

# Journal of Applied Biology & Biotechnology

Available online at <http://www.jabonline.in>

## Isolation and functional evaluation of probiotic bacteria from goat milk: A small ruminant-derived resource for animal and human health applications

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Doi: <http://doi.org/10.7324/JABB.2026.264555>

### SUPPLEMENTARY MATERIAL

**Table S1.** Selection of 14 strains based on common biochemical characterizations, including catalase, oxidase, and sugar fermentation tests.

Isolate ID	KOH Test	Catalase	Oxidase	Indole	Methyl Red	Voges- Proskauer	Citrate Utilization	Nitrate Reduction
1	+	-	-	-	+	+	-	-
2	+	+	-	-	+	+	-	-
3	+	+	-	-	-	+	+	+

4	+	+	−	−	+	−	−	−
<b>5</b>	+	−	−	−	−	−	+	+
<b>6</b>	+	+	−	−	+	+	+	+
<b>7</b>	+	+	−	−	+	−	+	+
<b>8</b>	+	+	−	−	−	+	−	−
<b>9</b>	+	−	−	−	+	+	+	+
10	+	+	−	−	−	+	−	+
<b>11</b>	+	+	−	−	+	−	+	−
<b>12</b>	+	+	+	−	+	+	+	+
13	+	−	−	−	−	−	+	+
<b>14</b>	+	+	−	−	+	+	+	−
<b>15</b>	+	+	−	−	−	−	−	−
<b>16</b>	+	−	−	−	+	+	+	+
<b>17</b>	+	+	−	−	+	−	−	+
18	+	+	+	−	+	+	+	+
<b>19</b>	+	+	+	−	+	+	+	+
20	+	−	−	−	+	+	+	+
21	+	+	−	−	+	+	−	+
22	+	+	−	−	−	+	+	+
23	+	−	−	−	−	−	−	−

**Table S2.** Selection of 5 potent probiotic strains based on specific biochemical characterizations, such as bile salt tolerance, acid resistance, and enzymatic activity.

ISOLATE ID	ACID TOLERANCE	BILE TOLERANCE	ADHESION	AGGREGATION
1	+	+	+	+
<b>2</b>	+	+	+	+
3	+	+	+	–
4	–	+	+	+
5	+	–	–	–
<b>6</b>	+	+	+	+
7	–	+	+	+
8	–	–	–	–
9	+	+	+	–
10	+	+	+	–
11	+	+	–	+
<b>12</b>	+	+	+	–
13	–	–	–	–
14	+	–	+	+
15	–	–	–	–
16	+	+	–	+
17	+	+	–	–

18	+	+	+	+
19	+	+	+	+
20	+	+	–	–
21	+	–	+	–
22	–	–	+	+
23	–	–	–	–

**Table S3.** 16S rRNA gene sequencing details of selected probiotic strains. The table presents the strain name, identified organism, 16S rRNA gene length (in base pairs), and corresponding accession and GI numbers from the NCBI database.

Strain Name	Organism	16S rRNA Gene Length	Accession Number	GI Number
<i>Bacillus subtilis</i> strain GMI2	<i>Bacillus subtilis-II</i>	1,500 bp	PP472426.1	2697401519
<i>Bacillus subtilis</i> strain GMI1	<i>Bacillus subtilis-I</i>	1,362 bp	PP472092.1	2697401173
<i>Lactobacillus helveticus</i> strain GM-3	<i>Lactobacillus helveticus</i>	1,003 bp	PQ270253.1	2796986130
<i>Bacillus aerolacticus</i> strain GM-1	<i>Bacillus aerolacticus</i>	1,396 bp	PQ270249.1	2796986124
<i>Heyndrickxia coagulans</i> strain GM-2	<i>Heyndrickxia coagulans</i>	1,062 bp	PQ270252.1	2796986129

**Table S4.** Technological properties of dominant lactic acid bacteria isolated from raw goat milk.

Strains	Proteolytic activity	Lipolytic activity	Diacetyl production	Exopolysaccharide production (mg/L)
<i>Bacillus subtilis</i> (Strain 1)	+	+	+	85.0 ±2.0c
<i>Bacillus subtilis</i> (Strain 2)	+	+	+	20.2 ±1.7h
<i>Lactobacillus helveticus</i>	+a	+wb	+	58.0 ±2.6e
<i>Bacillus aerolacticus</i>	+	+w	+	22.3 ±2.5gh
<i>Heyndrickxia coagulans</i>	+	+w	+	93.0 ±1.1a
<b>P-value</b>	<b>0.000</b>	-	-	-

*a* Signs indicated as ‘+’ = positive halo formation in the colony; ‘-’ = no halo formed in the colony.

*b* +w, weak halo formation in the colony.

*c* The signs indicated red ring formation.

*d* Exopolysaccharide means with different superscripts are significantly different ( $P < 0.05$ ).