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Isolation, Identification and Phylogenetic Analysis of Some Bacteriocins Producing *Paenibacillus* Species from Sheep Milk and Locally Fermented Alcoholic Beverage

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ABSTRACT

Samples of sheep milk and locally fermented alcoholic beverage (burukutu) were investigated for the presence of bacteriocin producing *Paenibacillus* species. Serial dilutions were made and the appropriate dilutions inoculated on MRS agar using spread plate method. Gram positive rods bacteria were isolated from the samples and identified as *Paenibacillus yanchengensis* from burukutu sample and *Paenibacillus yonginensis* from sheep milk sample. The cell free extracts from *Paenibacillus yanchengensis* grown in a broth had antimicrobial activity against *Listeria monocytogenes*, *Staphylococcus aureus* and *Salmonella* sp. but inactive against *E. coli* while cell free extract from *Paenibacillus yonginensis* grown in MRS broth had antimicrobial activity against *Listeria monocytogenes* and *Staphylococcus aureus* but inactive against *Salmonella* sp. and *E. coli*. The bacteria universal primer (27F, 1492R) for 16S rRNA gene was used to confirm the bacteria status of these isolates and the PCR products of 16S rRNA gene primer was sequenced using Sanger method. The BLAST analysis of the sequences also identified the two isolates as *Paenibacillus yanchengensis* from burukutu sample and *Paenibacillus yonginensis* from sheep milk sample. Consequently, the BLAST result was used to construct the phylogenetic trees for the two *Paenibacillus* species and phylogenetic analysis.

Key words: *Paenibacillus*, sheep milk, burukutu, bacteriocin, phylogenetic tree

INTRODUCTION

Paenibacillus species are rod shaped, Gram-positive, motile, facultative anaerobic or strictly aerobic bacteria which mostly show optimum growth at neutral pH within the temperature range of 28–40°C (Patowary and Deka, 2020). *Paenibacillus* species were originally included in the genus *Bacillus*, which historically was defined based on morphological characteristics in common with the *Bacillus subtilis* (Tiwari *et al*., 2019). A study using numerical taxonomy based on 188-unit characters suggested a framework for splitting *Bacillus* into several genera based on observed similarities and differences (Priest *et al*., 1998). A more accurate representation of phylogenetic relationships among these bacteria was attained in 1991, when 16S rRNA gene sequences were determined for standard strains of 51 species then defined as *Bacillus* (Priest *et al*., 1998; Ash *et al*., 1991). Phylogenetic analyses showed that these sequences segregated into at least five distinct clusters, one of which was reassigned to the novel genus *Paenibacillus* in 1993 (Ash *et al*., 1993) and includes the type species *Paenibacillus polymyxa* (Trüper, 2005).

Recent studies have identified over 20 species within the genus *Paenibacillus*, with some of the most well-known species being *Paenibacillus polymyxa*, *Paenibacillus larvae*, *Paenibacillus thiaminolyticus*, and *Paenibacillus macerans* These species are known for their robust metabolic pathways and the wide variety of bioactive compounds they produce (Li *et al*., 2024). *Paenibacillus* species are widely distributed in natural environments such as soil, water, and decaying organic matter, where they play important roles in nutrient cycling and decomposition (Grady *et al*., 2016). Isolated from a wide range of sources, the genus *Paenibacillus* comprises bacteria species relevant to humans, animals, plants, and the environment (Saez-Nieto *et al*., 2017). Members of *Paenibacillus* species are known for their ability to produce various

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bioactive compounds, including bacteriocins, which are antimicrobial peptides or proteins that inhibit the growth of other bacteria, often in a species-specific manner. *Paenibacillus* species produces bacteriocin molecules that are gaining huge interest (Arumugam *et al*., 2023). *Paenibacillus* species are known to produce at least two of the three classes of bacteriocins, being lantibiotics and pediocins. Lantibiotics are usually expressed at the late exponential phase or early stationary phase of bacterial growth, and are encoded in a cluster along with genes required for their extensive post-translational modifications (Huang and Yousef, 2015). The bacteriocins produced by *Paenibacillus* species have garnered significant attention due to their diverse range of antimicrobial activities, particularly against Gram-positive pathogens, and their potential applications in food preservation, agriculture, and medicine. Several *Paenibacillus* species are known to produce bacteriocins, which have shown promise in various applications (Grady *et al*., 2016). *Paenibacillus polymyxa* is one of the most studied species within this genus. It produces a range of antimicrobial compounds, including bacteriocins like paenibacillin, a novel bacteriocin with antimicrobial properties (El-Sharoud *et al*., 2022). According to Aramugam *et al*. (2023), one of the peptide antibiotic isolated from a *Paenibacillus* species, is paenibacillin, a lantibiotic molecule. The broad-spectrum antimicrobial activity, less possibility for resistance development, high stability, and so on make this novel lantibiotic an interesting molecule for applications in antimicrobial therapy and other industrial purposes.

Although, bacteriocin producing *Paenibacillus* species have been isolated from soil and other sources, isolation from ruminant milk and locally fermented alcoholic beverage (burukutu) have not been reported. This study therefore, tried to isolate bacteriocin producing *Paenibacillus* species from sheep milk and a locally fermented alcoholic beverage made from millet grains.

MATERIALS AND METHODS

Sheep milk samples were collected in sterile containers from Modibbo Adama University, Yola campus while burukutu was obtained from Girei Market, Adamawa state, Nigeria. All the samples were taken to the Microbiology Department of the Modibbo Adama University, Yola, for analysis. The sheep milk samples were allowed to ferment for 24 hours at 30^0C. Serial dilutions were prepared as described by Owuama, (2015). Spread plate method as described by Tomasello *et al*. (2023) was used in the inoculation of the samples. The plates were then incubated at 30^0C for 24 to 48hours. Discrete colonies that appeared on the MRS agar were counted using colony counter as described by Tomasello *et al*. (2023), and the result recorded as colony forming units per milliliter (cfu/ml). The discrete colonies were further sub-cultured to obtained pure cultures and were stored on agar slants at -4^0C. The colony morphology of the bacteria isolates was determined as described by Sharma *et al*. (2023), using size, shape, colour and texture of the colonies. The microscopic identification was done using Gram staining as described by Owuama (2015). The biochemical identification was done as described by Sjahril *et al*, (2021) and Went *et al*, (2022). In screening for bacteriocin production, crude bacteriocins was prepared as described by Sharma *et al*. (2019). Ten ml of nutrient broth was inoculated with 0.1ml (McFarland standard of 0.5) of freshly prepared cultures of each isolate and then incubated at 30^0C for 24 hours. The 24-hour cultures were centrifuged at 6000 rpm for 20minutes. The supernatants were filtered using cellulose acetate paper of pore size of 0.02µm to obtain cell free extracts (Refay *et al*., 2020; Chaithra *et al*., 2023). The pH of the extract was adjusted to 5.5 (optimal pH for the growth of test organisms) with 1% sodium hydroxide solution. To determine the bacteriocin activity, agar well diffusion method as described by Akbar *et al*. (2019) and Hossain *et al*. (2022) were used. The four test organisms, *Listeria monocytogenes*, *Staphylococcus aureus*, *E. coli* and *Salmonella* species, obtained from Microbiology Department of Modibbo Adama University, Yola, were authenticated by sub-culturing and proper identification. The McFarland standard of 0.5 of the test organisms were then inoculated on Muller Hilton agar (Merck, Germany) and a sterile cork borer (6mm diameter) was used to bore holes on the agar surfaces. Subsequently, 50µl of the cell free extract earlier prepared was pipetted into the appropriate wells and allowed to adsorb, then incubated for 24 hours at 30^0C. After 24 hours of incubation, zones of inhibition, where present, were measured and recorded in millimeter (mm).

The molecular identification of the bacteria isolates was done using the bacteria universal 16S rRNA gene primers (27F, 1492R) as described by Kai *et al*, (2019) and Winand *et al*, (2019). The PCR products of the 16S rRNA primers of the bacteria isolates were then sequenced as described by (Akacin *et al*., 2022). After the sequencing, a Basic Local Alignment search Tool (BLAST) was conducted on the result.

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RESULTS

Results of isolation, macroscopic, microscopic and biochemical identification of the bacteriocin producing bacteria associated with sheep milk and fermented alcoholic beverage (burukutu) examined are shown in Table 1. Macroscopic examination showed the size, shape, colour and texture of the bacteria isolates on the isolation media while microscopic examination showed that the bacteria isolates were both Gram positive rods. Vitek 2 compact system identified isolate B as *Paenibacillus yanchengensis* and isolate S as *Paenibacillus yonginensis* (Table 1)

Table 1. Macroscopic, microscopic and biochemical characteristics of the two bacteria isolates

S/N		Isolate	Size(mm)	Shape	Colour	Texture	G/R	Vitek 2
1		B	1-2	Circular	Cream	Smooth	GPR	*Paenibacillus yanchengensis*
2		S	2-3	Circular	White	Smooth	GPR	*Paenibacillus yonginensis*

Key: B = Isolate from Burukutu, S = Isolate from Sheep Milk, G/RX = Gram Reaction, GPR = Gram Positive Rod

From this study, screening for the presence of bacteriocin from the bacteria isolates was done using 4 bacteria species (*S. aureus*, *Listeria monocytogenes*, *E. coli* and *Salmonella* sp) as test organisms. Results of the screening showed that, isolates S and B produced the inhibitory substance (bacteriocin) against the test organisms. Isolate S from sheep milk produced substance that inhibited the growth of *Listeria monocytogenes* and *S. aureus* but no inhibition against *E. coli* and *Salmonella* sp. Isolate B from burukutu however, produced substance that inhibited the growth of *Listeria monocytogenes*, *S. aureus*, and *Salmonella* sp but no inhibition was observed against *E. coli* (Table 2)

Table 2. The inhibitory pattern in (mm) of the bacteriocins produced by *Paenibacillus yanchengensis* (isolate B) and *Paenibacillus yonginensis* (isolate S) against the test organisms

S/N	Isolate	Test organisms			
		List	S.aureus	*E. coli*	Sal
1.	*P. yanchengensis*	22	23	-	19
2.	*P. yonginensis*	20	17	-	-

Key: List = *Listeria monocytogenes*, S. aureus = *Staphylococcus aureus*, Sal = *Salmonella* species, - = no inhibition

The molecular identification of the bacteria isolates from this study was carried out using the bacteria universal primer (27F, 1492R) for 16S rRNA gene to confirm the presence of 16S rRNA gene. Figure 1 indicate that, the PCR products of the 2 bacteria isolates showed bands at 1500 of the 1kb ladder after running the electrophoresis gel.

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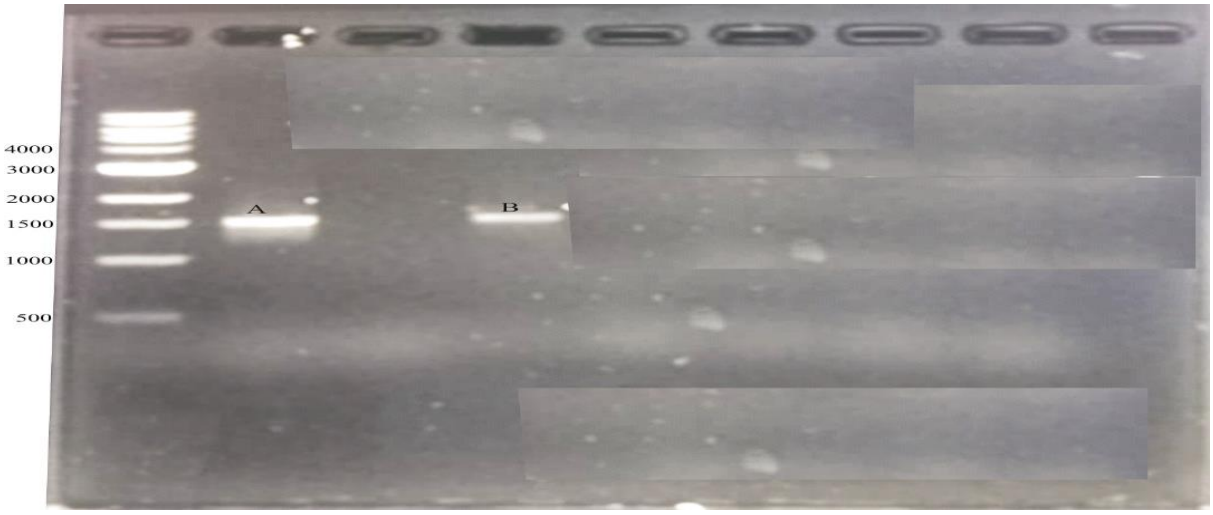

Figure 1. Agarose Gel Electrophoresis of 16S rRNA Gene Primer PCR Products Showing Bands at 1500bp from Bacteria Isolates from Burukutu (A) and Sheep milk (B)

The results of the partial sequencing of 16S rRNA gene and the BLAST prediction report of isolate B and S is shown in Table 3.

Table 3. Sequence BLAST Prediction Report of Isolates B and S

S/N	Isolate	Highest query coverage (%)	Sequence Length (bp)	% identity	Accession no of Blast hit	E-value	Alignment score	Organism
1	B	99%	1518	98.03%	MF9477121	0.0	≥200	*Paenibacillus yanchengensis*
2	S	98%	1250	99.20%	NR_148743	0.0	≥200	*Paenibacillus yonginensis*

The results of Phylogenetic tree of *Paenibacillus yanchengensis*, (isolate B) *P. yonginensis* (isolate S) and other *Paenibacillus* species is shown in figure 2 below.

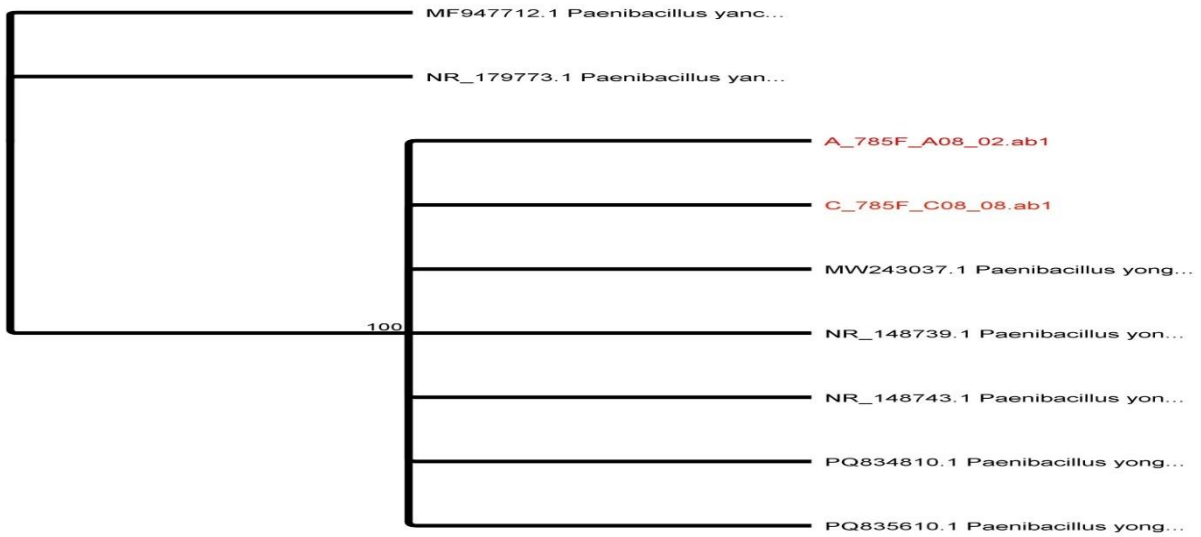

Figure 2. Phylogenetic tree of *Paenibacillus yanchengensis*, *P. yonginensis* and other *Paenibacillus* species

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DISCUSSION

The colony morphology of these isolates shows that, isolate B has small size (1-2mm), circular shape, creamy colour and smooth texture. Similarly, those of isolate S had small size (2-3mm), circular shape, white colour and smooth texture (Table 1). This result shows that colonies of isolate B were slightly smaller than those of isolate S. Both have circular shape and smooth texture but while B is creamy, S is slightly whitish. The result agreed with the report of Sukweenadhi *et al*, (2014) and Lu *et al*, (2018) who reported that, colonies of *Paenibacillus* species are small in size, circular in shape, creamy or white in colour and have a smooth texture. Microscopic identification revealed both the isolates as Gram positive rods while Vitek 2 compact system identified the isolates as *Paenibacillus yanchengensis* (B) and *P aenibacillus yonginensis* (S). *Paenibacillus* are rod shaped, Gram positive, motile, facultative anaerobic or strictly aerobic bacteria which mostly show optimum growth at neutral pH within the temperature range of 28-40^{0}C (Patowary and Deka, 2020).

From this study, screening for the presence of bacteriocin from the bacteria isolates was done using 4 bacteria species (*S. aureus, Listeria monocytogenes, E. coli* and *Salmonella* sp) as test organisms. Results of the screening showed that, 2 bacteria isolates (*P. yanchengensis*) from burukutu and (*P. yonginensis*) from sheep milk produced the inhibitory substance (bacteriocin) against the test organisms (Table 2). *P. yonginensis* produced substance that inhibited the growth of *Listeria monocytogenes* and *S. aureus* but no activity against *E. coli* and *Salmonella* sp. *P. yanchengensis* however, produced substance that inhibited the growth of *Listeria monocytogenes, S. aureus*, and *Salmonella* sp but no inhibition against *E. coli*. The bacteriocin produced by isolate *P. yonginensis* demonstrates promising antimicrobial activity, particularly against *Listeria monocytogenes* and *Staphylococcus aureus*, but shows no inhibition against *Escherichia coli* or *Salmonella* sp. Bacteriocins are antimicrobial peptides or proteins produced by bacteria that inhibit the growth of other microorganisms. Their spectrum of activity can vary significantly depending on their structure, mode of action, and the target bacteria (An *et al*., 2015; Mercado and Olmos *et al*., 2022). In this case, the bacteriocin shows good inhibition against Gram positive bacteria (*Listeria monocytogenes* and *Staphylococcus aureus*) but no activity against Gram-negative bacteria (*E. coli* and *Salmonella* sp.). This selective inhibition is commonly observed with bacteriocins and can be attributed to fact that Gram positive bacteria like *L. monocytogenes* and *S. aureus* have a thick peptidoglycan layer in their cell walls, which can be more susceptible to the action of bacteriocins that target cell membrane integrity or cell wall biosynthesis (Simons *et al*., 2020). On the other hand, Gram negative bacteria have an outer membrane that acts as a barrier, making them more resistant to many bacteriocins (Prudencio *et al*., 2015). This outer membrane contains lipopolysaccharides (LPS), which can prevent the bacteriocin from reaching the cell membrane. Second, some bacteriocins are specific to certain genera or species of bacteria (Yuang and Yousef, 2015). This could explain the lack of inhibition against *E. coli* and *Salmonella*, as these bacteria may possess mechanisms that either limit the access of bacteriocins or degrade them before they can act. *E. coli* and *Salmonella* sp. are known to have various defence mechanisms like efflux pumps, which could actively expel bacteriocins from the cell (Kumariya *et al*., 2019; Sengkhui, *et al*., 2023; Gauba and Rahma, 2023).

The fact that *P. yanchengensis* produced inhibitory substance against *Listeria monocytogenes, Staphylococcus aureus*, and *Salmonella* sp., but not *E. coli*, indicates a possible difference in the susceptibility of these pathogens to the bacteriocin. *Listeria monocytogenes*, *Staphylococcus aureus*, and *Salmonella* sp. possess peptidoglycan-rich cell walls, which are a common target for many bacteriocins. However, *E. coli* has an outer membrane composed of lipopolysaccharides (LPS) that can act as a barrier to some bacteriocins, especially those that need to penetrate the outer membrane before interacting with the inner membrane or cell wall (Shu and Mi, 2022). In contrast, *Listeria monocytogenes* and *Staphylococcus aureus* lack an outer membrane, which may make them more susceptible to this bacteriocin (Budic *et al*., 2011; Simons *et al*., 2020). Recent studies by Budic *et al*. (2011), show that some bacteriocins are designed to target specific pathways or receptors that are unique to certain species. Bacteriocins like nisin and pediocin (a class I and II bacteriocins) have been shown to target cell wall synthesis in Gram positive bacteria like Genus of *Listeria* and *Staphylococcus* (Zimina *et al*., 2020). In contrast, Gram negative bacteria like *E. coli* possess an outer membrane that requires bacteriocins to have a specific mode of action or a higher concentration to overcome this protective barrier (Prudencio *et al*., 2015). A recent study on bacteriocins produced by lactic acid bacteria demonstrated that bacteriocins can disrupt the cell membrane integrity of *Listeria* and *Staphylococcus aureus* by forming pores or binding to cell wall components like peptidoglycan (da Costa *et al*., 2019). For *Salmonella*, a similar

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mechanism has been suggested, where bacteriocins act on the cell wall and membrane. These mechanisms may explain the inhibition observed for these pathogens. However, for *E. coli*, bacteriocins typically need to traverse the outer membrane, a process that requires either specific receptor binding or outer membrane disruption (Bastos *et al*., 2015; Telhig *et al*., 2020).

The molecular identification of the bacteria isolates from this study was carried out using the bacteria universal primer (27F, 1492R) for 16S rRNA gene to confirm the presence of 16S rRNA gene. Figure 1 showed that the 2 bacteria isolates produced bands at 1500 of the 1kb ladder after running the agarose gel electrophoresis. This is the genotypic confirmation of the bacteria status of the isolates. The traditional identification of bacteria on the basis of phenotypic characteristics is generally not as accurate as identification based on genotypic methods (Matsumoto and Sugano, 2013). Nucleic acid sequencing of the bacterial 16S rRNA gene (here designated 16S) has been used for several decades to identify clinical and environmental isolates and to assign phylogenetic relationships. The 16S rRNA gene has been a mainstay of sequence-based bacteria analysis for decades. Analysis of the 16S rRNA gene sequence can more accurately identify bacteria with abnormal phenotypes, infrequent isolations, or inadequate descriptions (Johnson *et al*., 2019; Church *et al*., 2020).

Molecular identification of bacteria using the 16S rRNA gene primer is a widely used technique in microbiology to identify and classify bacteria species (Johnson *et al*., 2019). The 16S rRNA gene is often used in bacteria identification and phylogeny due to its highly conserved regions interspersed with variable regions, allowing for taxonomic discrimination. The 16S rRNA gene is approximately 1500 bp in size, which aligns with the expected fragment size from the PCR amplification. Uniformity of the bands across these isolates (showing the same band at 1500 bp) indicate that the isolates come from a similar taxonomic group or that the PCR conditions were optimal, ensuring uniform amplification (Yoon *et al*., 2017; Li *et al*. 2024).

The PCR products of 16S rRNA gene primer of isolates ‘B’ and ‘S’ were sequenced using Sanger method and the predicted BLAST results shown in Table 3. The PCR results obtained for the bacteria isolate ‘B’ show a sequence length of 1518 bp while ‘S’ show sequence length of 1250bp from a 16S rRNA gene fragment, which aligns with *Paenibacillus yanchengensis* with 98.03% identity and *Paenibacillus yonginensis* with 99% identity respectively. The alignment has a high query coverage of 99% and 98%, a perfect E-value of 0.0 each, and a high alignment score (≥ 200) each, suggesting a very strong match between the sample and the reference sequences. The organisms identified were *Paenibacillus yanchengensis* and *Paenibacillus ynginensis*, members of the *Paenibacillus* genus, which is widely known for its role in soil, plant, and industrial environments (Lu *et al*., 2018). The genus *Paenibacillus* comprises a diverse group of Gram-positive, rod-shaped bacteria known for their ecological versatility and ability to form spores (Potawary and Deka, 2020). The high sequence identity (98.03%, 99.20%) and coverage (99%, 98%) from the 16S rRNA gene sequence strongly suggest that the isolates belong to the reference species or a closely related strain. A 98% or greater identity generally indicates a close taxonomic relationship, with the 16S rRNA gene often providing reliable species-level identification, especially when query coverage is so high. A study by Johnson *et al*., (2019) and Li *et al.* (2024), highlights the importance of 16S rRNA gene sequencing for accurately identifying strains within the bacteria genus, especially when sequence identity reaches 98% or higher. Also, Caldeira *et al.* (2024), emphasized how the use of 16S rRNA gene sequencing allows for differentiation among *Paenibacillus* species and provides a rapid, reliable method for bacteria classification. The sequence lengths of 1518 bp and 1250bp are typical for 16S rRNA gene sequencing, providing sufficient resolution for genus and species-level identification. The alignment score being greater than or equal to 200, with a query coverage of 99%, further indicates a strong match between the PCR product and the reference sequences. This high coverage and alignment score reinforce the accuracy of the identification. *Paenibacillus* species are well characterized in public sequence databases, which supports the confidence of the identification (Yao *et al*., 2025). The sequence length (around 1500 bp or 1250bp) is often optimal for achieving clear taxonomic classifications when comparing against high-quality reference sequences. The E-value of 0.0 is extremely low, which means that the observed match is highly statistically significant, implying that the alignment is not due to random chance. The alignment score of ≥ 200 further solidifies this, indicating that the match is a strong one. E-values near 0.0 typically denote that the match is highly reliable and not an artifact of sequence comparison. The E-value of 0.0 in BLAST results indicates a perfect or near-perfect match, and this is a strong indicator of the accuracy of isolate ‘B’ and ‘S’ identification as well as emphasized that such low E-values suggest a well-defined and reliable taxonomic placement, reducing ambiguity in microbial classification. A 98.03% and 99.20% identity

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to *Paenibacillus yanchengensis* and *Paenibacillus yonginensis* is very high, but it is important to note that some *Paenibacillus* species are known to have closely related 16S rRNA sequences, which may lead to challenges in distinguishing between them at the species level based purely on this marker (Saez-Nieto *et al*., 2017). However, this level of identity (98% or more) typically confirms that the isolate is most likely *P. yanchengensis* and *Paenibacillus yonginensis* or a very closely related strain, provided that the database used for BLAST is comprehensive and up-to-date.

The bacteria isolates identified as *Paenibacillus yanchengensis* from burukutu and *Paenibacillus yonginensis* from sheep milk after sequencing of the 16S rRNA gene of the two isolates agreed with biochemical identification using Vitek 2 compact system. *Paenibacillus* species can be conveniently identified using Vitek 2 compact system (Fahim *et al*., 2022)

Figure 2. shows phylogenetic tree of *P. yanchengensis*, *P. Yonginensis* and other *Paenibacillus* species. This phylogenetic tree represents the evolutionary relationships between query sequences (*A_785F_A08_02.ab1* and *C_785F_C08_08.ab1*) and reference sequences from the *Paenibacillus* genus. The placement of these sequences provides insights into their classification and evolutionary divergence. The tree has two major clades: One contains sequences classified as *Paenibacillus yanchengensis* (P. yanc), the other consists of sequences classified as *Paenibacillus yonginensis* (P. yong). The query sequences are aligned within these respective groups: A_785F_A08_02.ab1 clusters with *P. yanc* (MF947712.1, NR_179773.1). C_785F_C08_08.ab1 clusters with *P. yong* (MW243037.1, NR_148739.1, NR_148743.1, PQ834810.1, PQ835610.1). The node separating *Paenibacillus yanchengensis* from *Paenibacillus yonginensis* has 100 bootstrap supports, indicating a very strong evolutionary distinction. This supports previous taxonomic classifications that distinguish these species based on 16S rRNA gene sequences.

The alignment of A_785F_A08_02.ab1 with *P. yanc* and C_785F_C08_08.ab1 with *P. yong* suggests that the sequencing results are in agreement with prior classifications of these bacteria species. This confirms that: A_785F_A08_02.ab1 is genetically more similar to *Paenibacillus yancengensis*. C_785F_C08_08.ab1 shares a closer evolutionary relationship with *Paenibacillus yonginensis*. This classification aligns with previous phylogenetic studies, which have established clear boundaries between these two *Paenibacillus* species based on genomic and phenotypic characteristics. Studies using 16S rRNA sequencing and genome-wide comparisons have demonstrated that *Paenibacillus yancengensis* and *Paenibacillus yonginensis* form distinct clusters within the genus (Kim *et al*., 2017; Xue *et al*., 2023).

CONCLUSION

Paenibacillus species are present in locally fermented alcoholic beverage, burukutu as well as sheep milk. *Paenibacillus yanchengensis* isolated from burukutu produced inhibitory substance that have antimicrobial activity against *Listeria monocytogenes*, *Staphylococcus aureus* and *Salmonella* sp. but inactive against *E. coli*. But, *Paenibacillus yonginensis* isolated from sheep milk produced inhibitory substance that had antimicrobial activity against *Listeria monocytogenes* and *Staphylococcus aureus*, but inactive against *Salmonella* sp. and *E. coli*. This further suggests that *Paenibacillus* species are present in most environments and do produce bacteriocins. The phylogenetic tree and phylogenetic analysis show that, the node separating *Paenibacillus yanchengensis* from *Paenibacillus yonginensis* on the phylogenetic tree has 100 bootstrap supports, indicating a very strong evolutionary distinction. This supports previous taxonomic classifications that distinguish these species based on 16S rRNA gene sequences.

Paenibacillus yanchengensis have never been isolated from burukutu before just like *Paenibacillus yonginensis* have never been isolated from sheep milk. The discovery that these bacteria produce bacteriocin has contributed greatly to knowledge as search for more microbial based products as alternative to chemical-based preservatives in the food industry intensifies.

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