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Research Article

STUDY OF UV MUTATED PROTEASE ENZYME FROM VARIOUS SOURCES

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ABSTRACT

Proteases have wide variety of functions and commercial applications. Protease is the third largest groups of industrial enzymes and is used in detergents production, leather industry, food industry, pharmaceutical industry and bioremediation processes. Protease and amylase are well known industrially important enzymes. To study the UV mutated protease enzyme from various sources. Microbes were isolated from curd, buccal cavity are probiotics were maintained under optimum conditions, the microbial growth and enzyme production carried out at 37 ˚C for at 24 hours and pH 7.0. The isolated microbes were mutated using UV radiation exposure which enhanced protease and amylase enzymes production. The protease enzyme was confirmed using protease and amylase assay. The isolated bacteria have the ability to produce the industrially valuable enzymes which have various industrial applications. The protease enzyme has wide applications in many industries and therefore the UV mutated enzyme will have more thermostability.

Keywords: amylase, protease, UV radiation, mutation

INTRODUCTION

Proteases have wide variety of functions and commercial applications[1].Protease is the third largest groups of industrial enzymes and is used in detergents production, leather industry, food industry, pharmaceutical industry and bioremediation processes[2,3]. They are classified into various groups such as alkaline protease, serine protease, cysteine protease, aspartic protease and metallo protease[4]. Currently13 billion tons of alkaline proteases find their applications in house hold laundry as detergents. Alkaline proteases were the first enzyme produced in bulk. Plant, animal and microbial sources are employed in there enzyme production. Microbial proteases are preferred to plant and animal sources to various advantages. A variety of microorganisms such as bacteria, fungi, yeast and Actinomycetes are known to produces enzymes[5]. Among all the microorganisms, *Bacillus* species produces a wide variety of extra-cellular enzymes like protease, amylase and lipase[6,3] e.g. *B. cereus, B. sterothermophilus, B. mojavensis, B. megaterium* and *B. Subtilis*.[7,8] Mutant B. cereus has the ability to sporulate in the presence of a high concentration of amino acids and glucose to produced excessive amounts of extracellular protease[9]. Amylases are extracellular enzymes that have the ability to hydrolyze the glycosidic linkages of starch and glycogen. Amylase enzyme were obtained from various sources, but amylases from fungi are the most important in biotechnology and food technology applications.[1] The present study was focused for the production of curd, buccal and probiotics isolated from Trichy Research Institute for Biotechnology at Trichy in Tamil Nadu.

MATERIALS AND METHODS

Collection of samples

The curd and probiotics sample were collected from Trichy Research Institute for Biotechnology at Tamil Nadu in India and the buccal cavity sample was collected from buccal cavity of a volunteer. The reagents and chemicals used for this project were taken from SRL India.

Isolation and Identification

Curd

A loop full of curd was taken and it was dissolved in 5 ml of Nutrient broth and incubated at 37 ˚C for 24 h with pH 6.8 in control incremental condition for enrichment. Subculture was prepared using spread plate technique[10].

Buccal cavity

A sample was swabbed from the buccal cavity of a human volunteer and it was dissolved in 5 ml of Nutrient broth and incubated at 37 ˚C for 24 h with pH 6.8 in control incremental condition for enrichment plate[11].

Probiotics

The pre stored probiotic sample was taken from *Bacillus cereus* and mixed with 5 ml of nutrient broth. After inoculation of the sample, the mixture was incubated at 37˚C for 24 h with pH 6.8. The nutrient agar was prepared and a loop full of sample was streaked in the plate by quadrant streaking method and incubated at 37 ºC for 24 h[12].

Biochemical activity analysis for microbes

Grams' staining was performed to identify grams reaction[13]. Motility test was carried out for the spontaneous movement of a cell from one location to another by consumption of energy. Morphology was used to determine the shape of the bacteria. Indole test was performed on bacterial species to determine the

ability of the organism to convert tryptophan into Indole by the deamination of tryptophan. Methyl red test was used to identify bacteria producing stable acid by mechanisms of mixed acid fermentation of glucose. The above biochemical activity tests were carried out for all selected microorganisms[7].

Mutant colony selection

The isolated pure culture was spread over the nutrient agar medium on the plate. The plate was separated into two portions; one half of the portion was covered with aluminum foil that protects microbes from the UV exposure. The plate was placed under UV exposure for different time periods of (10, 20, 30 min) and incubated at 37 ˚C for 24 h, mutant colony were used for future study[14].

Screening for proteolytic activity

Milk solids (w/v), 1.5% agar (w/v), 0.5% beef extract (w/v), 0.3% yeast extract (w/v) were incubated at 37°C for 24 h with pH6[15].

Protease production

The culture medium used in the protease production contained 0.5% glucose (w/v), 0.75% peptone (w/v), 0.5% (w/v) $MgSO_4.7H_2O$, 0.5% (w/v), KH_2PO_4 (w/v), and 0.01% (w/v) $FeSO_4.7H_2OS$ (w/v) maintained at 37°C for 24 h with pH 6.8 in a shaking incubator at (140 rpm). At the end of each fermentation period, the entire fermentation broth was centrifuged at 10,000 rpm at 4°C for 15 min and the clear supernatant was used for crude enzyme preparation[16].

SDS Page

The SDS page technique was carried out protocol[17]. The protein samples such as buccal cavity, curd and probiotic extracts were subjected to 10 % SDS PAGE with 200 KDa protein markers.

RESULTS

Isolation and identification

The bacteria isolated were identified based on their morphology, growth conditions, gram stain, motility and biochemical tests [Table 1].

The probiotic microbe isolated was characterized by the cocci shape, gram negative bacteria were positive for motility, Indole, methyl red similarly the buccal cavity 2 microbe isolated was characterized by rod shape gram negative bacteria which were positive for motility, Indole, methyl red. The Buccal cavity 1 microbe isolated was characterized by the rod shape, gram positive bacteria, presence of motility, methyl red and absence of Indole. The Curd 1 microbe isolated was characterized by rod shape, gram negative bacteria, presence of methyl red, Indole, and absence of motility. The Curd 2 microbe isolated was characterized by rod shape, gram positive bacteria, and presence of Indole and absence of motility, methyl red.

Mutant colony selection

After 24 h of incubation the mutant colony was observed in UV exposure portion of the plate. The Figure 1 showed more colonies in the UV unexposed portion of the plate. In Figure 4, UV exposed portion decreases with increasing time [Figure 1] [Figure 2] [Figure 3] [Figure 4].

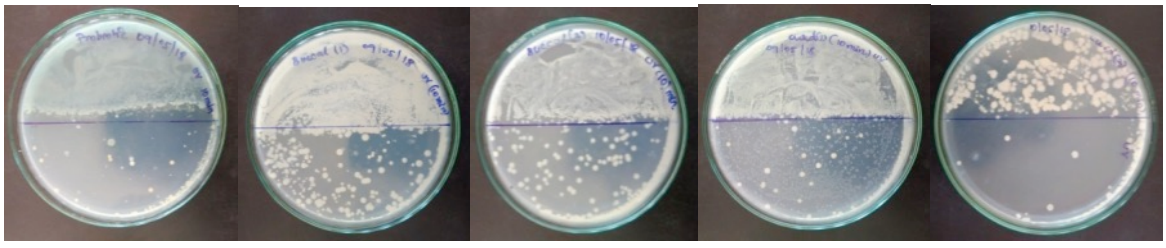
The microbes isolated from buccal cavity 2 was shown maximum UV exposure with stability similarly the microbes isolated from curd was 2 shown minimum UV exposure.

Screening for proteolytic activity

The zone formation represents the lysis of skim milk agar medium. A clear zone of skim hydrolysis indicates of protease producing organism *Bacillus cereus* [Figure 5].

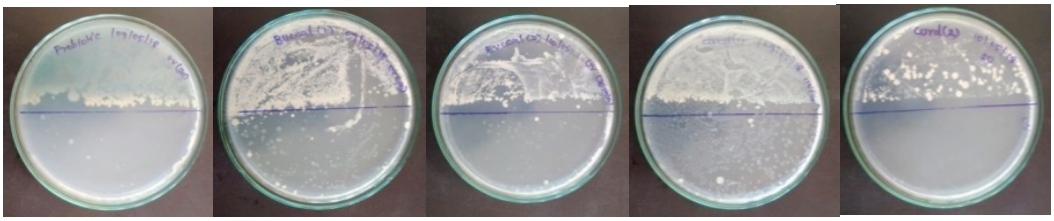
Table 1

S. No	Microorganism	Morphology	Gram Positive / Negative	Motility	Indole	Methyl red
1	Probiotic	Cocci	Gram negative	+	+	+
2	Buccal 1	Rod	Gram positive	+		+
3	Buccal 2	Rod	Gram positive	+	+	+
4	Curd 1	Rod	Gram negative	_	+	+
5	Curd 2	Rod	Gram positive	_	+	+



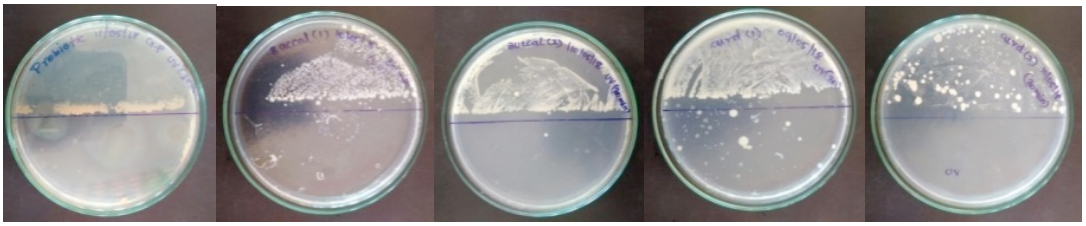
Probiotic Buccal -1 Buccal -2 Curd -1 Curd -2

Figure 1: Mutant colony selection at 10 min UV exposure



Probiotic Buccal -1 Buccal -2 Curd -1 Curd -2

Figure 2: Mutant colony selection at 20 min UV exposure



Probiotic Buccal -1 Buccal -2 Curd -1 Curd -2

Figure 3: Mutant colony selection at 30 min UV exposure

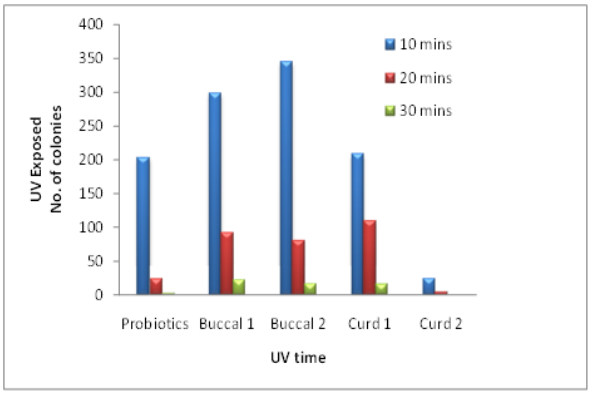


Figure 4: Effect of mutant colony selection under UV exposure

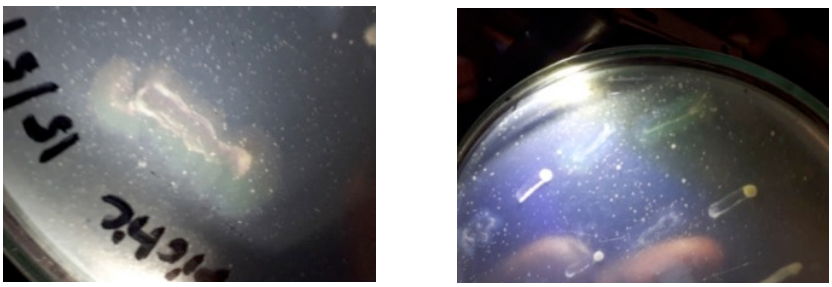
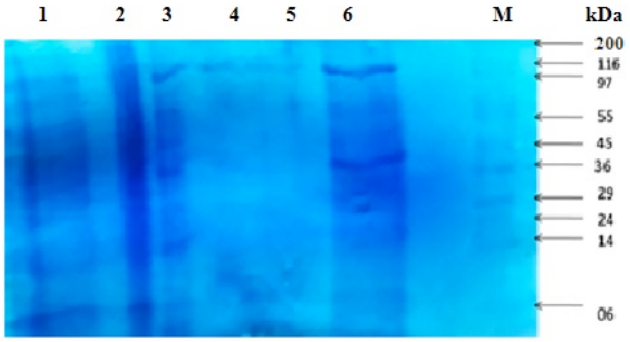


Figure 5: Screening for proteolytic activity of *Bacillus cereus*



1-Probiotic 1, **2**-Probiotic 2, **3**-Buccal 1,

4-Buccal 2, **5**-Buccal soup, **6**-probiotic soup

Figure 6: Conformation test for protease using SDS-PAGE

SDS-Page

The presence of protease enzyme was observed only in probiotic supernatant at 45 kDa compare to curd and buccal. Furthermore, from the present study the isolated microbes in probiotic show their presence on SDS-Page [Figure 6].

DISCUSSION

The probiotic bacteria have beneficial effects due to factors includes regulation of intestinal microbial homeostasis, changes in the availability of nutrients bring and modulation of local and systemic immune response[18].

The previous study reported that protease enzyme from probiotics showed a molecular weight of 37 kDa[19]. A study conducted in probiotics showed a molecular weight of 30 kDa[20].

A study conducted in probiotics showed the three predominant protein molecules from the SDS-Page of Hi Trap ion exchange[21]. The loss of non-protein compound of protease enzymes seen with weight of 34 kDa, 17 kDa, 13 kDa. The protease enzyme was observed in 45th band.

CONCLUSION

The identified *Bacillus cereus* has the ability to produce protease enzyme. Protease enzyme shows higher activity at UV mutated conditions. The confirmation of protease enzyme was identified by SDS-Page. In industry, the protease enzyme plays a vital role in the production of detergents, leather industry, food industry, pharmaceutical industry and bio remediation processes.

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