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ISOLATION AND GROWTH PATTERN STUDY OF PROSPECTIVE PROBIOTIC SPECIES OF PEDIOCOCCUS FROM FERMENTED FOODS

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Abstract: Members of Genus *Pediococcus* belong to Family Lactobacillaceae and are homolactic fermenters.These were isolated from various fermented foods of South Gujarat like Locha batter, Khaman batter and Jalebi batter. All isolates were subjected to morphological assay by Gram staining. Isolates were screened for acid production from sugar by zone of solubilization around colonies on Glucose Yeast Pepton agar with $CaCO_3$ after 24-48 hour of incubation. Selected isolates were subjected to various biochemical tests as per Bergey's Manual of Systematic Bacteriology. Isolates were tested for their capacity of producing extracellular hydrolytic enzymes like gelatinase, lipase, amylase and casienase. They were also studied for their antibiotic susceptibility profile using modified Kirby-Bauer's disc diffusion assay. Growth kinetics of selected isolates was studied in batch culture. Many isolates were identified as members of Genus Pediococcus by biochemical tests. Most isolates were not able to produce extracellular hydrolytic enzymes. Exponential phase of around 12 hours was reported on GYP medium.

INTRODUCTION

Probiotics have gained increasing attention due to several health benefits related to the human digestive and immune system .Lactic acid bacteria are major contribution in probiotic product.Lactic acid bacteria (LAB) are commonly used in foods as preservatives and texture, flavor and scent enhancers. The ability of these bacteria to produce different types of sugars and metabolites such as lactic acid , acetic acid , ethanol , diacetyl , acetone , exopolysaccharide by fermentation(Barbosa et al., 2017; Cotter et al., 2013; Gaspar et al., 2013;
Gudina et al., 2015; Mazzoli et al., 2014; Papagianni, 2012; Saad et al. 2013). LAB from the genera Lactobacillus, Bifidobacterium andPediococcus are commonly found in the mammalian gut microbiota, and some strains are classified as probiotic (WHO/WHO, 2002). The applicationof this bacterial group in the pharmaceutical and food industries has been increasing given that bacteriocin synthesis often occursin several LAB strains, resulting in the protection of fermentation products against spoilage and/or pathogenic bacteria.

Pediococcus is one of the important LAB which divides alternatively in two perpendicular directions in a single plane to form peculiar tetrads. They are characterized as Gram-positive,

catalase negative, non-motile, homofermentative (Singla et al. 2018). Pediococcus pentosaceus and P. acidilactici are among the two most widely occurring species in food and dairy environments (Banwo et al. 2013).
Growth curve analysis of probiotic LAB strain, Pediococcus is help to determine the different phase of bacteria mainly three are following: (1) lag phase (2) log phase (3) stationary phase. This growth curve analysis also give idea about time period at which becteriocin compound is produce and help in application part of Pediococcus strain used as probiotic product which are mixed with pre-biotic component like inulin (polysaccharide), some sugar component and ascorbic acid as protective agent .

Material and Methods

Isolation of bacteria: The tetrad-forming bacteria were isolated from the fermented foods obtained at the markets in south region of Gujarat like khaman batter, locha's batter and jalebi batter by agar plate culture after 3 to 5 days' incubation at 30°. The medium (GYP-CaCO3) was composed of 10.0 g, glucose; 10.0 g, yeast extract ;10.0 g, peptone; 10.0 g, CaC03; 12.0 g, agar; and 1,000 ml, distilled water; adjusted to pH 6.8. Acid-forming bacteria were detected by the formation of colonies with clear zone dissolving CaCO3. As colonies appeared they were stabbed in GYP-CaCO3 agar, and tetrad-forming bacteria were observed microscopically. Working cultures were maintained on GYP broth composed of 10.0 g, glucose; 10.0 g, yeast extract; 10.0 g, peptone, 10.0 g, and 1,000 ml, distilled water; adjusted to pH 6.8. The cultures were maintained by striking into GYP-CaCO3 agar slant plate/ Petriand preserved at 4°.

Identification methods:

1. **Morphological characteristics:-** Cell form, cell size, cell arrangement, and gram staining and negative staining

2**. Cultural characteristics:-**The colonial appearance was observed by the use of GYP agar plate after 1, 3, and 5 days' incubation at 30°. Turbidity and final pH of broth culture were observed by using GYP broth.

3**. Biochemical characteristics:-** The following biochemical characteristics were tested by the methods described in the reference: Nitrate reduction test , carbohydrate fermentation test , Reaction to litmus milk and gelatin liquefaction test , indol test , MR-test, V-P reaction, utilization of citrate on Simmon citrate agar, hydrogen sulfide test, uric acid and urea hydrolysis test , starch and casein hydrolysis was performed in different media like Nutrient sugar broth for carbohydrate fermentation test , GPB broth for MR and V-P test, 1% tryptone broth for indol test, 2% pepton for hydrogen sulfide test, PNB broth for nitrate reduction test, litmus milk medium for litmus milk test, starch agar plate for starch hydrolysis , skim milk agar for casein hydrolysis , tributyrene agar for lipase activity (R. whittenbury,1965) .

4. Microbial growth curve analysis:-

The probiotic potential of the LAB strain was previously isolated and characterized from south Gujarat local food locha, khaman and jalebi batter and identified as Pediococcus.
For growth curve analysis, the stock culture was inoculation (2% v/v) into GYP broth (100 ml) and incubation at 30 c. At regular 1 hr interval take the absorbance of the three flask at 600 nm until the stationary phase achieved .

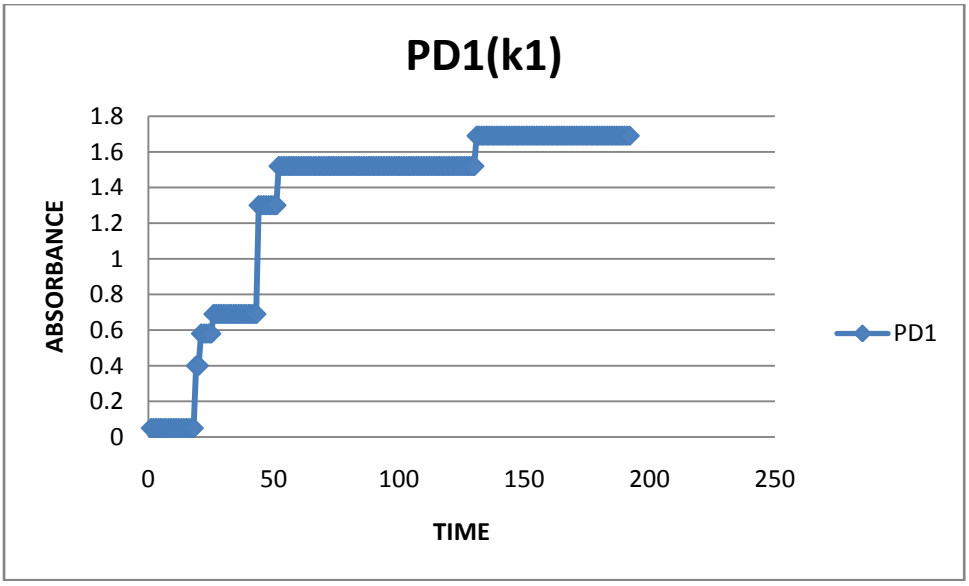
RESULT:-

TEST NAME	PD1 STRAIN	PD2 STRAIN	PD3 STRAIN
Carbohydrate fermentation test			
1. Glucose	+	-	-
2. Maltose	+	-	-
3. Sucrose	+	+	+
4. Lactose	-	+	-
5. Xylose	-	+	+
6. manitol	+	-	-
MR test	+	+	-
V-P test	-	-	-
SCT	-	-	-
1% Trypton	-	-	-
2% pepton	-	-	-
Nitrate reduction	-	-	-
Litmus milk test	+	+	+
UA test	+	+	+
Urea test	-	-	-
Gelatin liquefaction test	-	-	-
starch hydrolysis	-	-	-
Casein hydrolysis	-	-	-
Lipase	-	-	-

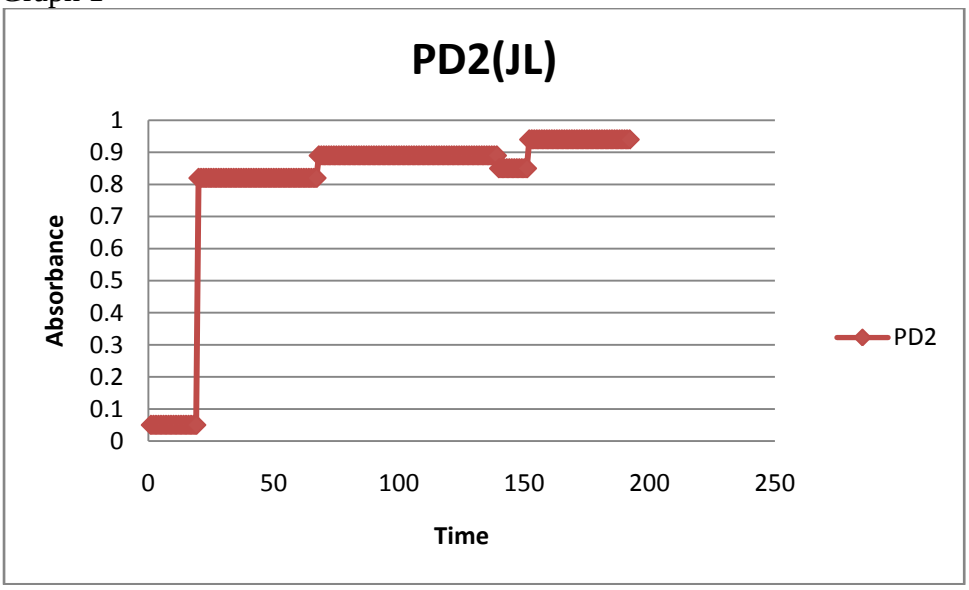
the tetrad forming bacteria was successfully isolated from south Gujarat local food like locha , khaman , and jalebi batter. The strains tested produced cocci forms and occurred in tetrads, in pairs, and rarely singly. Cell size was approximately 0.4 to 1.0 micro meter diameter. They were all gram positive, Whitish yellow, raised, and circular colonies appeared on GYP caco3agar plate after 24 -48 hours and clear zone of solubilization around colonies suggested that the colonies metabolize sugar and form lactic acid.

Identification and characterization of isolated strains done on the basis of Bergey's Manual of Systematic Bacteriology. In biochemical test , carbohydrate fermentation test PD1 strain give positive result in glucose maltose and manitol fermentation, PD2 strain metabolize lactose and form acid and give positive result, xylose was metabolized by strain PD2 and PD3, all 3 stain produce acid form sucrose as sugar source and the color change of media yellow to pink was observed . In MR test PD1 and PD2 give red color after adding MR reagent which means positive indication. V-P, indol, hydrogen sulfide, citrate agar test is negative for all three strains. Red color change in litmus milk liquefaction test was negative for all three strains and protease profile was negative for all three strains which include starch and casein hydrolysis and lipase test.

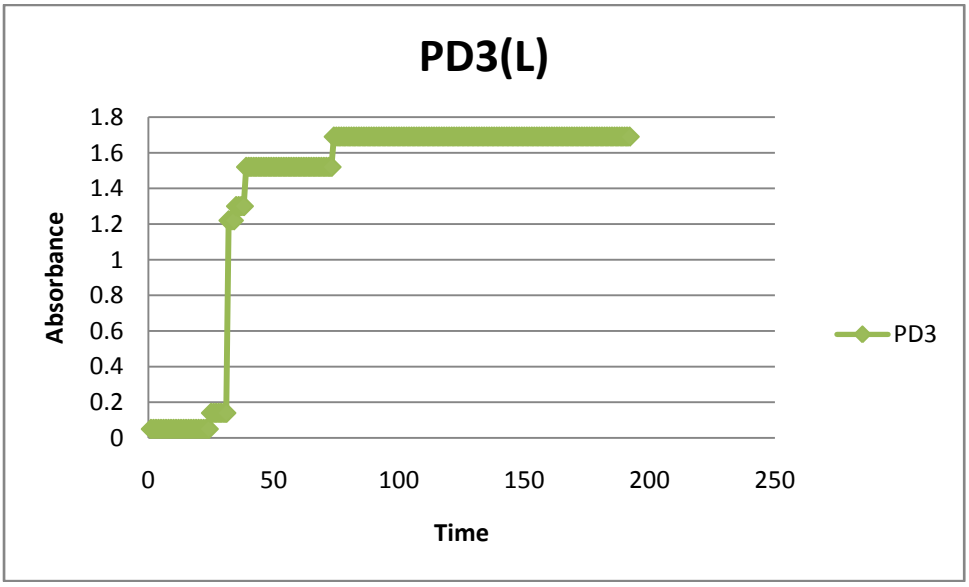
Growth curve of Pediococcus Determination of pre stationary viable cells of a bacterium is pre-requisite to its shelf life study. Growth curve study revealed that the Pediococcus reached to its pre stationary phase 12 hr after inoculation of 2% of active culture. By this process made growth curve of three different strains which are following:



Graph 1



Graph 2



Graph 3

Future prospective:-

After identification of three strains isolated from south Gujarat region by biochemical and morphological.In future , characterization will be done by 16s r-RNA test and also doing further production of cell free supernatant (CFS) from active culture of bacteria when they reached at their stationary phase which contain antimicrobial compound , exopolysaccharide and perform physical and biochemical characterization of cell free supernatant (CFS) by check different parameters like heat resistance ,pH sensitivity , salt tolerance capacity. after characterization of CFS then of then check the probiotic potential of Pediococcus species which are isolated from different source.

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