

**ISOLATION IDENTIFICATION AND CHARACTERIZATION OF
PROBIOTIC BACTERIA ASSOCIATED WITH FERMENTED FISH
PRODUCT**

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Abstract

Probiotics are live microorganisms that have beneficial effects on health of their host. Fish is an important source of nutrients which include essential proteins, unsaturated fatty acids, vitamins and minerals. The objective of this research is isolate, characterize (morphological and biochemical) and identify the viable probiotic bacteria in the fermented fish product. Serial dilutions were prepared up to 10^{-6} with sterilized saline from fermented sample. 0.1 ml from each dilution was spread on sterile de Man Rogosa and Sharpe agar (MRS agar) plates and incubate at 37 °C for 24 hrs. Nine isolates were purified by streaking the each separated colony on freshly prepared MRS agar. All the nine purified isolates were subjected to Gram staining, endospore staining and motility was investigated by hanging drop method. The morphological characteristics of isolates were noted by observing their texture, color, size, elevation margine and consistency. Bio chemical characterization (Indole test, Methyl red test, Voges proskauer's test, citrate utilization test, H₂S production test and Urease test) of the nine isolates were performed. Sugar fermentation pattern (Glucose, Lactose, Arabinose, Mannitol, Sorbitol, Maltose, Dextrose, Sucrose, Fructose) of nine isolates were studied by inoculating isolates to MRS broth which was contained phenol red pH indicator and respective sugar. Tolerance of nine isolates to different concentrations of salt (3 %, 6 % and 9 %), different temperatures (30 °C, 37 °C and 40 °C) and different pH values (2, 3 and 4). The molecular level characterization of isolates was investigated. DNA of the isolate was extracted using an in-house optimized SDS protinaseK 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 (BLAST). Among the nine isolates seven isolates were Gram positive and two isolates were gram negative. All the nine isolates were observed to be non spore foaming and non motile. All the nine isolates were negative for Indole test, Catalase test, H₂S production test and citrate utilization test. Nine isolates could ferment fructose sugar. All the isolates show optimum tolerance to 37 °C and 40 °C when an optimum tolerance pH was 3-4. At pH 2 the growth is weak. All of the isolates were showing the optimum growth at 6 % salt concentration. However they can tolerate 3 % and 9 % salt concentrations. By molecular level identification, the isolates were identified as *Pediococcus acidilactici* (AB680157.1), *Lactococcus lactis* (CP006766.1), *Lactococcus lactis* (KJ690920.1) and *Weissella paramesenteroides* (HQ009793.1). The isolates are commercially used

probiotic organisms. Therefore the fermented fish product may have functional properties.

Key words: Fermented fish, Probiotic bacteria