

Table 5 Acid tolerance of isolates

Isolate	pH 2.5	pH 3.0	pH 3.5	pH 4	pH 7
<i>Bacillus</i> spp.	1.8±0.2	2.2±0.4	2.6±0.2	2.8±0.3	3.4±0.2
Cocci bacteria	0.4±0.1	0.7±0.2	0.9±0.1	1.1±0.2	1.8±0.3

Statistical analysis showed significantly higher acid tolerance ($p < 0.05$) for *Bacillus* isolates across all pH levels.

Table 6 Bile tolerance

Isolate	0.2%	0.4%	0.6%	1.0%
<i>Bacillus</i> spp.	2.8±0.3	2.2±0.1	1.9±0.2	1.6±0.1
Cocci bacteria	2.7±0.1	2.1±0.3	1.6±0.1	1.3±0.2

The superior tolerance exhibited by *Bacillus* spp. indicates their adaptation to intestinal bile conditions.

3.4 Antimicrobial activity

As presented in table 7 and figure 2, the inhibitory activity of the tested probiotic isolates against *Escherichia coli* isolates showed clear differences between *Cocci* spp. and *Bacillus* spp., as demonstrated by their respective zones of inhibition. *Bacillus* spp exhibited the highest antagonistic effect with a mean inhibition zone of 34 mm, while *Cocci* spp. produced a comparatively smaller zone of 26 mm. The significantly larger inhibition zone produced by *Bacillus* spp. indicates a stronger antimicrobial potency against the target pathogen.

Table 7 Zone of inhibition against pathogens

Pathogen	<i>Cocci</i> spp. (mm)	<i>Bacillus</i> spp. (mm)
<i>Escherichia coli</i>	26	34
<i>Salmonella typhi</i>	29	29
<i>Staphylococcus aureus</i>	39	39

Bacillus isolates demonstrated strong antagonistic activity, suggesting production of antimicrobial compounds.

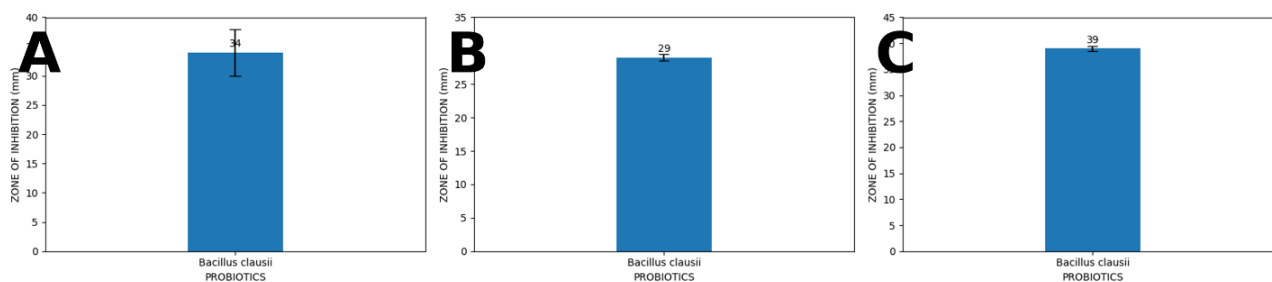


Figure 2 Diameter of inhibition zone of *Bacillus clausii* tested against A=*Escherichia coli* B=*Salmonella typhi* C=*Staphylococcus aureus* isolates.

Mean radii with different superscripts indicate significant ($\alpha_{0.05}$) difference according to DMRT.

3.5 Molecular identification

PCR amplification of the 16S rRNA gene produced single bands of approximately 1500 bp, confirming successful amplification. Sequencing analysis identified the isolate as *Bacillus clausii*. Phylogenetic analysis revealed a close evolutionary relationship between the identified strain and previously reported *Bacillus clausii* strains retrieved from the NCBI GenBank database (Figure 3). The identified strain showed close clustering with *Bacillus clausii* PRA25, *Bacillus clausii* AK31, and *Bacillus clausii* XJU2. Bootstrap values ranged from 86% to 98%, indicating strong support for the phylogenetic clustering.