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Isolation, characterization and identification of lactic acid bacteria from fermented barley

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Lactic acid bacteria (LAB) from food sources are extensively studied for its diversified role in human health. Their function extends from normalization of intestinal microbiota composition and immunomodulation to metabolic effects such as reduced serum cholesterol effects, reduced toxic effects and lactose hydrolysis. Barley (*Hordeum vulgare*) is a member of the grass family. This study attempts to isolate and characterize bacteria associated with fermented barley. An Indian variety of barley seeds (a sample drawn from composite mix) were purchased locally, milled to passed through a 0.5 mm sieve, mixed with sterilized distilled water (1:2 ratio, w/v) and allowed to ferment at 30 °C for 24 h. Serial dilutions were prepared up to 10⁻³ with sterilized saline from the fermented sample, 0.1 ml from each dilution were spread on sterile MRS agar plates (Hi-Media, India) and incubated (37 °C, 24 h.) Each colony forming unit isolated was streaked on fresh MRS plates to examine the purity, followed by gram staining. All purified isolates were inoculated in MRS broth and incubated (37 °C, 24h.). Biochemical tests, indole, methyl red, vogus–prosker, citrate utilization, gelatin liquefaction, H₂S production, starch hydrolysis, urease and catalase were performed. DNA of the isolates was extracted using an in-house optimized SDS proteinaseK DNA extraction method. The 16S rRNA sequencing was carried out at Macrogen-South Korea and sequence alignment was carried out using Basic Local Alignment Search Tool. Three isolates, *Enterococcus faecium* AUS0004, *Enterococcus durans* strain 98D and *Enterococcus faecium* strain LMG 11423 were identified. All strains were Gram positive, cocci, catalase negative and negative for citrate utilization test, indole test, starch hydrolysis test, H₂S production test and urease test. All strains were positive for methyl red test. This is the first report of isolation and biochemical characterization of *Enterococcus spp.* from fermented Indian barley. However, *Enterococcus faecium* has been used in probiotics combinations of food for over twenty years. Further investigations need to be conducted to assess the functional and safety of these potential probiotic isolates.

Keywords: Lactic acid bacteria, barley, fermentation