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Isolation of bacteria tolerant to high ammonium sulphate and ammonium chloride from activated sludge of poultry slaughterhouse wastewater and their evaluation as ammonia oxidizing agents

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ABSTRACT

Activated sludge contains numerous advantageous microorganisms that have the ability to stabilize organic waste. The purpose of this study is to isolate and identify microbial strains capable of oxidizing ammonia in poultry slaughterhouse wastewater to diminish nitrogen levels in wastewater. Diluted activated sludge samples were inoculated onto nutrient agar plates with varying concentrations of $(NH_4)_2SO_4$ and NH_4Cl to isolate tolerant bacterial strains. Eight isolates, namely SO1K, SO3K, SO1OR, SO4PB, SO3P, SO4PM, CL3P, and CL5P, cultivated in a medium containing 50 g/L $(NH_4)_2SO_4$ or NH_4Cl were recovered from the activated sludge sample. Morphological and biochemical methods were employed to identify all of the bacterial isolates. The 16S rRNA genetic sequence was employed to identify four isolates using the molecular approach. *Brevibacterium* sp. was genetically related to strain SO1K, as evidenced by a 99.86% similarity in the 16S rRNA gene sequence. Strain SO3K exhibited 99.43% similarity to *Rothia koreensis*. Meanwhile, strain SO1OR and strain SO4PB demonstrated 100% sequence similarity to *Staphylococcus aureus* and *Staphylococcus epidermidis*, respectively, indicating a very close phylogenetic relationship. The examination of bacterial growth and ammonium reduction measurements demonstrated that strains SO1K, SO3K, SO1OR, and SO4PB were capable of growing in medium with elevated nitrogen concentrations and efficiently diminished the nitrogen levels in the medium. Specifically, SO1K reduced ammonium by 92.15%, SO3K by 95.77%, SO1OR by 98.63%, and SO4PB by 99.78%, indicating their potential as ammonia-oxidizing agents.

Keywords: indigenous bacteria, ammonia-oxidizing agent, activated sludge, slaughterhouse wastewater, heterotrophic nitrification, aerobic denitrification.

INTRODUCTION

The poultry slaughterhouse industry generates substantial amounts of organic matter in wastewater, commonly releasing ammonia as air pollutants (Ngobeni et al., 2022). Inadequate treatment of these wastes can lead to significant health hazards, unpleasant odor, and environmental contamination, prompting complaints from nearby populations (Bist et al., 2023). The wastewater generated by poultry slaughterhouse operations comprises blood, feathers, flesh, and sanitation agent from the production process. The management of poultry slaughterhouse wastewater is conducted biologically via aerobic and anaerobic processes, aided by decomposing microorganisms. The treatment intends to diminish the organic matter of wastewater during processing. Thus, its disposal into aquatic systems or the surrounding environment can be considered safe (Khusnuryani et al., 2014). The preliminary phase of conventional poultry slaughterhouse

wastewater treatment entails physicochemical methods, utilising screening to eliminate coarse materials. Subsequently, secondary treatment utilises a combination of anaerobic and aerobic biological processes to break down most dissolved organic contaminants and minerals efficiently. The final stage involves the application of tertiary treatment procedures, including filtration and disinfection, to enhance water purity, guaranteeing that the resultant effluent meets safety and environmental discharge standards. (Fatima et al., 2021).

Inadequate waste management can lead to environmental contamination, particularly through odor pollution from ammonia compounds. Ammonia is produced by the ammonification process, where decomposing bacteria metabolize organic substances from the remnants of deceased organisms, including proteins and amino acids, thereby deriving ammonia from organic nitrogen. The environmentally sustainable approach for ammonia decomposition involves the use of biological agents (Fitriyanto et al., 2017, 2019). The process is referred to as heterotrophic nitrification and aerobic denitrification. These bacteria possess the capability to utilize ammonia via the nitrification-denitrification process (Azkarahman et al., 2021; Prasetyo et al., 2018). This approach could optimize the decomposition of ammonia compounds in the poultry slaughterhouse wastewater. This research aimed to isolate and identify bacteria from the activated sludge of poultry slaughterhouse wastewater treatment systems, primarily focusing on strains that facilitate heterotrophic nitrification and aerobic denitrification process to diminish ammonia concentrations. Poultry slaughterhouse wastewater is characterized by elevated concentration of nitrogenous compounds, especially ammonia (Gržinić et al., 2023), rendering it a pertinent and selective environment for the isolation of resilient ammonia-oxidizing bacteria. Utilizing this waste as a source of isolation is warranted due to its nutrient-dense and microbially varied characteristics, which can promote the enrichment of functional strains with viable bioremediation capabilities (Ayilara and Babalola, 2023). The novelty of this study lies in exploring indigenous bacterial strains from poultry slaughterhouse waste treatment sludge that possesses dual functionality in heterotrophic nitrification and aerobic denitrification. This study emphasizes the potential application of these isolates for ammonia removal and their conversion into value-added products such as smart fertilizers, thereby enhancing environmental sustainability and supporting circular economy initiatives in the livestock industry.

METHODS

Bacterial isolation from activated sludge

Activated sludge samples were collected from the poultry slaughterhouse wastewater treatment facility at the Agrotechnology Innovation Center (PIAT) Universitas Gadjah Mada. The isolation process involved culturing the activated sludge on 1/100 diluted nutrient agar supplemented with 50 g/L of either ammonium sulfate ($(NH_4)_2SO_4$) or ammonium chloride (NH_4Cl). Dominant bacterial colonies have been isolated and re-streaked on the identical agar medium for obtaining pure isolates. Aerobic incubation then was conducted at 30 °C for 7 days. Isolated colonies were then transferred to slanted agar tubes for maintenance and preservation. Selected colonies were suspended in 9 mL of sterile distilled water, vortexed, and cultured on nutrient agar with and without the addition of $(NH_4)_2SO_4$ and NH_4Cl to assess their growth tolerance to ammonium salts. Isolates were subsequently transferred to liquid medium and cultured in conditions of continuous shaking to assess ammonium consumption.

Screening for ammonium-tolerant bacteria

Screening for ammonium-tolerant bacteria was carried out using agar medium. The medium was prepared by combining 1 mL of a stock solution containing 1% meat extract, 1% microbiological peptone, and 0.5% NaCl with 99 mL of distilled water and 1.5 g of agar powder. Various concentrations of $(NH_4)_2SO_4$ and NH_4Cl were added, including 0.1% (1 g/L), 1% (10 g/L), 3% (30 g/L), and 5% (50 g/L). Ammonium salts were used as the ammonia substrate in the prepared agar plates, and 100 µL of the 10^5 dilution was inoculated onto the prepared agar plates containing ammonium salts as the ammonia substrate. The plates were then incubated to observe bacterial growth under different ammonium concentrations.

Identification of bacterial isolates

Based on the guidelines outlined in Bergey's Manual of Systematic Bacteriology, the

morphological, biochemical, and physiological characteristics of the ammonium-responsive bacterium were investigated (Brenner et al., 2005). Four isolates were selected for further identification based on their tolerance to high nitrogen levels and nitrogen-reducing ability. Molecular identification was conducted to ensure accurate taxonomic classification of the selected isolates. Genomic DNA was collected using the Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research D6005). The 16S rRNA gene was amplified with the universal primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′). The PCR reaction was conducted in a total volume of 30 µL with 2× MyTaq HS Red Mix (Bioline, BIO-25048). The thermal cycling protocol included an initial pre-denaturation at 94 °C for 2 minutes, followed by denaturation at 92 °C for 300 seconds, annealing at 55 °C for 30 seconds, and extension at 72 °C for 1 minute, repeated for 30 cycles. A final extension was performed at 75 °C for 20 minutes, followed by a final hold at 4 °C. The PCR products were subsequently subjected to bidirectional sequencing. The obtained sequences were analyzed using the basic local alignment search tool (BLAST) to determine sequence homology based on available data in the NCBI GenBank database. Phylogenetic analysis was conducted utilizing the Neighbor-Joining method in the ClustalX2 software, comparing the isolates to both closely and distantly related reference strains (outgroups). Bootstrap analysis with 1000 replicates was used to assess the stability of the resulting phylogenetic tree, which was then visualized and edited using MEGA 11.

Bacterial growth profile and ammonium reduction assessment

A single loopful of a bacterial colony was inoculated into 5 mL of stock solution and the mixture was then cultured for 12 hours at room temperature on a rotary shaker set at 120 rpm in order to prepare a bacterial preculture. Subsequent to incubation, 1 mL of the preculture was inoculated into 100 mL of a fresh stock solution enriched with 5% $(NH_4)_2SO_4$ and NH_4Cl as substrate. As a control, 1 mL preculture was inoculated into 100 mL of stock solution devoid of ammonium salt supplementation. The cultures were cultured for 16 hours, and 1 mL samples were collected hourly to assess optical density (OD) at 625 nm using a spectrophotometer. At the same time points, 2 mL of culture was taken for the determination of ammonium concentration using the Nessler method (Anupong et al., 2022).

RESULTS AND DISCUSSION

Identification of bacterial isolates

The bacterial colonies were successfully isolated from activated sludge in the medium with various concentrations of ammonium as a selective stressor (Figure 1). At a concentration of 5% ammonium, eight distinct bacterial isolates were observed, namely SO3P, SO3K, SO1OR, SO4PB, SO4PM, CL3P, CL5P, and SO1K (Figure 2). The morphological characterization of bacterial isolates showed that all isolates were Gram-positive, as summarized in Table 1. Further observation revealed that SO1OR and SO4PB exhibited a *coccus* morphology, while the remaining six isolates displayed *diplococcal* forms. Antibacterial susceptibility assays demonstrated that SO1OR and SO4PB were resistant to the antibacterial agent, whereas the other six isolates were sensitive.

The morphological test revealed that all bacterial isolates belonged to the Gram-positive group, characterized by the existence of a dense peptidoglycan layer coating in the bacterial cell wall. This observation is supported by a statement from (Pasquina-Lemonche et al., 2020) who reported that Gram-positive bacteria possess significantly thicker peptidoglycan layers compared to Gram-negative bacteria, serving as a key distinguishing structural feature.

The bacterial shape analysis indicated that two isolates, SO1OR and SO4PB, demonstrated *coccus* shape, indicatice of a spherical configuration, while the remaining six isolates presented a *diplococcus* form, defined by pairs of spherical cells. These morphologies are characteristic of Gram-positive bacteria, commonly appear as short rods, chains, *cocci*, or *diplococci*, as noted by Ullberg and Özenci (2020). Antibiotic susceptibility tests revealed that six isolated were susceptible to antibiotics, whereas two isolates shwon resistance. This phenomenon may be ascribed to the lack of an outer membrane in Gram-positive bacteria, which enhances permeability to antibiotics. These structural characteristics have been associated with increased antibiotic susceptibility in Gram-positive organisms (Pasquina-Lemonche et al., 2020).

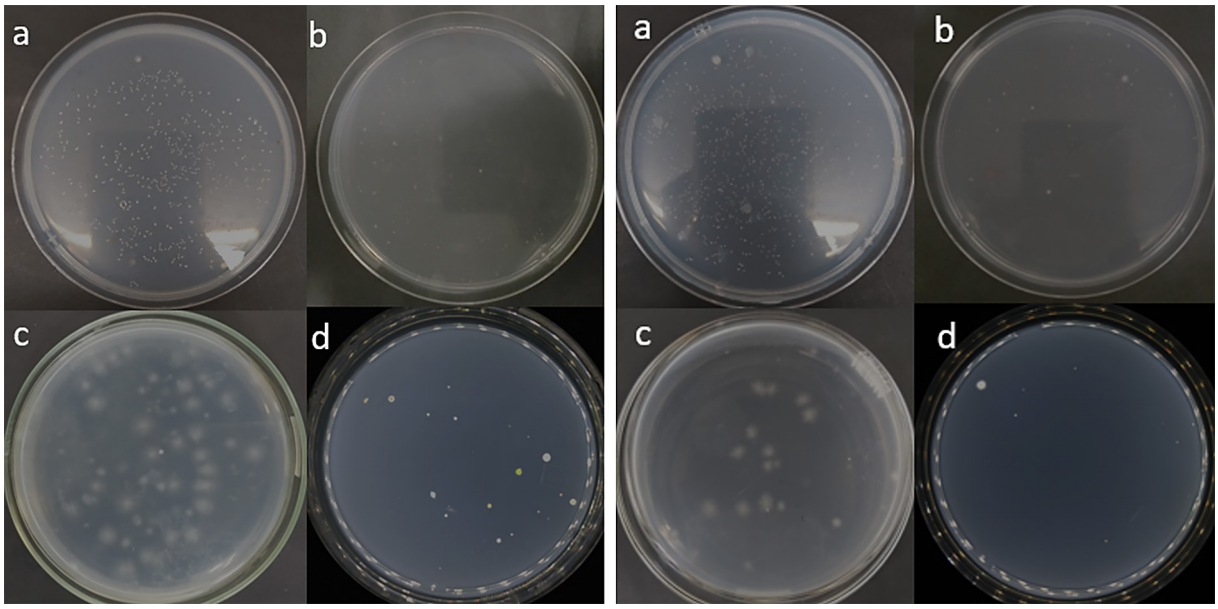

Figure 1. The results of inoculation on the substrate $(NH_4)_2SO_4$ (left) and NH_4Cl (right) with a concentration of: (a) 0.01% (b) 1% (c) 3% (d) 5%

Table 1. Isolate morphology test results

Isolate code	Indicator		
	Shape of colony	Gram stain	Antibacterial test
SO1OR	Round	Positive *coccus*	Resistant
SO1K	Round	Positive *diplococcus*	Sensitive
SO3P	Round	Positive *diplococcus*	Sensitive
SO3K	Round	Positive *diplococcus*	Sensitive
SO4PB	Round	Positive *coccus*	Resistant
SO4PM	Round	Positive *diplococcus*	Sensitive
CL3P	Round	Positive *diplococcus*	Sensitive
CL5P	Round	Positive *diplococcus*	Sensitive

The biochemical properties of the bacterial strain are presented in Table 2. Catalase activity was observed in all isolates, indicating their ability to produce the catalase enzyme. Fermentation tests for mannitol and various sugars, including glucose, lactose, maltose, and saccharose, yielded positive results only for isolates SO1OR and SO4PB, while the remaining six isolates tested negative. All isolates exhibited negative responses for sulfur reduction, indole production, and motility tests. The Simmons citrate test revealed negative citrate utilization in isolates SO1OR and SO4PB, whereas the remaining six isolates tested positive. Similarly, the urease test showed positive activity only in SO1OR and SO4PB, with the remaining isolates testing negative.

The uniform positive catalase test results among all isolates indicate that each bacterial strain possesses the ability to synthesize catalase, an enzyme responsible for decomposing hydrogen peroxide, a toxic byproduct of aerobic metabolism. Hydrogen peroxide induces oxidative stress in bacterial cells, and catalase plays a crucial role in mitigating this effect by converting it into water and oxygen. To counter this, catalase catalyzes the convertion of hydrogen peroxide into water and oxygen. This enzymatic activity is essential for maintaining redox homeostasis and protecting bacterial cells from oxidative damage (Hadwan et al., 2024).

The differences in sugar fermentation responses, where only the SO1OR and SO4PB isolates exhibited positive results for mannitol, glucose, lactose, maltose, and saccharose, may be attributed to the presence of specific metabolic enzymes in these strains. The ability to ferment

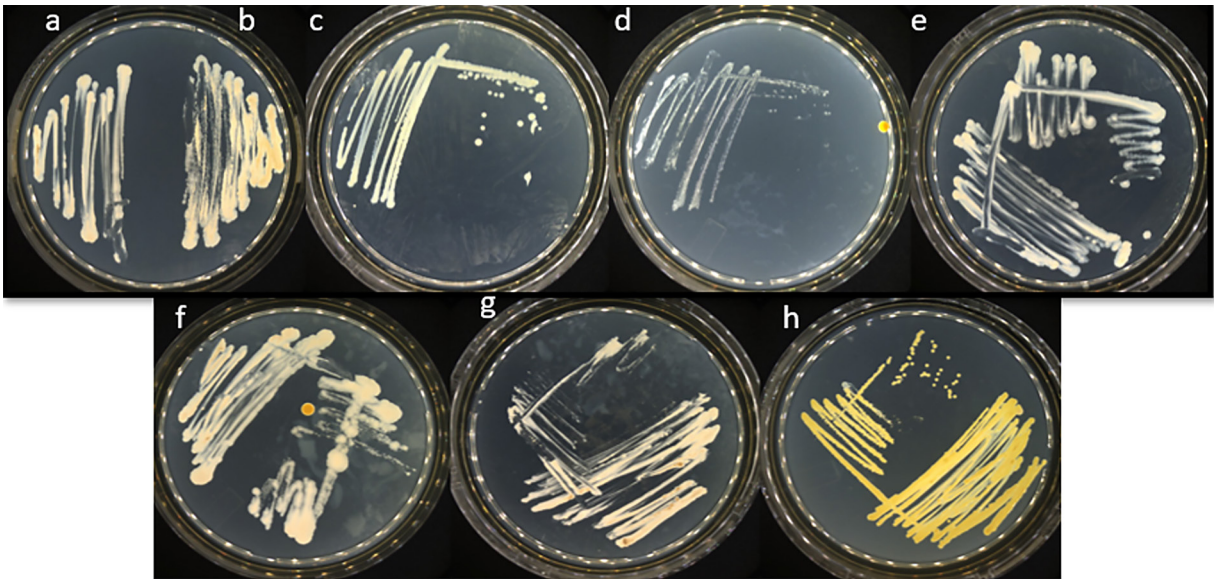

Figure 2. Growth of bacterial isolates with strain codes: (a) SO3P, (b) SO3K, (c) SO1OR, (d) SO4PB, (e) SO4PM, (f) CL3P, (g) CL5P, and (h) SO1K, on agar medium supplemented with 5% $(NH_4)_2SO_4$

Table 2. Isolate biochemical test results

Indicator	Isolate code							
	SO1OR	SO1K	SO4PM	SO4PB	SO3P	SO3K	CL3P	CL5P
Catalase	+	+	+	+	+	+	+	+
Mannitol	+	-	-	+	-	-	-	-
Glucose	+	-	-	+	-	-	-	-
Lactose	+	-	-	+	-	-	-	-
Maltose	+	-	-	+	-	-	-	-
Saccharose	+	-	-	+	-	-	-	-
Sulfur	-	-	-	-	-	-	-	-
Indole	-	-	-	-	-	-	-	-
Motility	-	-	-	-	-	-	-	-
Simmons citrate	-	+	+	-	+	+	+	-
Urea	+	-	-	+	-	-	-	-

particular sugars is often determined by the presence or absence of key enzymes such as mannitol dehydrogenase or beta-galactosidase. Variability in metabolic pathways across different bacterial genera or strains could explain the differential fermentation profiles observed. Similar observations have been reported in studies where only certain Gram-positive *cocci*, such as *Staphylococcus aureus* and *Staphylococcus epidermidis*, demonstrated mannitol fermentation activity (Namvar et al., 2014; Nguyen et al., 2019).

The sulfur, indole, and motility tests showed negative responses across all isolates. This indicates that none of the bacterial isolates were able to reduce sulfur compounds to hydrogen sulfide (H_2S), a trait commonly associated with certain Gram-negative bacteria possessing flagella (Dordević et al., 2021). The negative indole results suggest that the isolates lack the ability to utilize the amino acid tryptophan for the synthesis of pyruvic acid, ammonia, or indole. The indole test specifically detects the tryptophanase, which hydrolyzes tryptophan into indole and other by-products (Bhattacharyya, 2023).

Motility observations revealed that all isolates lacked flagella and therefore exhibited no motile behavior. Nonmotile characteristics are commonly found in coccus-shaped bacteria, which is consistent with previous reports indicating that nonmotile bacteria are often associated with coccoid

morphology (Zegadło et al., 2023). Positive responses were observed in the urease test results in strains SO1OR and SO4PB, indicating their ability to produce the urease. This enzyme catalyzes the hydrolysis of urea into ammonium and carbon dioxide (CO_2), which can serve as nitrogen sources for bacterial metabolism (Cui et al., 2022).

The findings of molecular identification of the isolates are presented in Figure 3. PCR products were visualized using 0.8% agarose gel electrophoresis. Based on the gel visualization, isolates SO1K, SO3K, SO1OR, and SO4PB produced amplicons with an approximate size of 1.000 base pairs. The sequencing results revealed that strain SO1K, SO3K, SO1OR, and SO4PB generated sequence lengths of 1.385 bp, 1.392 bp, 1.416 bp, and 1.414 bp, respectively.

The comparison of 16S rRNA gene homology of bacterial isolates is shown in Table 3. BLAST analysis revealed that isolate SO1K shared high similarity with *Brevibacterium* sp., SO3K with *Rothia koreensis*, SO1OR with *Staphylococcus aureus*, and SO4PB with *Staphylococcus epidermidis*. The 16S rRNA gene sequence of SO1K showed 99.86% similarity to the reference strain, while SO3K exhibited 99.43% similarity. In contrast, SO1OR and SO4PB both showed 100% identity with their respective reference strains.

These identity percentages indicate the degree of sequence alignment between the query and reference sequences. The common consensus in 16S rRNA gene analysis-based microbial identification is that a similarity result more than 97.5% indicates species-level identity, while values above 95% suggest genus-level identity (Stackebrandt and Goebel, 1994). The phylogenetic tree illustrating the relationships of isolates SO1K, SO3K, SO1OR, and SO4PB is presented in Figure 4.

Growth profile and ammonium reduction ability of bacterial isolates

All the isolates, SO1K, SO3K, SO1OR, and SO4PB exhibited notable growth and ammonium concentration reduction, as shown in Figure 4. The SO1K isolate, identified as *Brevibacterium* sp. showed a slight increase of absorbance between hour 0 and hour 4, indicating the beginning of the lag phase. The absorbance values increased steadily from hour 4 to hour 7, corresponding to the exponential growth phase. From hour 7 to hour 16, the absorbance values remained constant, indicating that the isolate had reached the stationary phase. A continuous decrease in ammonium concentration was observed over the 16 hours observation period, as reflected by a 92.15% reduction in ammonium concentration, from 310.99 mg/L at hour 0 to 24.41 mg/L at hour 16.

The SO3K isolate, taxonomically aligned with *Rothia koreensis*, began to show a slightly increasing absorbance from hour 0 to hour 3. Absorbance values were then increased rapidly from hour 3 to hour 8 and then remained relatively stable from hour 9 to hour 14. A decrease in absorbance was observed starting at hour 15, indicating the beginning of the death phase. Based on this pattern, the SO3K isolate was in the adaptation phase from hour 0 to hour 3, the exponential phase from hour 3 to hour 8, the stationary phase from hour 9 to hour 14, and the death phase at

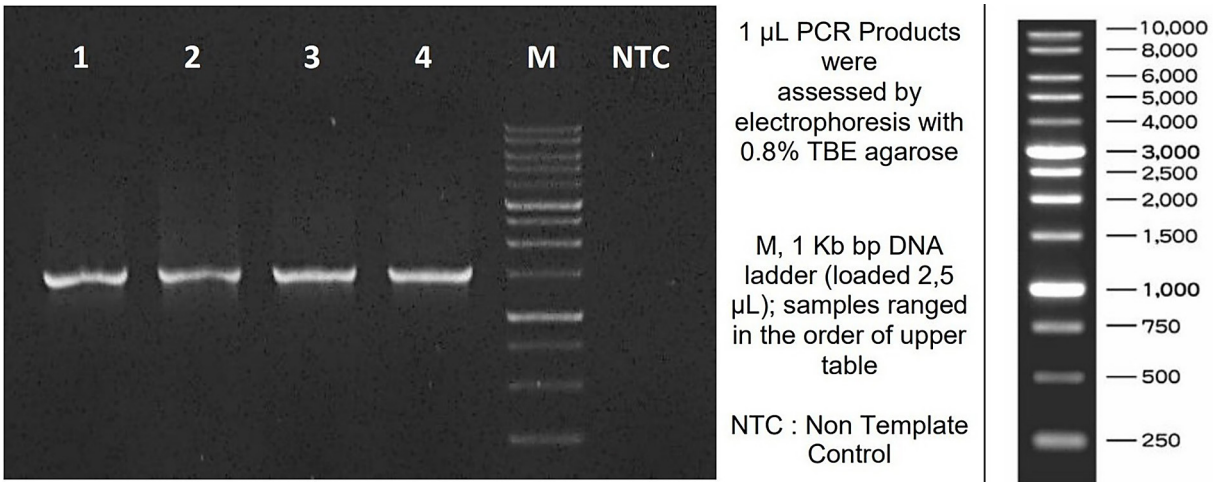

Figure 3. Results of amplification of bacterial isolates with codes: (1) SO1K, (2) SO3K, (3) SO1OR, (4) SO4PB, (M) DNA marker, and (NTC) non template control

Table 3. Comparison of 16S rRNA marker homology of bacterial isolates

No	Isolate	Comparison sequence access number	Species	Similarity (%)
1	SO1K	JF927301.1	*Brevibacterium* sp.	99.86
2	SO3K	MG557684.1	*Rothia koreensis*	99.43
3	SO1OR	CP102976.1	*Staphylococcus aureus*	100.00
4	SO4PB	CP102565.1	*Staphylococcus epidermidis*	100.00

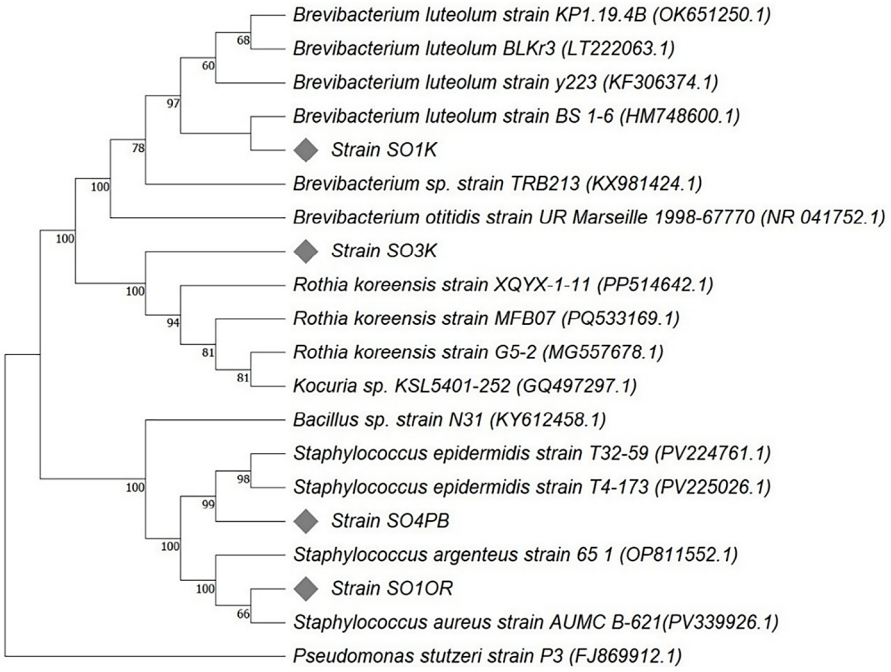

Figure 4. Neighbor-joining dendrogram based on 16S rRNA marker sequences in SO1K, SO3K, SO1OR and SO4PB isolates against comparison sequences

hour 15. A notable decrease in ammonium concentration was indicated during the 16 hours observation period, as shown by a 95.77% decrease in ammonium concentration, from 375.00 mg/L at hour 0 to 15.86 mg/L at hour 16.

Isolate SO1OR, confirmed to be *Staphylococcus aureus* based on molecular identification, began to exhibit measurable absorbance at hour 0 on both the control medium and the substrate. A slight increase in absorbance was observed from hour 0 to hour 3, followed by a rapid increase in absorbance from hour 3 to hour 8. A relatively constant absorbance values were then observed from hour 8 to hour 15. The SO1OR isolate was in the adaptation phase between hour 0 and hour 3, entered the exponential phase between hour 3 and hour 8, and reached the stationary phase between hour 9 and hour 15. A significant reduction in ammonium concentration was observed during the 16 hours incubation period, as indicated by a 98.63% decrease in ammonium concentration, from 317.40 mg/L at hour 0 to 4.36 mg/L at hour 16.

Identified as *Staphylococcus epidermidis,* the SO4PB isolate displayed an initial absorbance hour 0 and slightly increased until hour 3 Absorbance values was rapidly increased from hour 3 to hour 9, indicating the exponential growth phase. From hour 10 to hour 15, the absorbance values remained stable, suggesting that the culture had entered the stationary phase. At hour 16, a decrease in absorbance was observed, indicating the onset of the death phase. A reduction in ammonium concentration was observed under the tested conditions, as indicated by a 99.78% decrease in ammonium concentration, from 333.67 mg/L at hour 0 to 0.74 mg/L at hour 16. In addition to its growth activity, the SO4PB isolate exhibited the ability to lower ammonium levels during the incubation period (Figure 5).

All isolates underwent an adaptation phase that generally occurred between hour 0 and hour 4, although the exact timing varied depending on the bacterial strain. During this phase, the cells adjusted to the available nutrients and environmental conditions. The exponential phase of each bacterial isolate is different due to different types of bacteria including the ability to utilize nutrients so that the absorbance value produced is different. The exponential phase is the life cycle of a microorganism by multiplying by utilizing the synthesis of cell material that occurs in a short and constant time (Madigan et al., 2012). The stationary phase of each bacterial isolate occurred under different conditions, which can be attributed to variations in bacterial populations and the composition of the nutrient media, resulting in differences in the observed absorbance values. The bacterial growth will reach a stationary phase when the nutrients are depleted. The growth rate of the culture is zero when the medium is depleted or when other residues accumulate that inhibit bacterial growth. No significant decrease or increase in the number of cells occurs (Stanbury et al., 2016).

The growth profile of strain SO1K, identified as *Brevibacterium* sp., exhibited a faster growth rate compared to the growth profile of *Brevibacterium aureum* reported by Chen et al. (2013), which entered an adaptation phase from hour 0 to 12, followed by an exponential phase until hour 24, and reached a stationary phase that persisted until hour 96. The death phase began at hour 96, as indicated by a gradual decline in optical density

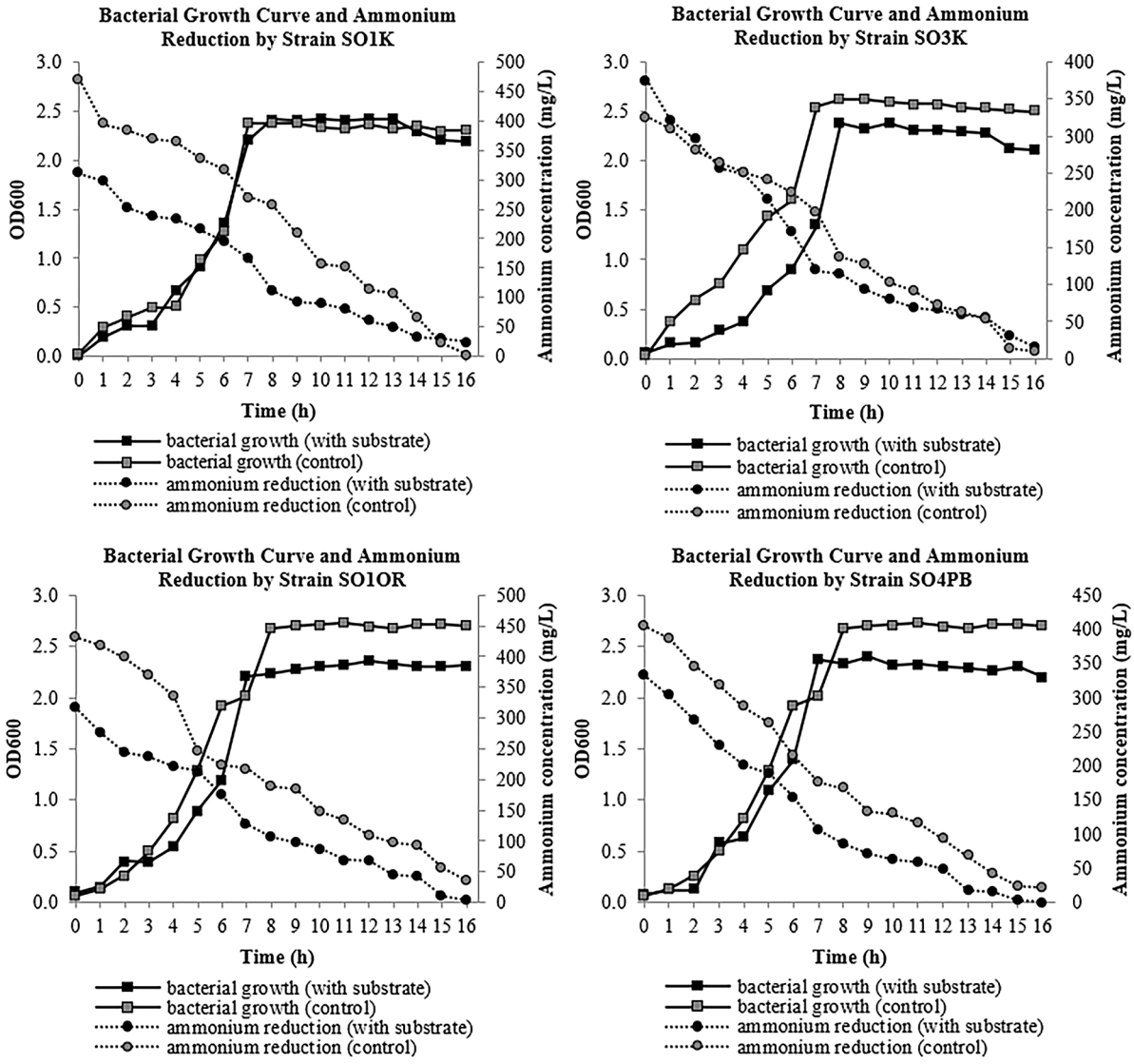

Figure 5. Bacterial growth curve and ammonium reduction by strain SO1K, SO3K, SO1OR, and SO4PB

observed until hour 120. The growth profile of strain SO3K, identified as *Rothia koreensis*, demonstrated a more rapid growth pattern compared to that of *Rothia mucilaginosa* as reported by Uranga et al. (2020). In their study, *Rothia mucilaginosa* exhibited an adaptation phase from hour 0 to 5, followed by exponential growth until hour 15, and entered the stationary phase which persisted up to hour 50. No decline in cell density was observed within the 50-hour observation period, indicating that the death phase had not yet begun. Strain SO1OR, identified as *Staphylococcus aureus*, exhibited a faster growth profile than the *Staphylococcus aureus* strain reported by Masood et al. (2020). In that study, the strain underwent an adaptation phase during the first 2 hours, followed by an exponential phase until hour 10, and remained in the stationary phase up to hour 23, with no clear onset of the death phase. Similarly, strain SO4PB, which was identified as *Staphylococcus epidermidis*, also demonstrated accelerated growth when compared to the growth pattern of *Staphylococcus epidermidis* described in the same study. The reference strain displayed a lag phase of approximately 1.7 hours, entered exponential growth until around hour 16.7, and maintained a stationary phase thereafter. No decline in optical density was observed up to hour 23. In contrast, SO4PB progressed more rapidly through the initial growth phases, indicating enhanced environmental adaptation and growth efficiency under the tested conditions.

All four bacterial strains demonstrated a reduction in ammonium concentration exceeding 90%, with the most substantial decrease observed in strain SO4PB, identified as *Staphylococcus epidermidis*, that accomplished a decrease of 99.78%. These results suggest that the strains have the capacity to degrade ammonia and may function as potential ammonia-oxidizing bacteria. Such bacteria are known to reduce ammonia levels in their environment through the activity of ammonia monooxygenase (AMO), a key enzyme in the oxidation of ammonium ions. AMO facilitates the transformation of ammonia (NH_4^+) into hydroxylamine (NH_2OH), which is subsequently oxidized to nitrite (NO_2^-) by hydroxylamine oxidoreductase (Farooq et al., 2022; Simon and Klotz, 2013).

The findings related to strain SO1K, identified as *Brevibacterium* sp. align with the previous study showing that bacterial genera such as *Brevibacterium ammoniilyticum* obtained from sewage sludge processing plants near agricultural areas, are capable of degrading ammonia (Kim et al., 2013). The isolate that was identified as *Rothia koreensis*, is a species previously described as *Kocuria koreensis* (Park et al., 2010) and later reclassified based on phylogenomic and phenotypic analyses (Nouioui et al., 2018). *Kocuria* sp. is an *Actinobacteria* capable of oxidizing ammonia to nitrite, this condition is in accordance with the statement from a previous study that *Kocuria* sp. belongs to the *Actinobacteria* group which is Gram-positive and arranged in pairs that can oxidize ammonia to nitrite (Huang and Jaffé, 2015; Kandi et al., 2016). The observed reduction in ammonia levels in this study suggests a potential ammonia-related metabolic activity by *Staphylococcus aureus*. Although no previous studies have confirmed its ability to oxidize ammonia, *Staphylococcus aureus* is known to possess nitrate reductase, which reduces nitrate to nitrite (Li et al., 2023), and nitric oxide synthase, which catalyzes the conversion of L-arginine to nitric oxide in the presence of oxygen and NADPH (Gøtterup et al., 2007). Ammonia assimilation in *Staphylococcus aureus* is mediated by glutamine synthetase (glnA), which facilitates the ATP-dependent synthesis of glutamine from glutamate and ammonia (Somerville and Proctor, 2009). These metabolic pathways suggest that *Staphylococcus aureus* has well-established mechanisms for nitrogen utilization, although its direct involvement in ammonia oxidation remains unconfirmed and may be specific to certain strains. The ability of *Staphylococcus epidermidis* strain SO4PB to oxidize ammonia is in opposition to what was found of Ohkubo et al. (2022) who states that the incorporation of ammonia to *Staphylococcus epidermidis* cultures resulted in elevated production of reactive oxygen species (ROS), which in turn inhibited bacterial growth. *Staphylococcus epidermidis* also tends to produce ammonia as part of its metabolic activity, as demonstrated by Lindgren et al. (2014). In contrast, strain SO4PB in the present study exhibited both robust growth and high ammonia removal efficiency, suggesting strain-specific differences in ammonia tolerance and metabolic response, potentially influenced by environmental adaptation or genetic variation. *Staphylococcus epidermidis* has also been reported to carry out denitrification, with the ability to reduce nitrate by up to 87% under anoxic condition within 120 hours (Zaffar et al., 2024), indicating its broader role in nitrogen transformation process.

CONCLUSION

This study successfully isolated eight bacterial strains from the activated sludge of poultry slaughterhouse wastewater namely SO1K, SO1OR, SO3K, SO3P, SO4PB, SO4PM, CL3P, and CL5P. Based on their tolerance to high ammonium levels and ammonium-reducing ability, four isolates were selected for further identification. Molecular identification revealed that SO1K shared 99.86% sequence identity with *Brevibacterium* sp., SO3K showed 99.43% similarity to *Rothia koreensis*, SO1OR was 100% identical to *Staphylococcus aureus*, and SO4PB was 100% identical to *Staphylococcus epidermidis*. These selected strains demonstrated significant ammonium reduction capacities, with SO1K reducing ammonium by 92.15%, SO3K by 95.77%, SO1OR by 98.63%, and SO4PB by 99.78%. These findings suggest that the selected strains have promising potential for application in the treatment of nitrogen-rich wastewater.

Acknowledgement

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REFERENCES

1. Anupong, W., Jutamas, K., On-uma, R., Sabour, A., Alshiekheid, M., Karuppusamy, I., Lan Chi, N. T., Pugazhendhi, A. (2022). Sustainable bioremediation approach to treat the sago industry effluents and evaluate the possibility of yielded biomass as a single cell protein (SCP) using cyanide tolerant Streptomyces tritici D5. *Chemosphere*, *304*, 135248. https://doi.org/10.1016/j.chemosphere.2022.135248
2. Ayilara, M. S., Babalola, O. O. (2023). Bioremediation of environmental wastes: The role of microorganisms. *Frontiers in Agronomy*, *5*, 1183691. https://doi.org/10.3389/fagro.2023.1183691
3. Azkarahman, A. R., Widodo, W., Fitriyanto, N. A., Yusiati, L. M., Erwanto, Y. (2021). Total ammonia and N2O emission characteristics from Alcaligenes sp. LS2T cultures and its application on laying hen manure associated with different pH conditions. *International Journal of Environment and Waste Management*, *27*(1), 1–20. https://doi.org/10.1504/ijewm.2021.10033670
4. Bhattacharyya S. (2023). overview on old and new biochemical test for bacterial identification. *Journal of Surgical Case Reports and Images*, *6*(5), 01–11. https://doi.org/10.31579/2690-1897/163
5. Bist, R. B., Subedi, S., Chai, L., Yang, X. (2023). Ammonia emissions, impacts, and mitigation strategies for poultry production: A critical review. *Journal of Environmental Management*, *328*, 116919. https://doi.org/10.1016/j.jenvman.2022.116919
6. Brenner, D. J., Krieg, N. R., Staley, J. T. (Eds.). (2005). *Bergey's Manual® of Systematic Bacteriology: Volume Two The Proteobacteria Part C The Alpha-, Beta-, Delta-, and Epsilonproteobacteria*. Springer US. https://doi.org/10.1007/0-387-29298-5
7. Chen, S., Dong, Y. H., Chang, C., Deng, Y., Zhang, X. F., Zhong, G., Song, H., Hu, M., Zhang, L.-H. (2013). Characterization of a novel cyfluthrin-degrading bacterial strain *Brevibacterium aureum* and its biochemical degradation pathway. *Bioresource Technology*, *132*, 16–23. https://doi.org/10.1016/j.biortech.2013.01.002
8. Cui, M.-J., Teng, A., Chu, J., Cao, B. (2022). A quantitative, high-throughput urease activity assay for comparison and rapid screening of ureolytic bacteria. *Environmental Research*, *208*, 112738. https://doi.org/10.1016/j.envres.2022.112738
9. Dordević, D., Jančíková, S., Vítězová, M., Kushkevych, I. (2021). Hydrogen sulfide toxicity in the gut environment: Meta-analysis of sulfate-reducing and lactic acid bacteria in inflammatory processes. *Journal of Advanced Research*, *27*, 55–69. https://doi.org/10.1016/j.jare.2020.03.003
10. Farooq, M. S., Uzair, M., Maqbool, Z., Fiaz, S., Yousuf, M., Yang, S. H., Khan, M. R. (2022). Improving nitrogen use efficiency in aerobic rice based on insights into the ecophysiology of archaeal and bacterial ammonia oxidizers. *Frontiers in Plant Science*, *13*, 913204. https://doi.org/10.3389/fpls.2022.913204
11. Fatima, F., Du, H., Kommalapati, R. R. (2021). Treatment of poultry slaughterhouse wastewater with membrane technologies: a review. *Water*, *13*(14), 1905. https://doi.org/10.3390/w13141905
12. Fitriyanto, N. A., Gutama, R., Wandita, T. G., Erwanto, Y., Hayakawa, T., Nakagawa, T. (2019). Isolation and characterization of Alcaligenes sp. LS2T from poultry farm at Yogyakarta city and the growth ability in animal's urine medium. *AIP Conference Proceedings*, *2099*(020006), 1–6. https://doi.org/10.1063/1.5098411
13. Fitriyanto, N. A., Winarti, A., Imara, F. A., Erwanto, Y., Hayakawa, T., Nakagawa, T. (2017). Identification and growth characters of nitrifying

pseudomonas sp., LS3K isolated from odorous region of poultry farm. *Journal of Biological Sciences*, *17*(1), 1–10. https://doi.org/10.3923/jbs.2017.1.10

14. Gøtterup, J., Olsen, K., Knöchel, S., Tjener, K., Stahnke, L. H., Møller, J. K. S. (2007). Relationship between nitrate/nitrite reductase activities in meat associated staphylococci and nitrosylmyoglobin formation in a cured meat model system. *International Journal of Food Microbiology*, *120*(3), 303–310. https://doi.org/10.1016/j.ijfoodmicro.2007.08.034
15. Gržinić, G., Piotrowicz-Cieślak, A., Klimkowicz-Pawlas, A., Górny, R. L., Ławniczek-Wałczyk, A., Piechowicz, L., Olkowska, E., Potrykus, M., Tankiewicz, M., Krupka, M., Siebielec, G., Wolska, L. (2023). Intensive poultry farming: A review of the impact on the environment and human health. *Science of The Total Environment*, *858*, 160014. https://doi.org/10.1016/j.scitotenv.2022.160014
16. Hadwan, M. H., Hussein, M. J., Mohammed, R. M., Hadwan, A. M., Saad Al-Kawaz, H., Al-Obaidy, S. S. M., Al Talebi, Z. A. (2024). An improved method for measuring catalase activity in biological samples. *Biology Methods and Protocols*, *9*(1), bpae015. https://doi.org/10.1093/biomethods/bpae015
17. Huang, S., Jaffé, P. R. (2015). Characterization of incubation experiments and development of an enrichment culture capable of ammonium oxidation under iron-reducing conditions. *Biogeosciences*, *12*(3), 769–779. https://doi.org/10.5194/bg-12-769-2015
18. Kandi, V., Palange, P., Vaish, R., Bhatti, A. B., Kale, V., Kandi, M. R., Bhoomagiri, M. R. (2016). Emerging bacterial infection: Identification and clinical significance of *Kocuria* species. *Cureus*, *8*(8). https://doi.org/10.7759/cureus.731
19. Khusnuryani, A., Martani, E., Wibawa, T., Widada, J. (2014). Molecular identification of phenol-degrading and biofi lm-forming bacteria from wastewater and peat soil. *Indonesian Journal of Biotechnology*, *19*(2), 99–110. https://doi.org/10.22146/ijbiotech.9299
20. Kim, J., Srinivasan, S., You, T., Bang, J. J., Park, S., Lee, S. S. (2013). *Brevibacterium ammoniilyticum* sp. Nov., an ammonia-degrading bacterium isolated from sludge of a wastewater treatment plant. *International Journal of Systematic and Evolutionary Microbiology*, *63*(PART3), 1111–1118. https://doi.org/10.1099/ijs.0.039305-0
21. Li, Y., Pan, T., Cao, R., Li, W., He, Z., Sun, B. (2023). Nitrate reductase NarGHJI modulates virulence via regulation of *agr* expression in methicillin-resistant *Staphylococcus aureus* strain USA300 LAC. *Microbiology Spectrum*, *11*(3), e03596-22. https://doi.org/10.1128/spectrum.03596-22
22. Lindgren, J. K., Thomas, V. C., Olson, M. E., Chaudhari, S. S., Nuxoll, A. S., Schaeffer, C. R., Lindgren, K. E., Jones, J., Zimmerman, M. C., Dunman, P. M., Bayles, K. W., Fey, P. D. (2014). Arginine Deiminase in Staphylococcus epidermidis Functions To Augment Biofilm Maturation through pH Homeostasis. *Journal of Bacteriology*, *196*(12), 2277–2289. https://doi.org/10.1128/JB.00051-14
23. Madigan, M. T., Martinko, J. M., Stahl, D. A., Clark, D. P. (2012). Brock Biology of Microorganism. In *Pearson-Benjamin Cummings* (13th Editi). Pearson Education, Inc. https://doi.org/10.1007/s13398-014-0173-7.2
24. Masood, S., Saleem, I., Smith, D., Chu, W.-K. (2020). Growth pattern of magnetic field-treated bacteria. *Current Microbiology*, *77*(2), 194–203. https://doi.org/10.1007/s00284-019-01820-7
25. Namvar, A. E., Bastarahang, S., Abbasi, N., Ghehi, G. S., Farhadbakhtiarian, S., Arezi, P., Hosseini, M., Baravati, S. Z., Jokar, Z., Chermahin, S. G. (2014). Clinical characteristics of Staphylococcus epidermidis: A systematic review [Text/html]. *GMS Hygiene and Infection Control; 9(3):Doc23*. https://doi.org/10.3205/DGKH000243
26. Ngobeni, P. V., Basitere, M., Thole, A. (2022). Treatment of poultry slaughterhouse wastewater using electrocoagulation: A review. *Water Practice and Technology*, *17*(1), 38–59. https://doi.org/10.2166/wpt.2021.108
27. Nguyen, T., Kim, T., Ta, H. M., Yeo, W. S., Choi, J., Mizar, P., Lee, S. S., Bae, T., Chaurasia, A. K., Kim, K. K. (2019). Targeting mannitol metabolism as an alternative antimicrobial strategy based on the structure-function study of Mannitol-1-Phosphate dehydrogenase in *Staphylococcus aureus*. *mBio*, *10*(4), e02660-18. https://doi.org/10.1128/mBio.02660-18
28. Nouioui, I., Carro, L., García-López, M., Meier-Kolthoff, J. P., Woyke, T., Kyrpides, N. C., Pukall, R., Klenk, H.-P., Goodfellow, M., Göker, M. (2018). Genome-based taxonomic classification of the phylum actinobacteria. *Frontiers in Microbiology*, *9*, 2007. https://doi.org/10.3389/fmicb.2018.02007
29. Ohkubo, T., Matsumoto, Y., Ogasawara, Y., Sugita, T. (2022). Alkaline stress inhibits the growth of Staphylococcus epidermidis by inducing TCA cycle-triggered ROS production. *Biochemical and Biophysical Research Communications*, *588*, 104–110. https://doi.org/10.1016/j.bbrc.2021.12.053
30. Park, E.-J., Roh, S. W., Kim, M.-S., Jung, M.-J., Shin, K.-S., Bae, J.-W. (2010). Kocuria koreensis sp. Nov., isolated from fermented seafood. *International Journal of Systematic and Evolutionary Microbiology*, *60*(1), 140–143. https://doi.org/10.1099/ijs.0.012310-0
31. Pasquina-Lemonche, L., Burns, J., Turner, R. D., Kumar, S., Tank, R., Mullin, N., Wilson, J. S., Chakrabarti, B., Bullough, P. A., Foster, S. J., Hobbs, J. K. (2020). The architecture of the Gram-positive bacterial cell wall. *Nature*, *582*(7811), 294–297. https://doi.org/10.1038/s41586-020-2236-6

32. Prasetyo, R. A., Pertiwiningrum, A., Erwanto, Y., Yusiati, L. M., Fitriyanto, N. A. (2018). Characterization of *Pseudomonas* sp. LS3K as Nitrate Removal Agent at Different C/N Ratios Under Aerobic Condition. In *Proceeding of the 2nd International Conference on Tropical Agriculture* 185–194. Springer International Publishing. https://doi.org/10.1007/978-3-319-97553-5_19
33. Simon, J., Klotz, M. G. (2013). Diversity and evolution of bioenergetic systems involved in microbial nitrogen compound transformations. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1827*(2), 114–135. https://doi.org/10.1016/j.bbabio.2012.07.005
34. Somerville, G. A., Proctor, R. A. (2009). At the crossroads of bacterial metabolism and virulence factor synthesis in staphylococci. *Microbiology and Molecular Biology Reviews*, *73*(2), 233–248. https://doi.org/10.1128/MMBR.00005-09
35. Stackebrandt, E., Goebel, B. M. (1994). Taxonomic note: A place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *International Journal of Systematic and Evolutionary Microbiology*, *44*(4), 846–849. https://doi.org/10.1099/00207713-44-4-846
36. Stanbury, P. F., Whitaker, A., Hall, S. J. (2016). Principles of Fermentation Technology: Third Edition. In *Principles of Fermentation Technology: Third Edition*.
37. Ullberg, M., Özenci, V. (2020). Identification and antimicrobial susceptibility testing of Gram-positive and Gram-negative bacteria from positive blood cultures using the Accelerate Pheno™ system. *European Journal of Clinical Microbiology & Infectious Diseases*, *39*(1), 139–149. https://doi.org/10.1007/s10096-019-03703-y
38. Uranga, C. C., Arroyo, P., Duggan, B. M., Gerwick, W. H., Edlund, A. (2020). Commensal oral *Rothia Mucilaginosa* produces Enterobacter, a metal-chelating siderophore. *mSystems*, *5*(2), e00161-20. https://doi.org/10.1128/mSystems.00161-20
39. Zaffar, R., Nazir, R., Hameed, J., Rather, M. A. (2024). Biofilm and extracellular polymeric substance (EPS) synergy: Revealing Staphylococcus's role in nitrate bioremediation. *World Journal of Microbiology and Biotechnology*, *40*(12), 391. https://doi.org/10.1007/s11274-024-04197-3
40. Zegadło, K., Gieroń, M., Żarnowiec, P., Durlik-Popińska, K., Kręcisz, B., Kaca, W., Czerwonka, G. (2023). Bacterial motility and its role in skin and wound infections. *International Journal of Molecular Sciences*, *24*(2), 1707. https://doi.org/10.3390/ijms24021707