)	NA
268	KN1A/4	(-)	Coccus	(+)	(+)	NA
269	KN1A/5	(+)	Coccus	(+)	(-)	NA
270	KN1A/6	(-)	Coccus	(+)	(+)	NA
271	KN1A/7	(-)	Cocobacillus	(++)	(+)	NA
272	KN1A/9	(-)	Cocobacillus	(+++)	(+)	NA
273	KN1A/10	(-)	Bacillus	(+++)	(-)	NA
274	KN1A/13	(+)	Bacillus	(+++)	(-)	
275	KN1A/14	(-)	Cocobacillus	(+)	(+)	NA
276	KN1A/16	(-)	Cocobacillus	(+++)	(+)	NA
277	KN1A/22	(-)	Bacillus	(++)	(+)	NA
278	KN1A/23	(+)	Bacillus	(+)	(-)	
279	KN1B/1	(-)	Coccus	(+++)	(-)	NA
280	KN1B/2	(-)	Bacillus	(++)	(+)	NA

APPENDIX-B: Continued.

281	KN1B/3	(-)	Coccus	(+)	(-)	NA	-
282	KN1B/5	(-)	Bacillus	(+)	(+)	NA	
283	KN1B/6	(+)	Bacillus	(++)	(-)		
284	KN1B/7	(-)	Bacillus	(+++)	(+)	NA	
285	KN1B/9	(-)	Coccus	(++)	(+)	NA	
286	KN1B/10	(+)	Bacillus	(++)	(+)	NA	
287	KN1B/11	(-)	Bacillus	(++)	(+)	NA	
288	KN1B/13	(-)	Cocobacillus	(++)	(-)	NA	-
289	KN2A/1	(-)	Bacillus	(++)	(+)	NA	
290	KN2A/2	(-)	Coccus	(+++)	(-)	NA	
291	KN2A/4	(-)	Cocobacillus	(+++)	(+)	NA	
292	KN2A/5	(-)	Bacillus	(+++)	(+)	NA	
293	KN2A/6	(-)	Bacillus	(+++)	(+)	NA	
294	KN2A/7	(-)	Cocobacillus	(+++)	(+)	NA	
295	KN2A/8	(+)	Bacillus	(++)	(-)		
296	KN2A/9	(-)	Bacillus	(+)	(+)	NA	
297	KN2A/10	(-)	Cocobacillus	(++)	(+)	NA	
298	KN2A/11	(-)	Coccus	(++)	(-)	NA	
299	KN2A/12	(+)	Coccus	(++)	(-)		
300	KN2A/13	(-)	Cocobacillus	(+++)	(+)	NA	
301	KN2A/14	(+)	Bacillus	(+)	(-)		
302	KN2A/15	(-)	Bacillus	(+++)	(+)	NA	
303	KN2A/16	(+)	Bacillus	(++)	(-)		
304	KN2A/18	(-)	Cocobacillus	(++)	(-)	NA	
305	KN2B/1	(-)	Coccus	(++)	(-)	NA	
306	KN2B/2	(-)	Coccus	(++)	(+)	NA	-
307	KN2B/3	(-)	Coccus	(++)	(-)	NA	
308	KN2B/4	(-)	Coccus	(+++)	(-)	NA	
309	KN2B/5	(+)	Bacillus	(++)	(+)		
310	KN2B/6	(-)	Coccus	(++)	(-)	NA	
311	KN2B/7	(-)	Bacillus	(++)	(-)	NA	
312	KN2B/8	(+)	Bacillus	(++)	(-)		
313	KN2B/9	(-)	Coccus	(++)	(+)	NA	
314	KN2B/10	(+)	Bacillus	(++)	(-)		
315	KN2B/11	(-)	Coccus	(++)	(-)	NA	
316	KN2B/12	(-)	Bacillus	(+++)	(+)	NA	
317	KN2B/13	(-)	Coccus	(++)	(-)	NA	
318	KN2B/14	(+)	Coccus	(++)	(-)	NA	
319	KN2B/15	(-)	Coccus	(+++)	(+)	NA	
320	KN2B/16	(+)	Bacillus	(+++)	(-)		
321	KN3A/1	(-)	Coccus	(+++)	(+)	NA	
322	KN3A/2	(+)	Coccus	(+)	(-)	NA	
323	KN3A/3	(-)	Bacillus	(+++)	(+)	NA	
324	KN3A/4	(-)	Coccus	(+++)	(+)	NA	
325	KN3A/5	(-)	Cocobacillus	(+++)	(+)	NA	
326	KN3A/6	(+)	Coccus	(++)	(-)	NA	
327	KN3A/7	(-)	Cocobacillus	(+++)	(+)	NA	
328	KN3A/8	(-)	Bacillus	(++)	(+)	NA	
329	KN3A/9	(-)	Coccus	(++)	(+)	NA	
330	KN3A/10	(+)	Coccus	(+++)	(+)	NA	
331	KN3A/11	(-)	Coccus	(++)	(+)	NA	
332	KN3A/12	(-)	Bacillus	(+++)	(+)	NA	
333	KN3A/13	(-)	Coccus	(++)	(-)	NA	
334	KN3A/14	(-)	Bacillus	(+)	(+)	NA	
335	KN3A/15	(-)	Coccus	(++)	(+)	NA	
336	KN3A/16	(-)	Coccus	(++)	(+)	NA	
337	KN3A/17	(-)	Bacillus	(++)	(+)	NA	
338	KN3A/21	(-)	Bacillus	(+)	(+)	NA	
339	KN3A/22	(+)	Bacillus	(+)	(-)		
340	KN3A/23	(-)	Bacillus	(+++)	(+)	NA	
341	KN3A/24	(-)	Bacillus	(++)	(+)	NA	
342	KN3B/1	(-)	Diplococcus	(++)	(-)	NA	
343	KN3B/2	(-)	Bacillus	(+)	(-)	NA	
344	KN3B/3	(-)	Coccus	(+)	(+)	NA	
345	KN3B/4	(-)	Coccus	(++)	(+)	NA	
346	KN3B/5	(-)	Bacillus	(+)	(+)	NA	
347	KN3B/6	(-)	Coccus	(++)	(-)	NA	
348	KN3B/7	(-)	Coccus	(++)	(-)	NA	
349	KN3B/8	(-)	Coccus	(+++)	(+)	NA	
350	KN3B/10	(-)	Bacillus	(+)	(-)	NA	

APPENDIX-B: Continued.

Isolate Number	Isolate Name	Preliminary Identification Tests				
		Gram Staining	Bacterial Morphology	Catalase Test	Oxidase Test	Spore Staining
351	KN3B/11	(-)	Bacillus	(++)	(-)	NA
352	KN3B/12	(-)	Bacillus	(++)	(+)	NA
353	KN3B/13	(-)	Coccus	(++)	(+)	NA
354	KN3B/14	(+)	Bacillus	(+++)	(-)	
355	KN3B/15	(-)	Coccus	(++)	(-)	NA
356	KY1A/2	(-)	Bacillus	(++)	(+)	NA
357	KY1A/3	(-)	Bacillus	(-)	(-)	NA
358	KY1A/4	(-)	Bacillus	(+)	(+)	NA
359	KY1A/6	(-)	Bacillus	(+++)	(+)	NA
360	KY1A/7	(-)	Bacillus	(-)	(-)	NA
361	KY1A/8	(-)	Bacillus	(-)	(-)	NA
362	KY1A/9	(-)	Bacillus	(+++)	(+)	NA
363	KY1A/10	(-)	Bacillus	(++)	(-)	NA
364	KY1A/11	(+)	Coccus	(+)	(-)	NA
365	KY1A/12	(-)	Coccus	(++)	(-)	NA
366	KY1A/13	(-)	Bacillus	(++)	(-)	NA
367	KY1A/14	(-)	Bacillus	(++)	(+)	NA
368	KY1B/1	(-)	Coccus	(+++)	(+)	NA
369	KY1B/2	(-)	Coccus	(++)	(-)	NA
370	KY1B/3	(-)	Coccus	(+)	(-)	NA
371	KY1B/4	(-)	Cocobacillus	(++)	(+)	NA
372	KY1B/5	(-)	Cocobacillus	(++)	(-)	NA
373	KY1B/6	(+)	Bacillus	(+++)	(+)	
374	KY1B/7	(-)	Cocobacillus	(++)	(+)	NA
375	KY1B/8	(-)	Coccus	(++)	(-)	NA
376	KY1B/9	(-)	Coccus	(++)	(-)	NA
377	KY1B/10	(-)	Coccus	(++)	(-)	NA
378	KY1B/11	(-)	Coccus	(++)	(-)	NA
379	KY1B/12	(-)	Coccus	(++)	(+)	NA
380	KY1B/13	(-)	Cocobacillus	(++)	(-)	NA
381	KY1B/14	(-)	Bacillus	(++)	(-)	NA
382	KY1B/16	(+)	Cocobacillus	(++)	(-)	
383	KY1B/17	(-)	Bacillus	(++)	(-)	NA
384	KY1B/18	(-)	Cocobacillus	(++)	(+)	NA
385	KY2A/1	(-)	Bacillus	(+++)	(+)	NA
386	KY2A/2	(-)	Bacillus	(+++)	(+)	NA
387	KY2A/3	(-)	Cocobacillus	(++)	(+)	NA
388	KY2A/4	(-)	Bacillus	(-)	(+)	NA
389	KY2A/5	(-)	Coccus	(++)	(+)	NA
390	KY2A/6	(-)	Bacillus	(++)	(+)	NA
391	KY2A/7	(-)	Bacillus	(++)	(+)	NA
392	KY2A/9	(-)	Bacillus	(+++)	(-)	NA
393	KY2A/10	(-)	Bacillus	(+++)	(+)	NA
394	KY2A/11	(+)	Bacillus	(+++)	(-)	
395	KY2A/12	(-)	Cocobacillus	(++)	(+)	NA
396	KY2A/13	(+)	Bacillus	(++)	(-)	
397	KY2A/14	(-)	Bacillus	(++)	(+)	NA
398	KY2A/15	(-)	Coccus	(++)	(+)	NA
399	KY2A/16	(-)	Bacillus	(+)	(-)	NA
400	KY2A/17	(-)	Bacillus	(++)	(+)	NA
401	KY2A/18	(-)	Coccus	(+++)	(-)	NA
402	KY2A/20	(-)	Cocobacillus	(++)	(+)	NA
403	KY2B/1	(+)	Bacillus	(++)	(-)	
404	KY2B/2	(-)	Bacillus	(+++)	(-)	NA
405	KY2B/3	(-)	Cocobacillus	(+)	(-)	NA
406	KY2B/4	(+)	Bacillus	(+)	(-)	
407	KY2B/5	(-)	Bacillus	(++)	(+)	NA
408	KY2B/6	(+)	Bacillus	(++)	(-)	
409	KY2B/7	(-)	Coccus	(+)	(-)	NA
410	KY2B/8	(-)	Coccus	(++)	(+)	NA
411	KY2B/9	(+)	Bacillus	(-)	(-)	
412	KY2B/10	(-)	Cocobacillus	(++)	(+)	NA
413	KY2B/11	(-)	Cocobacillus	(++)	(+)	NA
414	KY2B/12	(-)	Cocobacillus	(++)	(-)	NA
415	KY3A/1	(-)	Bacillus	(+++)	(+)	NA
416	KY3A/2	(-)	Bacillus	(++)	(-)	NA
417	KY3A/4	(-)	Bacillus	(-)	(+)	NA
418	KY3A/5	(-)	Coccus	(+++)	(-)	NA
419	KY3A/7	(-)	Bacillus	(-)	(+)	NA
420	KY3A/8	(-)	Bacillus	(-)	(-)	NA

APPENDIX-B: Continued.

Isolate Number	Isolate Name	Preliminary Identification Tests				
		Gram Staining	Bacterial Morphology	Catalase Test	Oxidase Test	Spore Staining
421	KY3A/9	(-)	Bacillus	(-)	(-)	NA
422	KY3A/10	(-)	Diplococcus	(++)	(+)	NA
423	KY3A/11	(-)	Bacillus	(+++)	(+)	NA
424	KY3A/12	(-)	Bacillus	(-)	(+)	NA
425	KY3A/13	(-)	Bacillus	(-)	(+)	NA
426	KY3A/14	(-)	Bacillus	(-)	(-)	NA
427	KY3A/15	(-)	Coccus	(++)	(-)	NA
428	KY3A/16	(-)	Bacillus	(++)	(+)	NA
429	KY3A/17	(-)	Diplococcus	(++)	(-)	NA
430	KY3A/18	(-)	Coccus	(+)	(-)	NA
431	KY3A/19	(-)	Bacillus	(+++)	(+)	NA
432	KY3A/20	(-)	Bacillus	(+++)	(+)	NA
433	KY3A/23	(-)	Bacillus	(-)	(-)	NA
434	KY3A/24	(-)	Cocobacillus	(-)	(-)	NA
435	KY3B/1	(-)	Bacillus	(+++)	(+)	NA
436	KY3B/2	(-)	Cocobacillus	(++)	(-)	NA
437	KY3B/3	(-)	Bacillus	(-)	(-)	NA
438	KY3B/4	(-)	Bacillus	(+++)	(+)	NA
439	KY3B/5	(-)	Bacillus	(++)	(+)	NA
440	KY3B/6	(-)	Bacillus	(++)	(+)	NA
441	KY3B/7	(-)	Coccus	(+)	(-)	NA
442	KY3B/9	(-)	Bacillus	(++)	(+)	NA
443	KY3B/10	(-)	Cocobacillus	(++)	(+)	NA
444	KY3B/13	(+)	Bacillus	(++)	(+)	
445	KY3B/14	(-)	Cocobacillus	(++)	(+)	NA
446	Iz1A/1	(-)	Bacillus	(+++)	(+)	NA
447	Iz1A/2	(-)	Coccus	(++)	(+)	NA
448	Iz1A/4	(-)	Bacillus	(+)	(-)	NA
449	Iz1A/5	(-)	Cocobacillus	(++)	(+)	NA
450	Iz1A/6	(-)	Cocobacillus	(+++)	(+)	NA
451	Iz1A/7	(-)	Bacillus	(++)	(+)	NA
452	Iz1A/8	(-)	Bacillus	(+++)	(+)	NA
453	Iz1A/9	(-)	Cocobacillus	(++)	(+)	NA
454	Iz1A/10	(-)	Cocobacillus	(++)	(+)	NA
455	Iz1A/12	(-)	Bacillus	(+)	(+)	NA
456	Iz1A/13	(-)	Cocobacillus	(++)	(-)	NA
457	Iz1A/14	(-)	Bacillus	(++)	(+)	NA
458	Iz1A/15	(-)	Bacillus	(+++)	(+)	NA
459	Iz1A/16	(-)	Diplococcus	(++)	(-)	NA
460	Iz1A/17	(-)	Bacillus	(+)	(+)	NA
461	Iz1A/18	(-)	Cocobacillus	(+++)	(+)	NA
462	Iz1A/19	(-)	Coccus	(++)	(+)	NA
463	Iz1A/20	(-)	Bacillus	(++)	(+)	NA
464	Iz1A/21	(+)	Bacillus	(++)	(-)	
465	Iz1A/23	(-)	Bacillus	(++)	(-)	NA
466	Iz1A/24	(-)	Bacillus	(+)	(+)	NA
467	Iz1B/1	(-)	Bacillus	(+++)	(-)	NA
468	Iz1B/2	(-)	Bacillus	(++)	(-)	NA
469	Iz1B/3	(-)	Bacillus	(++)	(+)	NA
470	Iz1B/4	(-)	Cocobacillus	(++)	(+)	NA
471	Iz1B/5	(-)	Bacillus	(+++)	(+)	NA
472	Iz1B/6	(-)	Coccus	(++)	(-)	NA
473	Iz1B/7	(-)	Bacillus	(++)	(+)	NA
474	Iz1B/8	(+)	Coccus	(+)	(-)	NA
475	Iz1B/9	(-)	Bacillus	(++)	(+)	NA
476	Iz1B/10	(-)	Bacillus	(+)	(-)	NA
477	Iz1B/11	(-)	Cocobacillus	(+++)	(-)	NA
478	Iz1B/13	(-)	Coccus	(++)	(-)	NA
479	Iz1B/14	(-)	Cocobacillus	(++)	(-)	NA
480	Iz1B/15	(-)	Bacillus	(+++)	(+)	NA
481	Iz1B/16	(-)	Bacillus	(+)	(-)	NA
482	Iz2A/1	(-)	Bacillus	(+)	(-)	NA
483	Iz2A/2	(-)	Cocobacillus	(+++)	(+)	NA
484	Iz2A/3	(-)	Diplococcus	(++)	(-)	NA
485	Iz2A/4	(-)	Diplococcus	(++)	(-)	NA
486	Iz2A/5	(-)	Bacillus	(+++)	(+)	NA
487	Iz2A/6	(-)	Bacillus	(+)	(+)	NA
488	Iz2A/7	(-)	Bacillus	(+)	(+)	NA
489	Iz2A/8	(-)	Bacillus	(+++)	(+)	NA
490	Iz2A/9	(-)	Bacillus	(++)	(+)	NA
491	Iz2A/10	(-)	Coccus	(++)	(+)	NA

APPENDIX-B: Continued.

Isolate Number	Isolate Name	Preliminary Identification Tests				
		Gram Staining	Bacterial Morphology	Catalase Test	Oxidase Test	Spore Staining
491	iZ2A/10	(-)	Coccus	(++)	(+)	NA
492	iZ2A/11	(-)	Bacillus	(+)	(+)	NA
493	iZ2A/12	(-)	Bacillus	(+)	(+)	NA
494	iZ2A/13	(-)	Bacillus	(++)	(+)	NA
495	iZ2A/14	(+)	Bacillus	(+)	(-)	
496	iZ2A/15	(-)	Coccus	(+)	(+)	NA
497	iZ2A/16	(+)	Bacillus	(++)	(-)	
498	iZ2A/17	(-)	Bacillus	(++)	(+)	NA
499	iZ2A/18	(-)	Bacillus	(++)	(+)	NA
500	iZ2A/19	(+)	Cocobacillus	(+++)	(+)	
501	iZ2A/20	(+)	Bacillus	(++)	(+)	
502	iZ2B/1	(+)	Bacillus	(+++)	(-)	
503	iZ2B/2	(-)	Bacillus	(+++)	(+)	NA
504	iZ2B/3	(-)	Coccus	(++)	(+)	NA
505	iZ2B/4	(-)	Coccus	(++)	(+)	NA
506	iZ2B/5	(-)	Coccus	(++)	(-)	NA
507	iZ2B/6	(-)	Bacillus	(++)	(+)	NA
508	iZ2B/7	(-)	Cocobacillus	(++)	(+)	NA
509	iZ2B/8	(-)	Bacillus	(+++)	(+)	NA
510	iZ2B/9	(-)	Coccus	(++)	(-)	NA
511	iZ2B/10	(-)	Coccus	(+++)	(+)	NA
512	iZ2B/11	(-)	Bacillus	(++)	(+)	NA
513	iZ2B/12	(-)	Bacillus	(++)	(+)	NA
514	iZ2B/13	(-)	Bacillus	(++)	(+)	NA
515	iZ2B/14	(-)	Bacillus	(++)	(+)	NA
516	iZ3A/1	(-)	Coccus	(++)	(+)	NA
517	iZ3A/2	(-)	Coccus	(++)	(-)	NA
518	iZ3A/3	(-)	Cocobacillus	(+++)	(+)	NA
519	iZ3A/4	(-)	Bacillus	(+++)	(+)	NA
520	iZ3A/5	(-)	Cocobacillus	(+++)	(+)	NA
521	iZ3A/6	(+)	Cocobacillus	(++)	(+)	
522	iZ3A/7	(-)	Bacillus	(-)	(+)	NA
523	iZ3A/8	(+)	Bacillus	(++)	(-)	
524	iZ3A/9	(+)	Cocobacillus	(+++)	(+)	
525	iZ3A/11	(-)	Bacillus	(++)	(+)	NA
526	iZ3A/12	(-)	Bacillus	(++)	(+)	NA
527	iZ3A/13	(-)	Bacillus	(+)	(+)	NA
528	iZ3A/15	(-)	Bacillus	(++)	(+)	NA
529	iZ3A/17	(-)	Diplococcus	(++)	(+)	NA
530	iZ3A/18	(-)	Cocobacillus	(++)	(-)	NA
531	iZ3A/19	(-)	Bacillus	(-)	(+)	NA
532	iZ3A/20	(-)	Coccus	(++)	(-)	NA
533	iZ3B/1	(-)	Coccus	(++)	(-)	NA
534	iZ3B/2	(-)	Bacillus	(+++)	(+)	NA
535	iZ3B/3	(+)	Cocobacillus	(++)	(-)	
536	iZ3B/4	(-)	Coccus	(++)	(+)	NA
537	iZ3B/5	(-)	Coccus	(++)	(-)	NA
538	iZ3B/6	(-)	Coccus	(++)	(+)	NA
539	iZ3B/7	(-)	Bacillus	(+++)	(+)	NA
540	iZ3B/8	(+)	Coccus	(+++)	(-)	NA
541	iZ3B/9	(-)	Cocobacillus	(++)	(+)	NA
542	iZ3B/10	(-)	Cocobacillus	(++)	(-)	NA

APPENDIX-C: Nfb agar medium results of 542 isolates.

Isolate Number	Isolate Name	Nfb Medium Results			Isolate Number	Isolate Name	Nfb Medium Results			Isolate Number	Isolate Name	Nfb Medium Results		
		Day 1	Day 2	Day 3			Day 1	Day 2	Day 3			Day 1	Day 2	Day 3
1	ND1A/2				30	ND1A/34				61	ND2A/7			
2	ND1A/3				31	ND1A/36				62	ND2A/8			
3	ND1A/4				32	ND1B/1				63	ND2A/9			
4	ND1A/5				33	ND1B/3				64	ND2A/10			
5	ND1A/6				34	ND1B/6				65	ND2A/11			
6	ND1A/7				35	ND1B/7				66	ND2A/12			
7	ND1A/8				36	ND1B/8				67	ND2A/13			
8	ND1A/10				37	ND1B/9				68	ND2A/14			
9	ND1A/11				38	ND1B/10				69	ND2A/15			
10	ND1A/12				39	ND1B/11				70	ND2A/16			
11	ND1A/13				40	ND1B/12				71	ND2A/17			
12	ND1A/15				41	ND1B/13				72	ND2A/34			
13	ND1A/16				42	ND1B/14				73	ND2A/36			
14	ND1A/17				43	ND1B/16				74	ND2A/37			
15	ND1A/18				44	ND1B/17				75	ND2A/38			
16	ND1A/19				45	ND1B/18				76	ND2A/39			
17	ND1A/20				46	ND1B/19				77	ND2A/40			
18	ND1A/21				47	ND1B/20				78	ND2A/41			
19	ND1A/22				48	ND1B/21				79	ND2A/42			
20	ND1A/23				49	ND1B/22				80	ND2A/43			
21	ND1A/24				50	ND1B/23				81	ND2A/44			
22	ND1A/25				51	ND1B/24				82	ND2A/45			
23	ND1A/26				52	ND1B/25				83	ND2A/46			
24	ND1A/27				53	ND1B/26				84	ND2A/47			
25	ND1A/28				54	ND1B/28				85	ND2A/48			
26	ND1A/29				55	ND1B/30				86	ND2A/49			
27	ND1A/31				56	ND1B/32				87	ND2A/61			
28	ND1A/32				57	ND1B/34				88	ND2A/62			
29	ND1A/33				58	ND2A/4				89	ND2A/63			
30	ND1A/34				59	ND2A/5				90	ND2A/65			
					60	ND2A/6								

APPENDIX-C: Continued.

Isolate Number	Isolate Name	Nfb Medium Results			Isolate Number	Isolate Name	Nfb Medium Results			Isolate Number	Isolate Name	Nfb Medium Results		
		Day 1	Day 2	Day 3			Day 1	Day 2	Day 3			Day 1	Day 2	Day 3
91	ND2B/1				121	ND3A/13				151	ND3B/14			
92	ND2B/2				122	ND3A/14				152	ND3B/15			
93	ND2B/3				123	ND3A/15				153	ND3B/16			
94	ND2B/4				124	ND3A/16				154	ND3B/17			
95	ND2B/5				125	ND3A/17				155	ND3B/18			
96	ND2B/6				126	ND3A/18				156	ND3B/19			
97	ND2B/7				127	ND3A/19				157	ND3B/20			
98	ND2B/8				128	ND3A/20				158	ND3B/22			
99	ND2B/9				129	ND3A/21				159	ND3B/23			
100	ND2B/11				130	ND3A/22				160	ND3B/24			
101	ND2B/12				131	ND3A/23				161	ND3B/25			
102	ND2B/13				132	ND3A/24				162	ND3B/26			
103	ND2B/14				133	ND3A/25				163	ND3B/27			
104	ND2B/15				134	ND3A/26				164	ND3B/28			
105	ND2B/16				135	ND3A/27				165	ND3B/29			
106	ND2B/17				136	ND3A/29				166	ND3B/30			
107	ND2B/18				137	ND3A/30				167	ND3B/31			
108	ND2B/19				138	ND3A/31				168	ND3B/32			
109	ND2B/20				139	ND3A/32				169	ND3B/33			
110	ND2B/21				140	ND3A/33				170	ND3B/34			
111	ND2B/22				141	ND3B/2				171	ND3B/35			
112	ND2B/23				142	ND3B/4				172	ND3B/36			
113	ND2B/24				143	ND3B/5				173	AF1A/1			
114	ND3A/2				144	ND3B/7				174	AF1A/2			
115	ND3A/4				145	ND3B/8				175	AF1A/3			
116	ND3A/5				146	ND3B/9				176	AF1A/4			
117	ND3A/8				147	ND3B/10				177	AF1A/9			
118	ND3A/9				148	ND3B/11				178	AF1A/12			
119	ND3A/11				149	ND3B/12				179	AF1A/13			
120	ND3A/12				150	ND3B/13				180	AF1A/14			

APPENDIX-C: Continued.

Isolate Number	Isolate Name	Nfb Medium Results				Isolate Number	Isolate Name	Nfb Medium Results				Isolate Number	Isolate Name	Nfb Medium Results			
		Day 1	Day 2	Day 3	Day 7			Day 1	Day 2	Day 3	Day 7			Day 1	Day 2	Day 3	Day 7
181	AF1A/15					211	AF2A/28					241	AF3A/25				
182	AF1A/17					212	AF2A/29					242	AF3A/26				
183	AF1A/18					213	AF2B/2					243	AF3A/29				
184	AF1A/20					214	AF2B/6					244	AF3A/31				
185	AF1A/21					215	AF2B/9					245	AF3A/32				
186	AF1A/22					216	AF2B/12					246	AF3A/33				
187	AF1A/23					217	AF2B/13					247	AF3B/1				
188	AF1B/1					218	AF2B/15					248	AF3B/3				
189	AF1B/2					219	AF2B/16					249	AF3B/4				
190	AF1B/3					220	AF2B/19					250	AF3B/5				
191	AF1B/4					221	AF2B/20					251	AF3B/7				
192	AF1B/5					222	AF3A/1					252	AF3B/10				
193	AF1B/7					223	AF3A/2					253	AF3B/11				
194	AF1B/8					224	AF3A/3					254	AF3B/14				
195	AF1B/11					225	AF3A/4					255	AF3B/15				
196	AF1B/13					226	AF3A/5					256	AF3B/17				
197	AF1B/15					227	AF3A/9					257	AF3B/18				
198	AF1B/16					228	AF3A/10					258	AF3B/19				
199	AF1B/17					229	AF3A/11					259	AF3B/20				
200	AF1B/18					230	AF3A/12					260	AF3B/21				
201	AF1B/21					231	AF3A/13					261	AF3B/22				
202	AF1B/25					232	AF3A/14					262	AF3B/23				
203	AF1B/26					233	AF3A/15					263	AF3B/24				
204	AF2A/1					234	AF3A/16					264	AF3B/25				
205	AF2A/2					235	AF3A/17					265	KN1A/1				
206	AF2A/3					236	AF3A/19					266	KN1A/2				
207	AF2A/13					237	AF3A/20					267	KN1A/3				
208	AF2A/16					238	AF3A/22					268	KN1A/4				
209	AF2A/19					239	AF3A/23					269	KN1A/5				
210	AF2A/23					240	AF3A/24					270	KN1A/6				

APPENDIX-C: Continued.

Isolate Number	Isolate Nam	Nfb Medium Results				Isolate Number	Isolate Nam	Nfb Medium Results				Isolate Number	Isolate Nam	Nfb Medium Results			
		Day 1	Day 2	Day 3	Day 7			Day 1	Day 2	Day 3	Day 7			Day 1	Day 2	Day 3	Day 7
271	KN1A/7					301	KN2A/14					331	KN3A/11				
272	KN1A/9					302	KN2A/15					332	KN3A/12				
273	KN1A/10					303	KN2A/16					333	KN3A/13				
274	KN1A/13					304	KN2A/18					334	KN3A/14				
275	KN1A/14					305	KN2B/1					335	KN3A/15				
276	KN1A/16					306	KN2B/2					336	KN3A/16				
277	KN1A/22					307	KN2B/3					337	KN3A/17				
278	KN1A/23					308	KN2B/4					338	KN3A/21				
279	KN1B/1					309	KN2B/5					339	KN3A/22				
280	KN1B/2					310	KN2B/6					340	KN3A/23				
281	KN1B/3					311	KN2B/7					341	KN3A/24				
282	KN1B/5					312	KN2B/8					342	KN3B/1				
283	KN1B/6					313	KN2B/9					343	KN3B/2				
284	KN1B/7					314	KN2B/10					344	KN3B/3				
285	KN1B/9					315	KN2B/11					345	KN3B/4				
286	KN1B/10					316	KN2B/12					346	KN3B/5				
287	KN1B/11					317	KN2B/13					347	KN3B/6				
288	KN1B/13					318	KN2B/14					348	KN3B/7				
289	KN2A/1					319	KN2B/15					349	KN3B/8				
290	KN2A/2					320	KN2B/16					350	KN3B/10				
291	KN2A/4					321	KN3A/1					351	KN3B/11				
292	KN2A/5					322	KN3A/2					352	KN3B/12				
293	KN2A/6					323	KN3A/3					353	KN3B/13				
294	KN2A/7					324	KN3A/4					354	KN3B/14				
295	KN2A/8					325	KN3A/5					355	KN3B/15				
296	KN2A/9					326	KN3A/6					356	KN1A/2				
297	KN2A/10					327	KN3A/7					357	KN1A/3				
298	KN2A/11					328	KN3A/8					358	KN1A/4				
299	KN2A/12					329	KN3A/9					359	KN1A/6				
300	KN2A/13					330	KN3A/10					360	KN1A/7				

APPENDIX-C:Continued.

Isolate Number	Isolate Name	Nfb Medium Results				Isolate Number	Isolate Name	Nfb Medium Results				Isolate Number	Isolate Name	Nfb Medium Results			
		Day 1	Day 2	Day 3	Day 7			Day 1	Day 2	Day 3	Day 7			Day 1	Day 2	Day 3	Day 7
361	KY1A/8					391	KY2A/7					421	KY3A/9				
362	KY1A/9					392	KY2A/9					422	KY3A/10				
363	KY1A/10					393	KY2A/10					423	KY3A/11				
364	KY1A/11					394	KY2A/11					424	KY3A/12				
365	KY1A/12					395	KY2A/12					425	KY3A/13				
366	KY1A/13					396	KY2A/13					426	KY3A/14				
367	KY1A/14					397	KY2A/14					427	KY3A/15				
368	KY1B/1					398	KY2A/15					428	KY3A/16				
369	KY1B/2					399	KY2A/16					429	KY3A/17				
370	KY1B/3					400	KY2A/17					430	KY3A/18				
371	KY1B/4					401	KY2A/18					431	KY3A/19				
372	KY1B/5					402	KY2A/20					432	KY3A/20				
373	KY1B/6					403	KY2B/1					433	KY3A/23				
374	KY1B/7					404	KY2B/2					434	KY3A/24				
375	KY1B/8					405	KY2B/3					435	KY3B/1				
376	KY1B/9					406	KY2B/4					436	KY3B/2				
377	KY1B/10					407	KY2B/5					437	KY3B/3				
378	KY1B/11					408	KY2B/6					438	KY3B/4				
379	KY1B/12					409	KY2B/7					439	KY3B/5				
380	KY1B/13					410	KY2B/8					440	KY3B/6				
381	KY1B/14					411	KY2B/9					441	KY3B/7				
382	KY1B/16					412	KY2B/10					442	KY3B/9				
383	KY1B/17					413	KY2B/11					443	KY3B/10				
384	KY1B/18					414	KY2B/12					444	KY3B/13				
385	KY2A/1					415	KY3A/1					445	KY3B/14				
386	KY2A/2					416	KY3A/2					446	IZ1A/1				
387	KY2A/3					417	KY3A/4					447	IZ1A/2				
388	KY2A/4					418	KY3A/5					448	IZ1A/4				
389	KY2A/5					419	KY3A/7					449	IZ1A/5				
390	KY2A/6					420	KY3A/8					450	IZ1A/6				

APPENDIX-C:Continued.

Isolate Number	Isolate Name	Nfb Medium Results				Isolate Number	Isolate Name	Nfb Medium Results			
		Day 1	Day 2	Day 3	Day 7			Day 1	Day 2	Day 3	Day 7
451	I21A/7					481	I21B/16				
452	I21A/8					482	I22A/1				
453	I21A/9					483	I22A/2				
454	I21A/10					484	I22A/3				
455	I21A/12					485	I22A/4				
456	I21A/13					486	I22A/5				
457	I21A/14					487	I22A/6				
458	I21A/15					488	I22A/7				
459	I21A/16					489	I22A/8				
460	I21A/17					490	I22A/9				
461	I21A/18					491	I22A/10				
462	I21A/19					492	I22A/11				
463	I21A/20					493	I22A/12				
464	I21A/21					494	I22A/13				
465	I21A/23					495	I22A/14				
466	I21A/24					496	I22A/15				
467	I21B/1					497	I22A/16				
468	I21B/2					498	I22A/17				
469	I21B/3					499	I22A/18				
470	I21B/4					500	I22A/19				
471	I21B/5					501	I22A/20				
472	I21B/6					502	I22B/1				
473	I21B/7					503	I22B/2				
474	I21B/8					504	I22B/3				
475	I21B/9					505	I22B/4				
476	I21B/10					506	I22B/5				
477	I21B/11					507	I22B/6				
478	I21B/13					508	I22B/7				
479	I21B/14					509	I22B/8				
480	I21B/15					510	I22B/9				

APPENDIX-C:Continued.

Isolate Number	Isolate Name	Nfb Medium Results				Isolate Number	Isolate Name	Nfb Medium Results			
		Day 1	Day 2	Day 3	Day 7			Day 1	Day 2	Day 3	Day 7
511	I2B/10					541	I2B/9				
512	I2B/11					542	I2B/10				
513	I2B/12										
514	I2B/13										
515	I2B/14										
516	I2A/1										
517	I2A/2										
518	I2A/3										
519	I2A/4										
520	I2A/5										
521	I2A/6										
522	I2A/7										
523	I2A/8										
524	I2A/9										
525	I2A/11										
526	I2A/12										
527	I2A/13										
528	I2A/15										
529	I2A/17										
530	I2A/18										
531	I2A/19										
532	I2A/20										
533	I2B/1										
534	I2B/2										
535	I2B/3										
536	I2B/4										
537	I2B/5										
538	I2B/6										
539	I2B/7										
540	I2B/8										

APPENDIX-D: Ammonium Detection Assay Results of ~150 Isolates.

30 °C										37 °C									
Ammonium Concentration					Ammonium Concentration					Ammonium Concentration					Ammonium Concentration				
Isolate No	Conc. (mg/L)	Isolate No	Conc. (mg/L)	Isolate No	Conc. (mg/L)	Isolate No	Conc. (mg/L)	Isolate No	Conc. (mg/L)	Isolate No	Conc. (mg/L)	Isolate No	Conc. (mg/L)	Isolate No	Conc. (mg/L)	Isolate No	Conc. (mg/L)		
<i>S. aureus</i> (-)	0.866	AF1A/21	1.330	KN2A/15	0.683	<i>S. aureus</i> (-)	0.276	AF1B/16	2.177	KN2B/2	2.923	KY1B/7	2.358						
	<i>K. pneumoniae</i> (+)					3.702													
ND1A/3	1.300	AF1A/22	2.027	KN3A/1	0.547	ND1B/19	1.624	AF1B/25	1.505	KN2B/6	1.533	KY1B/16	1.387						
ND1A/34	0.169	AF1A/23	3.346	KN3A/5	0.574	ND1B/22	1.071	AF1B/26	0.740	KN2B/15	0.631	KY1B/18	1.110						
ND2A/5	0.285	AF2A/2	1.520	KN3A/7	0.547	ND1B/26	0.708	AF2B/2	2.760	KN3B/8	1.547	KY2B/4	0.767						
ND2A/36	0.493	AF2A/3	1.612	KN3A/14	0.848	ND2B/4	1.451	AF2B/6	3.010	KN3B/12	2.227	KY2B/9	1.622						
ND2A/41	1.555	AF2A/29	2.457	KN3A/17	0.710	ND2B/12	2.051	AF2B/19	2.644	KN3B/14	1.555	KY2B/10	3.430						
ND2A/42	-0.263	AF3A/1	1.925	KN3A/23	0.502	ND2B/15	1.216	AF2B/20	2.657	IZ1B/4	1.303	KY2B/11	3.119						
ND2A/48	1.150	AF3A/2	0.564	IZ1A/6	0.628	ND2B/19	2.116	AF3B/1	2.531	IZ1B/14	0.947	KY2B/12	0.905						
ND3A/2	1.587	AF3A/4	3.282	IZ1A/15	0.537	ND2B/20	2.545	AF3B/7	0.268	IZ2B/4	1.179	KY3B/1	0.569						
ND3A/5	0.532	AF3A/5	1.323	IZ1A/18	0.384	ND2B/23	1.335	AF3B/10	2.079	IZ2B/6	1.261	KY3B/3	0.700						
ND3A/8	1.281	AF3A/9	2.167	IZ2A/2	0.883	ND2B/24	1.481	AF3B/17	0.903	IZ2B/7	0.530	KY3B/6	0.577						
ND3A/12	0.443	AF3A/16	2.501	IZ2A/17	1.599	ND3B/5	1.081	AF3B/21	1.864	IZ2B/11	1.636	KY3B/14	0.530						
ND3A/13	1.528	AF3A/17	2.202	KY1A/10	1.953	ND3B/8	1.118	AF3B/22	2.708	IZ2B/13	1.404								
ND3A/18	2.074	AF3A/19	0.379	KY1A/12	1.137	ND3B/11	8.114	AF3B/23	1.602	IZ3B/2	0.520								
ND3A/19	1.229	AF3A/22	0.982	KY2A/4	1.244	ND3B/13	2.222	AF3B/24	2.694	IZ3B/4	1.794								
ND3A/21	1.891	AF3A/25	2.439	KY2A/6	0.480	ND3B/23	5.861	AF3B/25	3.007	IZ3B/6	1.384								
ND3A/26	1.011	AF3A/26	2.904	KY2A/15	1.365	ND3B/27	1.785	KN1B/1	0.767	IZ3B/7	1.881								
ND3A/27	0.829	KN1A/2	0.964	KY2A/20	1.555	ND3B/29	4.126	KN1B/3	3.427	IZ3B/10	1.859								
ND3A/30	0.544	KN1A/3	0.999	KY3A/1	0.468	ND3B/30	2.832	KN1B/5	1.068	KY1B/1	1.404								
AF1A/2	0.977	KN1A/22	1.584	KY3A/11	0.779	ND3B/36	3.914	KN1B/6	0.982	KY1B/2	1.955								
AF1A/17	1.241	KN2A/7	0.544	KY3A/19	0.611	AF1B/5	4.714	KN1B/11	0.421	KY1B/4	1.787								
AF1A/20	1.412	KN2A/10	0.549	KY3A/20	0.898	AF1B/15	1.844	KN1B/13	3.343	KY1B/5	2.649								

APPENDIX-E1: Biochemical characterization test results of 30 isolates. IMViC and SIM agar test.

Isolate Number	Isolate Name	IMViC Tests				H ₂ S	Motility
		Indole	MR	VP	Citrate		
0	Controls	PV* (+) KP* (-)	PV (+) KP (-)	KP (-) PV (+)	KP (-) EF* (-)	PV (+) KP (-)	PV (+) KP (-)
109	ND2B/20	-	-	+	+	-	+
126	ND3A/18	-	-	+	+	-	+
148	ND3B/11	-	-	-	-	-	+
159	ND3B/23	-	-	+	+	-	+
165	ND3B/29	-	-	+	+	-	+
166	ND3B/30	-	-	+	+	-	+
172	ND3B/36	-	-	+	+	-	+
186	AF1A/22	-	-	+	+	-	+
187	AF1A/23	-	-	+	+	-	+
192	AF1B/5	-	-	-	+	-	+
212	AF2A/29	-	-	-	+	-	+
213	AF2B/2	-	-	+	+	-	+
214	AF2B/6	-	-	+	+	-	+
220	AF2B/19	-	-	+	+	-	+
221	AF2B/20	-	-	+	+	-	+
227	AF3A/9	-	-	-	+	-	+
234	AF3A/16	-	+	-	+	+	+
235	AF3A/17	-	+	-	+	+	+
241	AF3A/25	-	+	-	+	+	+
242	AF3A/26	-	+	-	+	+	+
247	AF3B/1	-	-	+	+	-	+
261	AF3B/22	-	-	+	+	-	+
263	AF3B/24	-	+	-	-	-	+
264	AF3B/25	-	-	+	+	-	+
281	KN1B/3	-	-	+	+	-	+
288	KN1B/13	-	-	+	+	-	+
306	KN2B/2	-	-	+	+	-	+
372	KY1B/5	-	-	+	+	-	+
412	KY2B/10	-	-	+	+	-	+
413	KY2B/11	-	-	+	+	-	+

*KP= K. pneumoniae (lab isolate), PV= P. vulgaris (lab isolate), EF= E. faecalis MCTC 8213, PA= P. aeruginosa ATCC 15692, EC= E. coli ATCC 25922

APPENDIX-E2: Biochemical characterization test results of 30 isolates. TSI agar and Phenylalanine deaminase agar tests.

Isolate Number	Isolate Name	TSI Tests					Phenylalanine Deaminase
		Glucose	Sucrose	Lactose	H ₂ S	Gas	
0	Controls	EC* (+) PA* (-)			PV (+) EC (-)	EC (+) PV (-)	PV (+) EC (-)
109	ND2B/20	+	-	-	-	+	-
126	ND3A/18	+	+	+	-	+	-
148	ND3B/11	+	+	+	-	-	-
159	ND3B/23	+	-	-	-	+	-
165	ND3B/29	+	+	+	-	+	-
166	ND3B/30	+	+	+	-	+	-
172	ND3B/36	+	+	+	-	+	-
186	AF1A/22	+	-	-	-	+	-
187	AF1A/23	+	-	-	-	+	-
192	AF1B/5	+	+	+	-	+	-
212	AF2A/29	-	-	-	-	-	-
213	AF2B/2	-	-	-	-	-	-
214	AF2B/6	+	+	+	-	+	-
220	AF2B/19	+	+	+	-	+	-
221	AF2B/20	+	+	+	-	+	-
227	AF3A/9	-	-	-	-	-	-
234	AF3A/16	+	+	+	+	+	-
235	AF3A/17	+	+	+	+	+	-
241	AF3A/25	+	+	+	+	+	-
242	AF3A/26	+	+	+	+	+	-
247	AF3B/1	+	+	+	-	+	-
261	AF3B/22	+	+	+	-	+	-
263	AF3B/24	+	-	-	-	-	-
264	AF3B/25	+	+	+	-	+	-
281	KN1B/3	+	+	+	-	+	-
288	KN1B/13	+	+	+	-	+	-
306	KN2B/2	+	+	+	-	+	-
372	KY1B/5	+	+	+	-	+	-
412	KY2B/10	-	-	-	-	-	-
413	KY2B/11	+	+	+	-	+	-

* PDA=Phenylalanine deaminase agar, OF= Oxidation/Fermentation, A= Aerobe, FA= Facultative anaerobe, I= Inert, PP= P. putida KT244

APPENDIX-E3: Biochemical characterization test results of 30 isolates. Decarboxylase, urease, ONPG tests.

Isolate Number	Isolate Name	Decarboxylase Tests						Urease	ONPG
		Lysine - 24h	Lysine - 48h	Ornithine - 24h	Ornithine - 48h	Arginine - 24h	Arginine - 48h		
0	Controls	KP (+) PV (-)		PV (+) KP (-)		PA (+) KP (-)		PV (+) KP (-)	EC (+) PV (-)
109	ND2B/20	-	-	+	+	+	+	-	+
126	ND3A/18	-	-	+	+	+	+	-	+
148	ND3B/11	-	-	-	-	+	+	-	-
159	ND3B/23	-	-	+	+	+	+	-	+
165	ND3B/29	-	-	+	+	+	+	-	+
166	ND3B/30	-	-	+	+	+	+	-	+
172	ND3B/36	-	-	+	+	+	+	-	+
186	AF1A/22	-	-	+	+	+	+	-	+
187	AF1A/23	-	-	+	+	+	+	-	+
192	AF1B/5	I	I	I	I	I	I	-	I
212	AF2A/29	I	+	I	+	I	+	-	+
213	AF2B/2	-	-	+	+	+	+	-	+
214	AF2B/6	-	-	+	+	+	+	-	+
220	AF2B/19	I	-	+	+	+	+	-	+
221	AF2B/20	-	-	+	+	+	+	-	+
227	AF3A/9	I	+	I	+	+	+	-	-
234	AF3A/16	-	-	+	+	+	+	-	+
235	AF3A/17	-	-	+	+	+	+	-	+
241	AF3A/25	-	-	+	+	+	+	-	+
242	AF3A/26	-	-	+	+	+	+	-	+
247	AF3B/1	-	-	+	+	+	+	-	+
261	AF3B/22	-	-	+	+	+	+	-	+
263	AF3B/24	-	-	-	-	-	-	-	-
264	AF3B/25	-	-	+	+	+	+	-	+
281	KN1B/3	-	-	+	+	+	+	-	+
288	KN1B/13	-	-	+	+	+	+	-	+
306	KN2B/2	-	-	+	+	+	+	-	+
372	KY1B/5	-	-	+	+	+	+	-	+
412	KY2B/10	-	-	+	+	+	+	-	+
413	KY2B/11	-	-	+	+	+	+	-	+

APPENDIX-E4: Biochemical characterization test results of 30 isolates. Carbonhydrate utilization tests.

Isolate Number	Isolate Name	Charbonhydrate Utilization Tests					
		Glucose - 24h	Glucose Gas -24h	Fructose	Lactose	Xylose	Mannitol
0	Controls	EC (+) PA (-)					
109	ND2B/20	+	+	+	-	+	+
126	ND3A/18	+	+	+	-	+	+
148	ND3B/11	+	-	+	-	-	-
159	ND3B/23	+	+	+	-	+	+
165	ND3B/29	+	+	+	-	+	+
166	ND3B/30	+	+	+	-	+	+
172	ND3B/36	+	+	+	+	+	+
186	AF1A/22	+	+	+	-	+	+
187	AF1A/23	+	+	+	-	+	+
192	AF1B/5	-	-	-	-	-	-
212	AF2A/29	-	-	+	-	+	-
213	AF2B/2	+	+	-	+	+	+
214	AF2B/6	+	+	-	+	+	+
220	AF2B/19	+	+	+	+	+	+
221	AF2B/20	+	+	-	+	+	+
227	AF3A/9	-*	-	+	-	-	-
234	AF3A/16	+	+	+	+	+	+
235	AF3A/17	+	+	+	+	+	+
241	AF3A/25	+	+	+	+	+	+
242	AF3A/26	+	+	+	+	+	+
247	AF3B/1	+	+	+	+	+	+
261	AF3B/22	+	+	+	+	+	+
263	AF3B/24	+	+	+	-	-	-
264	AF3B/25	+	+	+	+	+	+
281	KN1B/3	+	+	+	+	+	+
288	KN1B/13	+	+	+	+	+	+
306	KN2B/2	+	+	-	+	+	+
372	KY1B/5	+	+	-	+	+	+
412	KY2B/10	+	-	+	+	+	+
413	KY2B/11	+	+	+	+	+	-

*FRU= Fructose, LAC= Lactose, XYL= Xylose, MAT= Mannitol, MAN= Mannose, SUC= Sucrose, TRE=Trehalose, ARA= Arabinose, GAL= Galactose, CEL= Cellobiose

APPENDIX-E4: Continued.

Isolate Number	Isolate Name	Carbohydrate Utilization Tests					
		Mannose	Sucrose	Trehalose	Arabinose	Galactose	Cellobiose
0	Controls	EC (+) PA (-)				EF (+) PA (-)	KP (+) PA (-)
109	ND2B/20	+	+	+	-	+	+
126	ND3A/18	+	+	+	-	+	+
148	ND3B/11	+	+	-	-	-	-
159	ND3B/23	+	+	+	-	+	+
165	ND3B/29	+	+	+	-	+	+
166	ND3B/30	+	+	+	-	+	+
172	ND3B/36	+	+	+	-	+	+
186	AF1A/22	+	-	+	+	+	+
187	AF1A/23	+	-	+	+	+	+
192	AF1B/5	-	-	-	-	-	-
212	AF2A/29	+	-	+	-	-	+
213	AF2B/2	+	+	+	+	+	+
214	AF2B/6	+	+	+	+	+	+
220	AF2B/19	+	+	+	+	+	+
221	AF2B/20	+	+	+	+	+	+
227	AF3A/9	-	-	-	-	-	-
234	AF3A/16	+	-	+	+	+	+
235	AF3A/17	+	-	+	+	+	+
241	AF3A/25	+	-	+	+	+	+
242	AF3A/26	+	-	+	+	+	+
247	AF3B/1	+	+	+	-	+	+
261	AF3B/22	+	+	+	-	+	+
263	AF3B/24	+	-	-	-	-	-
264	AF3B/25	+	+	+	-	+	+
281	KN1B/3	+	+	+	-	+	+
288	KN1B/13	+	+	+	-	+	+
306	KN2B/2	+	+	+	-	+	+
372	KY1B/5	+	+	+	-	+	+
412	KY2B/10	+	+	+	-	+	+
413	KY2B/11	+	+	+	-	+	+

APPENDIX-F: Profiles of 30 Isolates.

16S rDNA Analysis		Enterobacter ludwigii Enterobacter cloacae Pantoea agglomerans	Carbohydrate Utilization Tests	Glucose	+	IMViC	Indole	-	Decarboxylase Tests	Lysine	24h	-
Isolate Number		109		Glucose Gas Production	+		MR	-			48h	-
Colony Morphologies	Isolate Name	ND2B/20		Fructose	+		VP	+		Ornithine	24h	+
	Pigmentation	White		Lactose	-		Citrate	+			48h	+
	Shape	Circular		Xylose	+	Glucose	+	Arginine		24h	+	
	Margin	Entire		Mannitol	+	Sucrose	-			48h	+	
	Elevation	Raised		Mannose	+	Lactose	-					
	Texture	Coarsely Granular		Sucrose	+	H2S	-					
	Size	Large		Trehalose	+	Gas	+					
	Appearance	Shiney		Arabinose	-	Urease	-					
	Optical Property	Translucent		Galactose	+	ONPG	+					
Preliminary Tests	Gram Staining	(-)		Cellobiose	+	Oxidation / Fermentation Tests	Aerobe					
	Morphology	Cocobacillus		Phenylalanine Deaminase	-		Facultative Anaerobic	+				
	Catalase Test	(++)					Inert					
	Oxidase Test	(-)		H2S (SIM)	-							
	Spore Staining			Motility (SIM)	-							

16S rDNA Analysis			Lelliottia amnigena Enterobacter sp. Klebsiella aerogenes		Carbohydrate Utilization Tests	Glucose		+	IMViC	Indole		-	Decarboxylase Tests	Lysine	24h	-
Isolate Number			126			Glucose Gas Production		+		MR		-			48h	-
Isolate Name			ND3A/18			Fructose		+		VP		+		Ornithine	24h	+
Colony Morphologies	Pigmentation		White			Lactose		-		Citrate		+			48h	+
	Shape		Irregular			Xylose		+	Glucose		+	Arginine	24h	+		
	Margin		Lobate			Mannitol		+	Sucrose		+		48h	+		
	Elevation		Raised			Mannose		+	Lactose		+					
	Texture		Finely Granular			Sucrose		+	H2S		-					
	Size		Small			Trehalose		+	Gas		+					
	Appearance		Shiney			Arabinose		-	Urease		-					
	Optical Property		Opaque			Galactose		+	ONPG		+					
	Preliminary Tests	Gram Staining		(-)		Cellobiose		+	Oxidation / Fermentation Tests	Aerobe						
Morphology		Cocobacillus		Phenylalanine Deaminase		-	Facultative Anaerobic			+						
Catalase Test		(++)					Inert									
Oxidase Test		(-)		H2S (SIM)		-										
Spore Staining		NA		Motility (SIM)		-										

16S rDNA Analysis			Curtobacterium sp		Carbonhydrate Utilization Tests	Glucose		+		IMVIC	Indole		-		Decarboxylase Tests	Lysine	24h		-			
Isolate Number			148			Glucose Gas Production		-			MR		-				48h		-			
Isolate Name			ND3B/11			Fructose		+			VP		-				Ornithine		24h		-	
Colony Morphologies	Pigmentation		Yellowish White			Lactose		-			Citrate		-				48h		-			
	Shape		Irregular			Xylose		-			Glucose		+			24h		+				
	Margin		Fimbrate			Mannitol		-			Sucrose		+			48h		+				
	Elevation		Raised			Mannose		+			Lactose		+									
	Texture		Arborescent			Sucrose		+			H2S		-									
	Size		Large			Trehalose		-			Gas		-									
	Appearance		Dull			Arabinose		-			Urease		-									
	Optical Property		Translucent			Galactose		-		ONPG		-										
Preliminary Tests	Gram Staining		(+)			Cellobiose		-		Oxidation / Fermentation Tests	Aerobe											
	Morphology		Cocobacillus			Phenylalanine Deaminase		-			Facultative Anaerobic											
	Catalase Test		(+)								Inert		+									
	Oxidase Test		(-)			H2S (SIM)		-														
	Spore Staining		NA			Motility (SIM)		-														

16S rDNA Analysis			<i>Enterobacter ludwigii</i> <i>Enterobacter cloacae</i>		Carbohydrate Utilization Tests	Glucose	+	IMViC	Indole	-	Decarboxylase Tests	Lysine	24h	-	
Isolate Number			159			Glucose Gas Production	+		MR	-			48h	-	
Isolate Name			ND3B/23			Fructose	+		VP	+		Ornithine	24h	+	
Colony Morphologies	Pigmentation	White		Lactose		-	Citrate		+	48h			+		
	Shape	Irregular		Xylose		+	Glucose		+			Arginine	24h	+	
	Margin	Wavy		Mannitol		+	Sucrose		-				48h	+	
	Elevation	Raised		Mannose		+	Lactose	-							
	Texture	Wavy Interlaced		Sucrose		+	H2S	-							
	Size	Large		Trehalose		+	Gas	+							
	Appearance	Shiny		Arabinose		-	Urease		-						
	Optical Property	Opaque		Galactose		+	ONPG		+						
	Preliminary Tests	Gram Staining	(-)			Cellobiose	+	Oxidation / Fermentation Tests	Aerobe						
Morphology		Bacillus		Phenylalanine Deaminase		-	Facultative Anaerobic		+						
Catalase Test		(+)					Inert								
Oxidase Test		(-)		H2S (SIM)		-									
Spore Staining		NA		Motility (SIM)		-									

16S rDNA Analysis			Enterobacter ludwigii Enterobacter cloacae		Carbonhydrate Utilization Tests	Glucose		+		IMVIC	Indole		-		Decarboxylase Tests	Lysine	24h		-	
Isolate Number			165			Glucose Gas Production		+			MR		-				48h		-	
Isolate Name			ND3B/29			Fructose		+			VP		+			Ornithine		24h		+
Colony Morphologies	Pigmentation		Yellowish White			Lactose		-		Citrate		+		48h		+				
	Shape		Irregular			Xylose		+		Glucose		+		24h		+				
	Margin		Wavy			Mannitol		+		Sucrose		+		48h		+				
	Elevation		Raised			Mannose		+		Lactose		+								
	Texture		Arborescent			Sucrose		+		H2S		-								
	Size		Medium			Trehalose		+		Gas		+								
	Appearance		Shiney			Arabinose		-		Urease		-								
	Optical Property		Translucent			Galactose		+		ONPG		+								
Preliminary Tests	Gram Staining		(-)			Cellobiose		+		Oxidation / Fermentation Tests	Aerobe									
	Morphology		Bacillus			Phenylalanine Deaminase		-			Facultative Anaerobic		+							
	Catalase Test		(+++)								Inert									
	Oxidase Test		(-)			H2S (SIM)		-												
	Spore Staining		NA			Motility (SIM)		-												

16S rDNA Analysis			<i>Enterobacter roggenkampii</i> <i>Enterobacter chengduensis</i> <i>Enterobacter asburiae</i>		Carbonhydrate Utilization Tests	Glucose		+	IMViC	Indole		-	Decarboxylase Tests	Lysine	24h	-
Isolate Number			166			Glucose Gas Production		+		MR		-			48h	-
Isolate Name			ND3B/30			Fructose		+		VP		+		Ornithine	24h	+
Colony Morphologies	Pigmentation	White		Lactose		-	Citrate		+	48h	+					
	Shape	Circular		Xylose		+	Glucose		+	Arginine	24h	+				
	Margin	Entire		Mannitol		+	Sucrose		+		48h	+				
	Elevation	Effuse		Mannose		+	Lactose		+							
	Texture	Smooth		Sucrose		+	H2S		-							
	Size	Large		Trehalose		+	Gas		+							
	Appearance	Shiney		Arabinose		-	Urease		-							
	Optical Property	Translucent		Galactose		+	ONPG		+							
	Preliminary Tests	Gram Staining	(-)			Cellobiose		+	Oxidation / Fermentation Tests		Aerobe					
Morphology		Bacillus		Phenylalanine Deaminase		-	Facultative Anaerobic				+					
Catalase Test		(++)					Inert									
Oxidase Test		(-)		H2S (SIM)		-										
Spore Staining		NA		Motility (SIM)		-										

16S rDNA Analysis			Enterobacter ludwigii Enterobacter cloacae		Carbohydrate Utilization Tests	Glucose		+		IMViC	Indole		-		Decarboxylase Tests	Lysine	24h		-	
Isolate Number			172			Glucose Gas Production		+			MR		-				48h		-	
Isolate Name			ND3B/36			Fructose		+			VP		+				24h		+	
Colony Morphologies	Pigmentation		White			Lactose		+		TSI	Citrate		+			Ornithine	48h		+	
	Shape		Irregular			Xylose		+			Glucose		+				24h		+	
	Margin		Lobate			Mannitol		+			Sucrose		+				Arginine	48h		+
	Elevation		Effuse			Mannose		+			Lactose		+							
	Texture		Smooth			Sucrose		+			H2S		-							
	Size		Large			Trehalose		+			Gas		+							
	Appearance		Shiney			Arabinose		-			Urease		-							
	Optical Property		Opaque			Galactose		+			ONPG		+							
	Preliminary Tests	Gram Staining		(-)		Cellobiose		+			Oxidation / Fermentation Tests	Aerobe								
Morphology		Cocobacillus		Phenylalanine Deaminase		-		Facultative Anaerobic		+										
Catalase Test		(++)						Inert												
Oxidase Test		(-)		H2S (SIM)		-														
Spore Staining				Motility (SIM)		-														

16S rDNA Analysis			Enterobacter cancerogenus Enterobacter asburiae		Carbohydrate Utilization Tests	Glucose		+		IMViC	Indole		-		Decarboxylase Tests	Lysine	24h		-	
Isolate Number			186			Glucose Gas Production		+			MR		-				48h		-	
Isolate Name			AF1A/22			Fructose		+			VP		+			Ornithine		24h		+
Colony Morphologies	Pigmentation		White			Lactose		-		TSI	Citrate		+			48h		+		
	Shape		Circular			Xylose		+			Glucose		+			24h		+		
	Margin		Wavy			Mannitol		+			Sucrose		-			48h		+		
	Elevation		Raised			Mannose		+			Lactose		-							
	Texture		Smooth			Sucrose		-			H2S		-							
	Size		Large			Trehalose		+			Gas		+							
	Appearance		Shiney			Arabinose		+			Urease		-							
	Optical Property		Opaque			Galactose		+			ONPG		+							
Preliminary Tests	Gram Staining		(-)			Cellobiose		+		Oxidation / Fermentation Tests	Aerobe									
	Morphology		Cocobacillus			Phenylalanine Deaminase		-			Facultative Anaerobic		+							
	Catalase Test		(+++)								Inert									
	Oxidase Test		(-)			H2S (SIM)		-												
	Spore Staining		NA			Motility (SIM)		-												

16S rDNA Analysis		Enterobacter cancerogenus Enterobacter cloacae		Carbonhydrate Utilization Tests	Glucose	+	IMViC	Indole	-	Decarboxylase Tests	Lysine	24h	-
Isolate Number		187			Glucose Gas Production	+		MR	-			48h	-
Isolate Name		AF1A/23			Fructose	+		VP	+		Ornithine	24h	+
Colony Morphologies	Pigmentation	White			Lactose	-	Citrate	+	Arginine			48h	+
	Shape	Circular			Xylose	+	Glucose	+			24h	+	
	Margin	Entire			Mannitol	+	Sucrose	-	48h		+		
	Elevation	Effuse			Mannose	+	Lactose	-	Gas				
	Texture	Smooth			Sucrose	-	H2S	-					
	Size	Medium			Trehalose	+	Gas	+					
	Appearance	Shiney			Arabinose	+	Urease		-				
	Optical Property	Translucent			Galactose	+	ONPG		+				
	Preliminary Tests	Gram Staining	(-)		Cellobiose	+	Oxidation / Fermentation Tests	Aerobe					
Morphology		Cocobacillus			Phenylalanine Deaminase	-		Facultative Anaerobic	+				
Catalase Test		(+++)				Inert							
Oxidase Test		(-)			H2S (SIM)	-							
Spore Staining		NA			Motility (SIM)	-							

16S rDNA Analysis		<i>Pseudomonas stutzeri</i> <i>Pseudomonas sp.</i>		Carbonhydrate Utilization Tests	Glucose	-	IMViC	Indole	-	Decarboxylase Tests	Lysine	24h	I		
Isolate Number		192			Glucose Gas Production	-		MR	-			48h	I		
Isolate Name		AF1B/5			Fructose	-		VP	-			Ornithine	24h	I	
Colony Morphologies	Pigmentation	Yellow			Lactose	-		Citrate	+				48h	I	
	Shape	Irregular			Xylose	-		Glucose	+				Arginine	24h	I
	Margin	Wavy			Mannitol	-		Sucrose	+					48h	I
	Elevation	Raised			Mannose	-		Lactose	+		24h			I	
	Texture	Arborescent			Sucrose	-		H2S	-						
	Size	Small			Trehalose	-		Gas	+						
	Appearance		Shiny		Arabinose	-		Urease			-	ONPG	I		
Optical Property		Translucent			Galactose	-									
Preliminary Tests	Gram Staining	(-)			Cellobiose	-	Oxidation / Fermentation Tests	Aerobe		Facultative Anaerobic					
	Morphology	Bacillus			Phenylalanine Deaminase	-									
	Catalase Test	(++)						Inert	+						
	Oxidase Test	(-)			H2S (SIM)			-							
	Spore Staining	NA			Motility (SIM)		-								

16S rDNA Analysis		<i>Enterobacter hormaechei</i> <i>Enterobacter cloacae</i>		Carbonhydrate Utilization Tests	Glucose	-	IMViC	Indole	-	Decarboxylase Tests	Lysine	24h	I		
Isolate Number		212			Glucose Gas Production	-		MR	-			48h	+		
Isolate Name		AF2A/29			Fructose	+		VP	-			Ornithine	24h	I	
Colony Morphologies	Pigmentation	White			Lactose	-		Citrate	+				48h	+	
	Shape	Irregular			Xylose	+		Glucose	-				Arginine	24h	I
	Margin	Lobate			Mannitol	-		Sucrose	-					48h	+
	Elevation	Raised			Mannose	+		Lactose	-		24h			I	
	Texture	Smooth			Sucrose	-		H2S	-						
	Size	Large			Trehalose	+		Gas	-						
	Appearance		Shiny		Arabinose	-		Urease			-	ONPG	+		
Optical Property		Opaque			Galactose	-									
Preliminary Tests	Gram Staining	(-)			Cellobiose	+	Oxidation / Fermentation Tests	Aerobe	+	Facultative Anaerobic					
	Morphology	Bacillus			Phenylalanine Deaminase	-									
	Catalase Test	(++)						Inert							
	Oxidase Test	(-)			H2S (SIM)			-							
	Spore Staining	NA			Motility (SIM)		-								

16S rDNA Analysis		<i>Enterobacter hormaechei</i>		Carbonhydrate Utilization Tests	Glucose	+	IMViC	Indole	-	Decarboxylase Tests	Lysine	24h	-		
Isolate Number		213			Glucose Gas Production	+		MR	-			48h	-		
Isolate Name		AF2B/2			Fructose	-		VP	+			Ornithine	24h	+	
Colony Morphologies	Pigmentation	Yellowish White			Lactose	+		Citrate	+				48h	+	
	Shape	Circular			Xylose	+		Glucose	-				Arginine	24h	+
	Margin	Entire			Mannitol	+		Sucrose	-					48h	+
	Elevation	Raised			Mannose	+		Lactose	-		24h			+	
	Texture	Smooth			Sucrose	+		H2S	-						
	Size	Small			Trehalose	+		Gas	-						
	Appearance		Shiney		Arabinose	+		Urease			-	ONPG	+		
Optical Property		Translucent			Galactose	+									
Preliminary Tests	Gram Staining	(-)			Cellobiose	+	Oxidation / Fermentation Tests	Aerobe		Facultative Anaerobic	+				
	Morphology	Cocobacillus			Phenylalanine Deaminase	-									
	Catalase Test	(++)						Inert							
	Oxidase Test	(-)			H2S (SIM)			-							
	Spore Staining	NA			Motility (SIM)		-								

16S rDNA Analysis			<i>Pseudomonas sp</i> <i>Pseudomonas putida</i>		Carbonhydrate Utilization Tests	Glucose		+	IMViC	Indole		-	Decarboxylase Tests	Lysine	24h	-	
Isolate Number			214			Glucose Gas Production		+		MR		-			48h	-	
Isolate Name			AF2B/6			Fructose		-		VP		+			Ornithine	24h	+
Colony Morphologies	Pigmentation	Yellowish White		Lactose		+	Citrate			+	48h	+					
	Shape	Irregular		Xylose		+	Glucose			+	Arginine	24h		+			
	Margin	Wavy		Mannitol		+	Sucrose			+		48h		+			
	Elevation	Effuse		Mannose		+	Lactose			+		24h		+			
	Texture	Smooth		Sucrose		+	H2S			-		48h		+			
	Size	Large		Trehalose		+	Gas			+							
	Appearance	Shiney		Arabinose		+	Urease			-							
	Optical Property	Translucent		Galactose		+	ONPG		+								
Preliminary Tests	Gram Staining	(-)		Cellobiose		+	Oxidation / Fermentation Tests	Aerobe									
	Morphology	Cocobacillus		Phenylalanine Deaminase		-		Facultative Anaerobic	+								
	Catalase Test	(++)							Inert								
	Oxidase Test	(-)		H2S (SIM)		-											
	Spore Staining	NA		Motility (SIM)		-											

16S rDNA Analysis			<i>Enterobacter sp</i> <i>Enterobacter cloacae</i> <i>Enterobacter hormaechei</i>		Carbonhydrate Utilization Tests	Glucose		+	IMViC	Indole		-	Decarboxylase Tests	Lysine	24h	I	
Isolate Number			220			Glucose Gas Production		+		MR		-			48h	-	
Isolate Name			AF2B/19			Fructose		+		VP		+			Ornithine	24h	+
Colony Morphologies	Pigmentation	White		Lactose		+	Citrate			+	48h	+					
	Shape	Circular		Xylose		+	Glucose			+	Arginine	24h		+			
	Margin	Entire		Mannitol		+	Sucrose			+		48h		+			
	Elevation	Raised		Mannose		+	Lactose			+		24h		+			
	Texture	Smooth		Sucrose		+	H2S			-		48h		+			
	Size	Small		Trehalose		+	Gas			+							
	Appearance	Shiney		Arabinose		+	Urease			-							
	Optical Property	Translucent		Galactose		+	ONPG		+								
Preliminary Tests	Gram Staining	(-)		Cellobiose		+	Oxidation / Fermentation Tests	Aerobe									
	Morphology	Cocobacillus		Phenylalanine Deaminase		-		Facultative Anaerobic	+								
	Catalase Test	(++)							Inert								
	Oxidase Test	(-)		H2S (SIM)		-											
	Spore Staining	NA		Motility (SIM)		-											

16S rDNA Analysis			<i>Enterobacter hormaechei</i>		Carbonhydrate Utilization Tests	Glucose		+	IMViC	Indole		-	Decarboxylase Tests	Lysine	24h	-	
Isolate Number			221			Glucose Gas Production		+		MR		-			48h	-	
Isolate Name			AF2B/20			Fructose		-		VP		+			Ornithine	24h	+
Colony Morphologies	Pigmentation	White		Lactose		+	Citrate			+	48h	+					
	Shape	Irregular		Xylose		+	Glucose			+	Arginine	24h		+			
	Margin	Lobate		Mannitol		+	Sucrose			+		48h		+			
	Elevation	Raised		Mannose		+	Lactose			+		24h		+			
	Texture	Smooth		Sucrose		+	H2S			-		48h		+			
	Size	Medium		Trehalose		+	Gas			+							
	Appearance	Shiney		Arabinose		+	Urease			-							
	Optical Property	Translucent		Galactose		+	ONPG		+								
Preliminary Tests	Gram Staining	(-)		Cellobiose		+	Oxidation / Fermentation Tests	Aerobe									
	Morphology	Coccus		Phenylalanine Deaminase		-		Facultative Anaerobic	+								
	Catalase Test	(++)							Inert								
	Oxidase Test	(-)		H2S (SIM)		-											
	Spore Staining	NA		Motility (SIM)		-											

16S rDNA Analysis			<i>Pseudomonas</i> sp <i>Pseudomonas putida</i> <i>Pseudomonas qingdaonensis</i>		Carbonhydrate Utilization Tests	Glucose	-*	IMViC	Indole	-	Decarboxylase Tests	Lysine	24h	I
Isolate Number			227			Glucose Gas Production	-		MR	-			48h	+
Isolate Name			AF3A/9			Fructose	+		VP	-			24h	I
Colony Morphologies	Pigmentation	Brownish White		Lactose		-	Citrate		+	Ornithine		48h	+	
	Shape	Irregular		Xylose		-	Glucose		-			Arginine	24h	+
	Margin	Wavy		Mannitol		-	Sucrose		-				48h	+
	Elevation	Raised		Mannose		-	Lactose		-					
	Texture	Smooth		Sucrose		-	H2S		-					
	Size	Medium		Trehalose		-	Gas		-					
	Appearance	Shiney		Arabinose		-	Urease		-					
Optical Property	Opaque		Galactose	-		ONPG		-						
Preliminary Tests	Gram Staining	(-)		Cellobiose		-	Oxidation / Fermentation Tests	Aerobe	+					
	Morphology	Cocobacillus		Phenylalanine Deaminase		-		Facultative Anaerobic						
	Catalase Test	(+++)						Inert						
	Oxidase Test	(-)					H2S (SIM)		-					
	Spore Staining	NA		Motility (SIM)		-								

16S rDNA Analysis			<i>Citrobacter ferundii</i> <i>Citrobacter braakii</i>		Carbonhydrate Utilization Tests	Glucose	+	IMViC	Indole	-	Decarboxylase Tests	Lysine	24h	-
Isolate Number			234			Glucose Gas Production	+		MR	+			48h	-
Isolate Name			AF3A/16			Fructose	+		VP	-			24h	+
Colony Morphologies	Pigmentation	White		Lactose		+	Citrate		+	Ornithine		48h	+	
	Shape	Irregular		Xylose		+	Glucose		+			Arginine	24h	+
	Margin	Lobate		Mannitol		+	Sucrose		+				48h	+
	Elevation	Effuse		Mannose		+	Lactose		+					
	Texture	Smooth		Sucrose		-	H2S		+					
	Size	Large		Trehalose		+	Gas		+					
	Appearance	Dull		Arabinose		+	Urease		-					
Optical Property	Translucent		Galactose	+		ONPG		+						
Preliminary Tests	Gram Staining	(-)		Cellobiose		+	Oxidation / Fermentation Tests	Aerobe						
	Morphology	Coccus		Phenylalanine Deaminase		-		Facultative Anaerobic	+					
	Catalase Test	(++)						Inert						
	Oxidase Test	(-)					H2S (SIM)		+					
	Spore Staining	NA		Motility (SIM)		+								

16S rDNA Analysis			<i>Citrobacter ferundii</i> <i>Citrobacter braakii</i>		Carbonhydrate Utilization Tests	Glucose	+	IMViC	Indole	-	Decarboxylase Tests	Lysine	24h	-
Isolate Number			235			Glucose Gas Production	+		MR	+			48h	-
Isolate Name			AF3A/17			Fructose	+		VP	-			24h	+
Colony Morphologies	Pigmentation	Pinkish White		Lactose		+	Citrate		+	Ornithine		48h	+	
	Shape	Circular		Xylose		+	Glucose		+			Arginine	24h	+
	Margin	Entire		Mannitol		+	Sucrose		+				48h	+
	Elevation	Raised		Mannose		+	Lactose		+					
	Texture	Smooth		Sucrose		-	H2S		+					
	Size	Medium		Trehalose		+	Gas		+					
	Appearance	Shiney		Arabinose		+	Urease		-					
Optical Property	Opaque		Galactose	+		ONPG		+						
Preliminary Tests	Gram Staining	(-)		Cellobiose		+	Oxidation / Fermentation Tests	Aerobe						
	Morphology	Bacillus		Phenylalanine Deaminase		-		Facultative Anaerobic	+					
	Catalase Test	(+++)						Inert						
	Oxidase Test	(-)					H2S (SIM)		+					
	Spore Staining	NA		Motility (SIM)		+								

16S rDNA Analysis		<i>Citrobacter ferundii</i> <i>Citrobacter braakii</i>		Carbohydrate Utilization Tests	Glucose	+	IMViC	Indole	-	Decarboxylase Tests	Lysine	24h	-
Isolate Number		241			Glucose Gas Production	+		MR	+			48h	-
Isolate Name		AF3A/25			Fructose	+		VP	-		Ornithine	24h	+
Colony Morphologies	Pigmentation	Yellowish White			Lactose	+	TSI	Citrate	+		48h	+	
	Shape	Circular			Xylose	+		Glucose	+		24h	+	
	Margin	Wavy			Mannitol	+		Sucrose	+		Arginine	48h	+
	Elevation	Effuse			Mannose	+		Lactose	+				
	Texture	Smooth			Sucrose	-		H2S	+				
	Size	Large			Trehalose	+		Gas	+				
	Appearance	Shiny			Arabinose	+	Urease		-				
Preliminary Tests	Optical Property	Opaque			Galactose	+	ONPG		+				
	Gram Staining	(-)			Cellobiose	+	Oxidation / Fermentation Tests	Aerobe					
	Morphology	Coccus			Phenylalanine Deaminase	-		Facultative Anaerobic	+				
	Catalase Test	(++)						Inert					
	Oxidase Test	(+)			H2S (SIM)								
	Spore Staining	NA			Motility (SIM)								

16S rDNA Analysis		<i>Yokenella sp.</i> <i>Enterobacter sp</i> <i>Citrobacter freundii</i>		Carbohydrate Utilization Tests	Glucose	+	IMViC	Indole	-	Decarboxylase Tests	Lysine	24h	-
Isolate Number		242			Glucose Gas Production	+		MR	+			48h	-
Isolate Name		AF3A/26			Fructose	+		VP	-		Ornithine	24h	+
Colony Morphologies	Pigmentation	Yellowish White			Lactose	+	TSI	Citrate	+		48h	+	
	Shape	Irregular			Xylose	+		Glucose	+		24h	+	
	Margin	Wavy			Mannitol	+		Sucrose	+		Arginine	48h	+
	Elevation	Raised			Mannose	+		Lactose	+				
	Texture	Smooth			Sucrose	-		H2S	+				
	Size	Medium			Trehalose	+		Gas	+				
	Appearance	Shiny			Arabinose	+	Urease		-				
Preliminary Tests	Optical Property	Opaque			Galactose	+	ONPG		+				
	Gram Staining	(-)			Cellobiose	+	Oxidation / Fermentation Tests	Aerobe					
	Morphology	Bacillus			Phenylalanine Deaminase	-		Facultative Anaerobic	+				
	Catalase Test	(+)						Inert					
	Oxidase Test	(-)			H2S (SIM)								
	Spore Staining	NA			Motility (SIM)								

16S rDNA Analysis		<i>Enterobacter ludwigii</i> <i>Enterobacter cloacae</i> <i>Pantoea agglomerans</i>		Carbohydrate Utilization Tests	Glucose	+	IMViC	Indole	-	Decarboxylase Tests	Lysine	24h	-
Isolate Number		247			Glucose Gas Production	+		MR	-			48h	-
Isolate Name		AF3B/1			Fructose	+		VP	+		Ornithine	24h	+
Colony Morphologies	Pigmentation	White			Lactose	+	TSI	Citrate	+		48h	+	
	Shape	Irregular			Xylose	+		Glucose	+		24h	+	
	Margin	Wavy			Mannitol	+		Sucrose	+		Arginine	48h	+
	Elevation	Raised			Mannose	+		Lactose	+				
	Texture	Smooth			Sucrose	+		H2S	-				
	Size	Large			Trehalose	+		Gas	+				
	Appearance	Shiny			Arabinose	-	Urease		-				
Preliminary Tests	Optical Property	Opaque			Galactose	+	ONPG		+				
	Gram Staining	(-)			Cellobiose	+	Oxidation / Fermentation Tests	Aerobe					
	Morphology	Cocobacillus			Phenylalanine Deaminase	-		Facultative Anaerobic	+				
	Catalase Test	(+++)						Inert					
	Oxidase Test	(-)			H2S (SIM)								
	Spore Staining	NA			Motility (SIM)								

16S rDNA Analysis	<i>Enterobacter ludwigii</i> <i>Enterobacter cloacae</i>	Carbohydrate Utilization Tests	Glucose	+	IMViC	Indole	-	Decarboxylase Tests	Lysine	24h	-
Isolate Number	261		Glucose Gas Production	+		MR	-			48h	-
Isolate Name	AF3B/22		Fructose	+		VP	+		Ornithine	24h	+
Colony Morphologies	Pigmentation		Lactose	+		Citrate	+			48h	+
	Shape		Xylose	+	TSI	Glucose	+		Arginine	24h	+
	Margin		Mannitol	+		Sucrose	+			48h	+
	Elevation		Mannose	+		Lactose	+				
	Texture		Sucrose	+		H ₂ S	-				
	Size		Trehalose	+		Gas	+				
	Appearance		Arabinose	-	Urease		-				
	Optical Property		Galactose	+	ONPG		+				
	Gram Staining		Cellobiose	+	Oxidation / Fermentation Tests	Aerobe					
Preliminary Tests	Morphology		Phenylalanine Deaminase	-		Facultative Anaerobic	+				
	Catalase Test					Inert					
	Oxidase Test		H ₂ S (SIM)	-							
	Spore Staining		Motility (SIM)	-							

16S rDNA Analysis	<i>Citrobacter ferundii</i> <i>Citrobacter braakii</i>	Carbohydrate Utilization Tests	Glucose	+	IMViC	Indole	-	Decarboxylase Tests	Lysine	24h	-
Isolate Number	264		Glucose Gas Production	+		MR	-			48h	-
Isolate Name	AF3B/25		Fructose	+		VP	+		Ornithine	24h	+
Colony Morphologies	Pigmentation		Lactose	+		Citrate	+			48h	+
	Shape		Xylose	+	TSI	Glucose	+		Arginine	24h	+
	Margin		Mannitol	+		Sucrose	+			48h	+
	Elevation		Mannose	+		Lactose	+				
	Texture		Sucrose	+		H ₂ S	-				
	Size		Trehalose	+		Gas	+				
	Appearance		Arabinose	-	Urease		-				
	Optical Property		Galactose	+	ONPG		+				
	Gram Staining		Cellobiose	+	Oxidation / Fermentation Tests	Aerobe					
Preliminary Tests	Morphology		Phenylalanine Deaminase	-		Facultative Anaerobic	+				
	Catalase Test					Inert					
	Oxidase Test		H ₂ S (SIM)	-							
	Spore Staining		Motility (SIM)	-							

16S rDNA Analysis	<i>Enterobacter mori</i>	Carbohydrate Utilization Tests	Glucose	+	IMViC	Indole	-	Decarboxylase Tests	Lysine	24h	-
Isolate Number	281		Glucose Gas Production	+		MR	-			48h	-
Isolate Name	KN1B/3		Fructose	+		VP	+		Ornithine	24h	+
Colony Morphologies	Pigmentation		Lactose	+		Citrate	+			48h	+
	Shape		Xylose	+	TSI	Glucose	+		Arginine	24h	+
	Margin		Mannitol	+		Sucrose	+			48h	+
	Elevation		Mannose	+		Lactose	+				
	Texture		Sucrose	+		H ₂ S	-				
	Size		Trehalose	+		Gas	+				
	Appearance		Arabinose	-	Urease		-				
	Optical Property		Galactose	+	ONPG		+				
	Gram Staining		Cellobiose	+	Oxidation / Fermentation Tests	Aerobe					
Preliminary Tests	Morphology		Phenylalanine Deaminase	-		Facultative Anaerobic	+				
	Catalase Test					Inert					
	Oxidase Test		H ₂ S (SIM)	-							
	Spore Staining		Motility (SIM)	-							

16S rDNA Analysis		<i>Enterobacter mori</i> <i>Enterobacter roggenkampii</i>		Carbonhydrate Utilization Tests	Glucose	+	IMViC	Indole	-	Decarboxylase Tests	Lysine	24h	-	
Isolate Number		288			Glucose Gas Production	+		MR	-			48h	-	
Isolate Name		KN1B/13			Fructose	+		VP	+			Ornithine	24h	+
Colony Morphologies	Pigmentation	White			Lactose	+		Citrate	+				48h	+
	Shape	Circular			Xylose	+		Glucose	+		Arginine		24h	+
	Margin	Entrire			Mannitol	+		Sucrose	+				48h	+
	Elevation	Raised			Mannose	+		Lactose	+			24h	+	
	Texture	Smooth			Sucrose	+		H2S	-			48h	+	
	Size	Small			Trehalose	+		Gas	+					
	Appearance	Shiney			Arabinose	-		Urease				-		
	Optical Property	Translucent			Galactose	+	ONPG		+					
Preliminary Tests	Gram Staining	(-)			Cellobiose	+	Oxidation / Fermentation Tests	Aerobe						
	Morphology	Cocobacillus	Phenylalanine Deaminase		-	Facultative Anaerobic		+						
						Inert								
	Catalase Test	(++)			H2S (SIM)			-						
	Oxidase Test	(-)			Motility (SIM)		-							
	Spore Staining	NA												

16S rDNA Analysis		<i>Enterobacter asburiae</i> <i>Enterobacter mori</i> <i>Enterobacter roggenkampii</i>		Carbonhydrate Utilization Tests	Glucose	+	IMViC	Indole	-	Decarboxylase Tests	Lysine	24h	-	
Isolate Number		306			Glucose Gas Production	+		MR	-			48h	-	
Isolate Name		KN2B/2			Fructose	-		VP	+			Ornithine	24h	+
Colony Morphologies	Pigmentation	White			Lactose	+		Citrate	+				48h	+
	Shape	Irregular			Xylose	+		Glucose	+		Arginine		24h	+
	Margin	Wavy			Mannitol	+		Sucrose	+				48h	+
	Elevation	Raised			Mannose	+		Lactose	+			24h	+	
	Texture	Smooth			Sucrose	+		H2S	-			48h	+	
	Size	Medium			Trehalose	+		Gas	+					
	Appearance	Dull			Arabinose	-		Urease				-		
	Optical Property	Opaque			Galactose	+	ONPG		+					
Preliminary Tests	Gram Staining	(-)			Cellobiose	+	Oxidation / Fermentation Tests	Aerobe						
	Morphology	Coccus	Phenylalanine Deaminase		-	Facultative Anaerobic		+						
						Inert								
	Catalase Test	(++)			H2S (SIM)			-						
	Oxidase Test	(+))			Motility (SIM)		-							
	Spore Staining	NA												

16S rDNA Analysis		<i>Enterobacter ludwigii</i> <i>Enterobacter cloacae</i>		Carbonhydrate Utilization Tests	Glucose	+	IMViC	Indole	-	Decarboxylase Tests	Lysine	24h	-	
Isolate Number		372			Glucose Gas Production	+		MR	-			48h	-	
Isolate Name		KY1B/5			Fructose	-		VP	+			Ornithine	24h	+
Colony Morphologies	Pigmentation	White			Lactose	+		Citrate	+				48h	+
	Shape	Circular			Xylose	+		Glucose	+		Arginine		24h	+
	Margin	Entrire			Mannitol	+		Sucrose	+				48h	+
	Elevation	Crateriform			Mannose	+		Lactose	+			24h	+	
	Texture	Smooth			Sucrose	+		H2S	-			48h	+	
	Size	Small			Trehalose	+		Gas	+					
	Appearance	Shiney			Arabinose	-		Urease				-		
	Optical Property	Translucent			Galactose	+	ONPG		+					
Preliminary Tests	Gram Staining	(-)			Cellobiose	+	Oxidation / Fermentation Tests	Aerobe						
	Morphology	Cocobacillus	Phenylalanine Deaminase		-	Facultative Anaerobic		+						
						Inert								
	Catalase Test	(++)			H2S (SIM)			-						
	Oxidase Test	(-)			Motility (SIM)		-							
	Spore Staining	NA												

16S rDNA Analysis			Acinetobacter sp Acinetobacter pittii Acinetobacter calcoaceticus		Carbonhydrate Utilization Tests	Glucose		+		IMViC	Indole		-		Decarboxylase Tests	Lysine	24h		-		
Isolate Number			412			Glucose Gas Production		-			MR		-				48h		-		
Isolate Name			KY2B/10			Fructose		+			VP		+				Ornithine	24h		+	
Colony Morphologies	Pigmentation		Yellowish White			Lactose		+			Citrate		+					48h		+	
	Shape		Circular			Xylose		+			Glucose		-			Arginine	24h		+		
	Margin		Entire			Mannitol		+			Sucrose		-				48h		+		
	Elevation		Raised			Mannose		+			Lactose		-								
	Texture		Smooth			Sucrose		+			H2S		-								
	Size		Small			Trehalose		+			Gas		-								
	Appearance		Shiney			Arabinose		-			Urease		-								
	Optical Property		Translucent			Galactose		+		ONPG		+									
Preliminary Tests	Gram Staining		(-)			Cellobiose		+		Oxidation / Fermentation Tests	Aerobe		+								
	Morphology		Cocobacillus			Phenylalanine Deaminase		-			Facultative Anaerobic										
	Catalase Test		(++)								Inert										
	Oxidase Test		(+))			H2S (SIM)		-													
	Spore Staining		NA			Motility (SIM)		-													

16S rDNA Analysis			Enterobacter ludwigii		Carbonhydrate Utilization Tests	Glucose		+		IMViC	Indole		-		Decarboxylase Tests	Lysine	24h		-		
Isolate Number			413			Glucose Gas Production		+			MR		-				48h		-		
Isolate Name			KY2B/11			Fructose		+			VP		+				Ornithine	24h		+	
Colony Morphologies	Pigmentation		White			Lactose		+			Citrate		+					48h		+	
	Shape		Circular			Xylose		+			Glucose		+			Arginine	24h		+		
	Margin		Wavy			Mannitol		-			Sucrose		+				48h		+		
	Elevation		Raised			Mannose		+			Lactose		+								
	Texture		Smooth			Sucrose		+			H2S		-								
	Size		Small			Trehalose		+			Gas		+								
	Appearance		Shiney			Arabinose		-			Urease		-								
	Optical Property		Translucent			Galactose		+		ONPG		+									
Preliminary Tests	Gram Staining		(-)			Cellobiose		+		Oxidation / Fermentation Tests	Aerobe										
	Morphology		Cocobacillus			Phenylalanine Deaminase		-			Facultative Anaerobic		+								
	Catalase Test		(++)								Inert										
	Oxidase Test		(+))			H2S (SIM)		-													
	Spore Staining		NA			Motility (SIM)		-													