microbiology. In order to reduce the time for curd formation the isolated micro-organism was mutated. The nutrient agar plates swabbed with bacteria were exposed to UV-radiation in order to induce the mutation. The plates were exposed to UV-irradiation for 5, 10, 15 and 20 min. The plates with less time exposure to UV-irradiation showed more colony count than the plates with longer time exposure

3.5 Comparative Study of Time of Curd Formation for Pure and Mutated Sample:

Single colony from each of the time exposure points were selected for curd formation study along with original strain. The strain which was exposed to UV-radiation for 10 min had shown maximum reduction in time for curd formation (i.e-77.41%) in comparison to original strain (i.e. 42.85%) (Fig. 3).

This result indicates that, exposure of bacteria for 10 min to UV radiation could have induced mutations in genes encoding enzymes responsible for curd formation since reduction in curd formation time was observed. By mutagenesis, we could able to successfully induce mutations in original strain to reduce the curd formation time.

3.6 Isolation and identification

The mutated bacterium was subjected to various biochemical tests for its identification up to species level. The biochemical results indicated that the bacteria could be a Lactobacillus species

3.6.1 Indole test: Bacteria showed positive result. After addition of Kovacs reagent, a cherry red colour appears in the top. The microbe is able to oxidized tryptophan to indole, pyruvic acid and ammonia present on medium because it contain tryptophanase enzyme.

3.6.2 Methyl-red test: Bacteria showed positive results. After addition of MR reagent the colour changed to red. This organism is capable of producing organic acid and is detected by addition of methyl red, a pH indicator.

3.6.3 Voges-Proskauer test: Bacteria showed negative result. After the addition of Barrets reagent the solution didn't change to pink. This is because this organism produce neutral product, acetoin, and colour remain unchanged.

3.6.4 Citrate utilization test: The bacteria showed the positive test where the colour changed from green to blue in bromothymol blue. This indicates the utilization of citrate and conversion of citric acid to sodium citrate (alkaline pH).

3.6.5 Catalase test: Bacteria showed the negative result. Effervescence was not observed after the addition of the H2O2.This indicates that the bacteria do not possess the catalase enzyme that degrades H2O2 to water and oxygen.

3.6.6 Oxidase test: The bacterium is oxidase positive. When a loopful of test bacteria was added on the oxidase disc it turned to blue colour. This is due to the oxidase enzyme present in bacteria.The bacteria oxidized the N, N-tetramethyl-p-phenylenediamine present on the disc and gives the blue colour.

3.6.7 Starch hydrolysis: Bacteria showed positive result. The clear zone was observed after the addition of iodine. This indicates that the bacteria produce the enzyme alpha-amylase and use starch as a source of carbon and energy.

Figure 1: Bacteria showed the Positive Result.

3.6.8 Casein hydrolysis: Casein, major protein found in milk, it is hydrolyzed by proteinase (caseinase) enzyme. On the agar plate the clear zone indicate positive result

3.6.9 Gelatin test: Bacteria showed positive result. Due to the presence of gelatinase enzyme in the bacteria the tubes content remain liquid after cooling.

3.6.10 H2S production: Bacteria showed negative test because organism are unable to produce H2S gas utilizing the ingredient of the medium. Production of H2S is detected by H2S indicators (heavy metal salt) which combine with H2S produced by reduction of sodium thiosulphate and colour changes to black.

3.6.11 Urease test: Bacteria showed negative result because it lack enzyme urease and colour remain unchanged. This indicates no production of the ammonia and remains yellow colour.

3.6.12 Carbohydrate (sucrose) fermentation test: Sucrose showed positive test for acid production indicated by the change in color of phenol red to yellow with gas was observed in durham tube. This indicates that the Sucrose is fermented to produce the acid end product so the medium changes to yellow from red.

3.6.13 Lactose fermentation test: Lactose showed positive result for acid production and no gas production has taken place because bacteria ferment the lactose and color of the media changed.

3.6.14 Fructose fermentation test: Fructose showed positive test for acid production indicated by the change in colour of phenol red to yellow and gas formation was observed in durham tube. This indicates that bacteria fermented the fructose and colour of the media changed to yellow from red.

3.6.15 Maltose fermentation test: Maltose showed positive test for acid production indicated by the change in color of phenol red to yellow but no gas was observed in durham tube. This indicates that the bacteria ferment the maltose to produce acid end product.

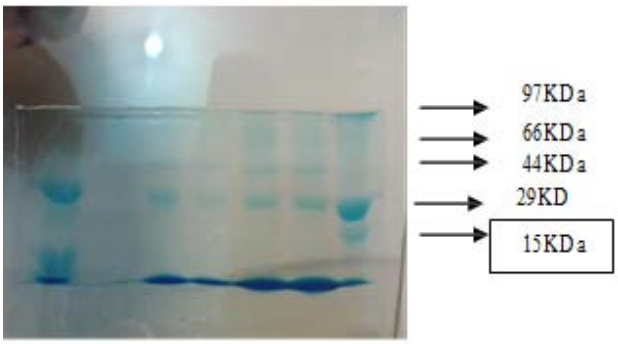


Figure 2: SDS-PAGE analysis of proteins

Marker all blue (Genei Banglore)
97KDa – Phospharylase B
66KDa – BSA
44KDa – Ovalbumin
29 KDa- Glutathione S-Transferase
15KDa - Lysozyme

3.7 RAPD

The same sample was used for PCR (RAPD). The smears reflects the amplification of protein bands, denser smear was seen for the mutated culture.

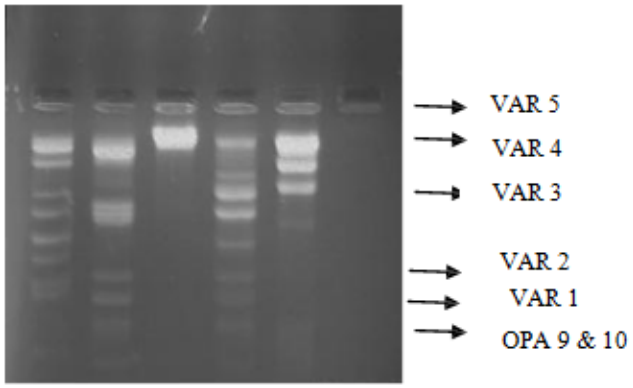
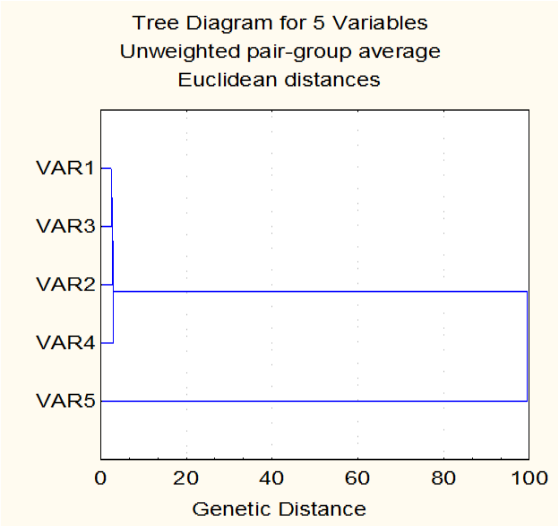


Figure 3: RAPD

VAR1- Pure culture
VAR2- 5 min mutated culture
VAR3- 10 min mutated culture
VAR4- 15 min mutated culture
VAR5- 20 min mutated culture



3.8 Antioxidant Activity

The DPPH and ABTS radical was scavenged by antioxidants by single electron and hydrogen atom transfer mechanism and transformed in to a yellow or colourless product. The scavenging activity of whey against these radicals was expressed as TE/L of whey. DDPH scavenging activity of the control milk lactobacillus acidophilus was found to be 0.28 mM of TE/L. The DPPH scavenging activity of mutated milk Lactobacillus acidophilus was found to be 0.71 mM of TE/L.

3.9 Enzyme Characterization

3.9.1Enzyme Purification

The enzyme from the mutated strain was isolated and was subjected to various purification steps such as salt precipitation, dialysis, and ion-exchange chromatography.

The amount of protein in different ellution buffer was determined by Lowry's method.

The protein content in the elution buffer of supernatant was estimated by Lowry's method

The sample with highest protein concentration was used for SDS Electrophoresis.

3.9.2 Protein Analysis by SDS-PAGE:

In order to test the presence of protein, SDS –PAGE was performed since the enzymes which are involved in curd formation (Fig. 2). The SDS-PAGE analysis indicated the presence of proteins which may also include the enzymes responsible for curd formation.

4. Future Prospective

Lactobacillus acidophilus has been reported to be beneficial probiotic organisms that provide excellent therapeutic benefits. The biological activity of probiotic bacteria is due in part to their ability to attach to enterocytes. This inhibits the binding of enteric pathogens by a process of competitive exclusion. Attachment of probiotic bacteria to cell surface receptors of enterocytes also initiates signalling events that result in the synthesis of cytokines. Probiotic bacteria also exert an influence on commensal micro-organisms by the production of lactic acid and bacteriocins. Lactobacillus strains can be good candidates as probiotic therapeutic agents for anti-inflammation.

Probiotic bacteria can modulate immune responses in the host gastrointestinal tract to promote health. The genomics era has provided novel opportunities for the discovery and characterization of bacterial probiotic effector molecules that elicit specific responses in the intestinal system. Nutrigenomic analyses of the response to probiotics have unravelled the signalling and immune response pathways which are modulated by probiotic bacteria. These approaches may lead to improved stratification of consumers and to subpopulation-level probiotic supplementation to maintain or improve health, or to reduce the risk of disease [21].

The crystal structure of tannase produced by *Lactobacillus plantarum*, as tannase catalyzes the hydrolysis of the galloyl ester bond of tannins to release gallic acid, although the enzyme is useful for various industries [22].

Some genetic features of *lactobacillar fructan* hydrolases were elucidated, information about their enzymology or mutational analyses were scarce. Lactobacillus casei IAM1045 exhibits extracellular activity degrading inulin. After partial purification of the inulin-degrading protein from the spent culture medium, several fragments were obtained by protease digestion. Based on their partial amino-acid sequences, oligonucleotide primers were designed, and its structural gene (levH1) was determined using the gene library constructed in the E. coli system.

The levH1 gene encoded a protein (designated as LevH1), of which calculated molecular mass and pI were 138.8-kDa and 4.66, respectively, that the variable domain and [beta]-sandwich module, besides the [beta]-propeller module, are important for inulin-degrading activity of LevH1 [23].

Major factors shaping codon and amino acid usage variation Lactobacillus sakei 23K were investigated. It included 13 other Lactobacillus species for a comparative analysis. Furthermore, 24 codons that were found to be optimally used by L. sakei and its comparative study with 13 Lactobacillus species might provide some useful information in their further study of molecular evolution and genetic engineering [24].

5. Conclusion

The bacteria form the curd more efficiently after improving the strain with the mutation. The bacteria isolated and identified was *Lactobacillus acidophillus* from the curd sample. The maximum reduction in time was obtained in 10 minute mutated sample of Lactobacillus acidophilus. Here, in this experiment increase in the activity of lactic acid after mutation decreases the time of curd formation. So, we can suggest that the mutated strain of *Lactobacillus acidophillus* can reduce the curd formation time more efficiently and the strain can be used commercially in the dairy industry.

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