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Original Research Article

Microbiology for Medical care

Isolation And Identification Of Amylase, Protease And Lipase Producing Bacteria From Soil Of Different Area Of Gujarat

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Abstract: Enzymes are being used in numerous new applications in the food, feed, agriculture, paper, leather, and textiles industries, resulting in significant cost reductions. At the same time, rapid technological developments are now stimulating the chemistry and pharma industries to embrace enzyme technology, a trend strengthened by concerns regarding health, energy, raw materials, and the environment , hence are reusable. For these present study different samples of soil were collected from Banaskantha (Bhabhar), Rajkot (Safar), Ahmedabad (Shilej), Surat (Chikhali), Sabarkatha (Palanpur) Gujarat India. The samples were serially diluted and inoculated on Nutrient agar by spread plate technique. The isolated bacteria were morphological and biochemical characterized and Gram's staining was performed using Gram's reagents (Hi-Media kit) and studied its metabolic activities by performing different biochemical tests. Bacteria were identified for amylase, protease and lipase activities using starch agar-1%, casein agar-1% and tributyrin agar-1% respectively. Isolate Strains could be compared with the previously found strains Bacillus cereus ATCC 1305, 5610, 1458 for the optimum production of amylase, protease and lipase.

Keywords: lipolytic, extracellular, inducible, constitutive.

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1. INTRODUCTION

Enzymes are biological catalysts, which initiate and accelerate thousands of biochemical reactions in living cells.[1,2] Certain enzymes are of special interest and are utilized as organic catalysts in numerous processes on an industrial scale. Special characteristics of microbial enzymes include their capability and appreciable activity under abnormal conditions, mainly of temperature and pH. Hence, certain microbial enzymes are categorized as thermophilic, acidophilic or alkalophilic. Low stability, high cost, and difficulty in reuse and recovery of free enzymes are the industrial application limits.[3] The immobilizing of enzymes can successfully resolve these difficulties.[4,5] Major sources of enzymes are - plants, animals and microorganisms (bacteria and fungi) of these sources, the microbial enzymes account for the major volume. [6,7,8] However, only less than 50 species are used to produce all of the microbial enzymes.[9,10] Enzymes produced by microorganisms are either inducible or constitutive[11]. The constitutive enzymes is always produced by the cell regardless of whether its substrate is present in the medium or not while the inducible enzymes are produced in response to the presence of its substrate[12,13]. *Bacillus* RV.B2.90 was found to be capable of producing an enzyme preparation possessing special characteristics such as being highly alkalophilic, moderately halophilic, thermophilic, and exhibiting the quality of a thermostable protease enzyme. Alkaline proteases possess the property of a great stability in their enzyme activity when used in detergents.[14,15]The alkaline protease produced from *Bacilli* and proteases from other microorganisms have found more applications overall in bio-industries such as: washing powders, tannery, food-industry, leather processing, pharmaceuticals, for studies in molecular biology and in peptide synthesis. [17,18] Our aim is to screen and study amylase, protease and lipase producing bacteria taken into consideration with aim using specific media. Our objective is to identify bacteria by performing gram's staining, to study morphologically and biochemical characteristics of selected isolates and to compare isolated with previously found strains Bacillus cereus ATCC 1305, 5610 and 1458 for the optimum production of amylase, protease and lipase.

2. MATERIALS AND METHODS

2.1 Sample Collection

Fig 1. Sites for sample collection

Soil samples were collected from Banaskantha (Bhabhar 24^{0}04'13.00"N, 71^{0}35'56.74"E), Rajkot (Safar 22^{0}17'59.02"N, 70^{0}45'41.14"E), Ahmedabad (Shilej 23^{0}01'38.85"N, 72^{0}29'12.94"E), Surat (Chikhali 21^{0}12'25.10"N, 73^{0}06'44.87"E), Sabarkatha (Palanpur 23^{0}29'01.07"N, 73^{0}17'16.66"E) Gujarat India (Fig 1). Samples were collected from 10cm depth from soil surface in a sterile polythene bag with a sterile spatula. Sample was stored at 4^{0}C until used.[12]

2.2 Enrichment and Isolation of bacteria

Enrichment of bacteria was done using different media such as nutrient broth. For isolation of bacteria, a loopfull culture from soil agar (Hi-media) as streaked on respective agar media by four sector method[2,15]. Isolates were preserved in respective medium at 4°C for further study.[7,11]

2.3 Morphological and Biochemical characterization

Isolates were purified by four sector method. Morphologically characterized by Gram's staining, Capsules staining by Hiss method and Colony is characterized by color, form, size, shape etc. and biochemical tests were done for the identification of bacteria. [12]

2.4 Screening of amylase producing bacteria

Amylase producers were screened on starch agar containing: Starch agar-30g/L, Agar-3%, PH- 7.2. After incubation for 2-3 days at 37°C, iodine solution (g/100ml: Iodine-0.33, KI- 0.66) was added. Clear zone surrounding the colonies against blue background showed amylolytic activity. [9]

2.5 Screening of lipase producing bacteria

Lipase producers were screened on tributyrin agar containing: tributyrin agar base- 23g/L, tributyrin oil - 10ml/L, Agar– 30g/L and pH- 7.2. Colonies showing clear zone at 37°C after 2-3 days was taken as evidence of lipolytic activity.[19]

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2.6 *Screening of protease producing bacteria*

Isolates were screened on casein agar containing: Casein-10g/L, Meat extract-5g/L, Agar- 30g/L and pH-7.2. Protease activity was measured by adding Frazier's reagent at 37°C after 2-3 days Clear zone surrounding the colonies showed proteolytic activity. [10]

2.7 *Optimization of growth conditions*

Bacterial isolates were evaluated at various pH (5-12), Nacl (1%-5%) temperature (25°C to 40°C) to find out the optimum growth conditions. Bacterial biomass in broth medium was measured after 24-48hrs absorption at 600 nm wavelength by using spectrophotometer[13]

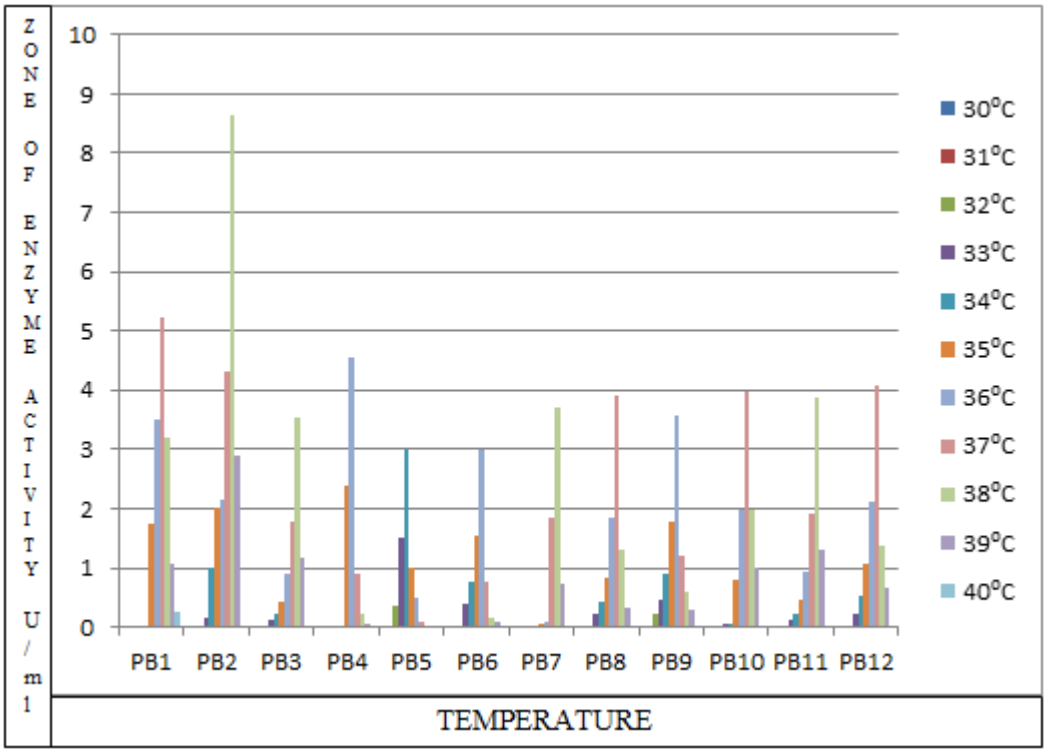

Fig 2. Effect of temperature on enzyme activity.

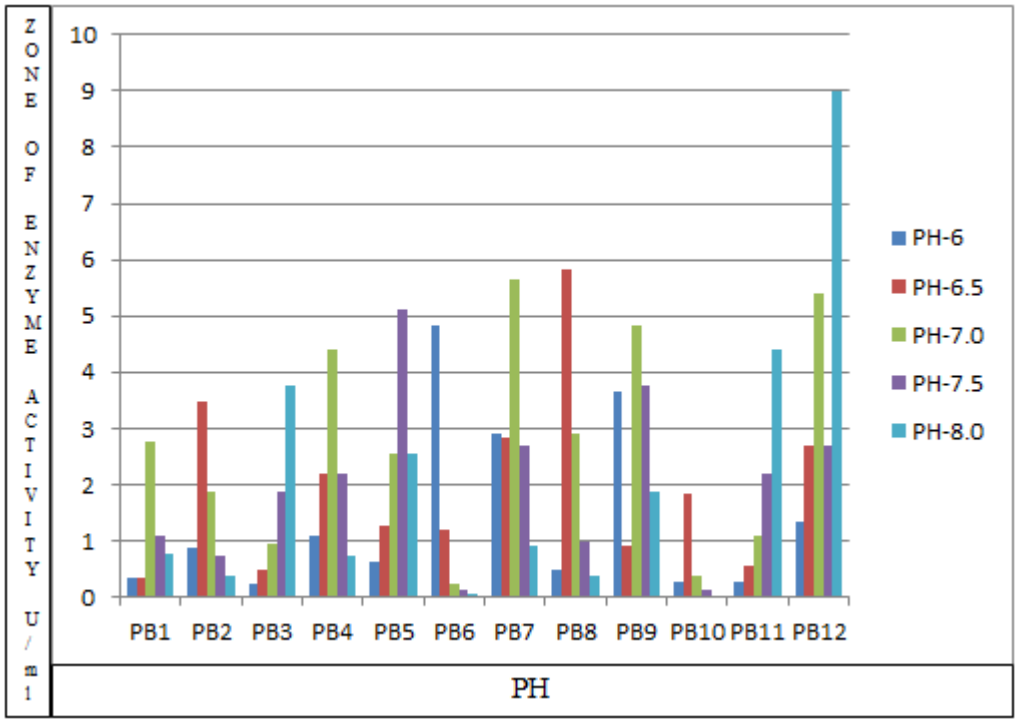

Fig 3. Effect of PH on enzyme activity.

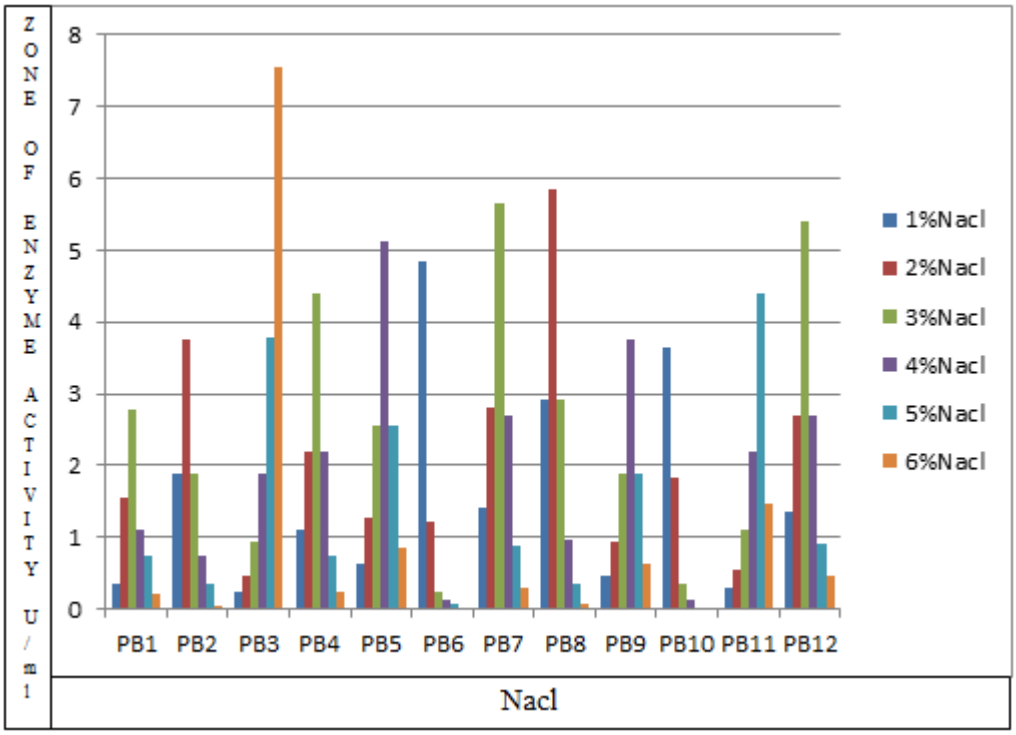

Fig 4. Effect of Nacl on enzyme activity.

3. RESULTS AND DISCUSSION

3.1 Enrichment and Isolation

From Soil samples total 12 isolates were obtained, they are designated as PB-1 to PB-12. They were able to grow at Nutrient agar at 37°C for 24 hrs.

3.2 Characterization of Isolates

3.2.1 Colony characterization

Colonies of isolates were characterized for features such as size, shape, surface texture, odor, pigment production and observed results are summarized in. Table No 1

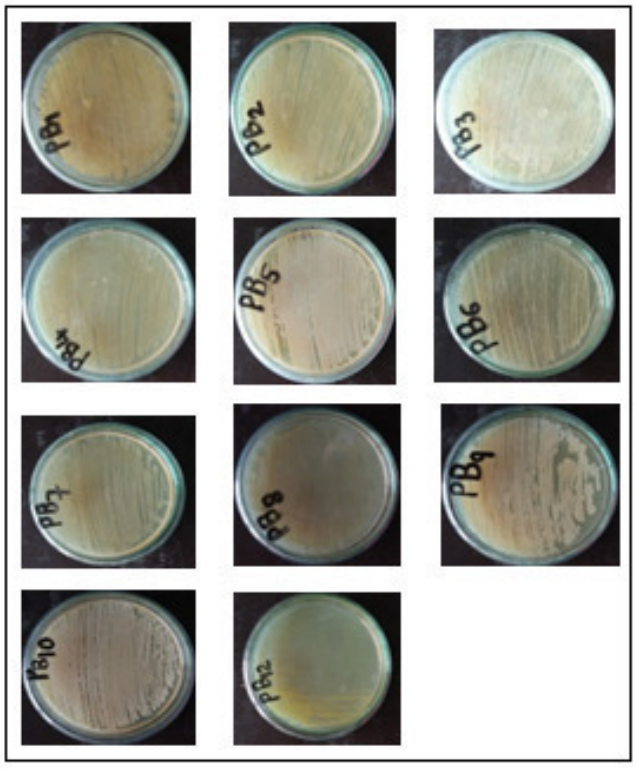

Fig 5. Isolated bacterial colony

3.3 Colony Characterization of Isolates

Table 1. Colony characteristics of isolates

Isolates	Size	Shape	Margin	Elevation	Surface Texture	Consistency	Opacity	Pigmentation
PB1	Small	Round	Entire	Convex	Smooth	Moist	Translucent	Cream
PB2	Small	Round	Entire	Flat	Smooth	Moist	Translucent	Cream
PB3	Medium	Round	Entire	Flat	Smooth	Moist-	Translucent	Creamy white
PB4	Small	Round	Entire	Flat	Smooth	Moist	Transparent	Cream
PB5	Large	Round	Entire	Flat	Smooth	Moist	Translucent	Cream
PB6	Puntiform	Round	Entire	Convex	Bullet	Moist	Translucent	Light pink
PB7	Puntiform	Round	Entire	Convex	Bullet	Moist	Translucent	Cream
PB8	Large	Round	Entire	Convex	Smooth	Moist	Transparent	Light cream
PB9	Puntiform	Round	Irregular	Flat	Bullet	Moist	Translucent	Off white
PB10	Large	Round	Entire	Flat	Bullet	Moist	Translucent	White
PB11	Large	Round	Entire	Convex	Smooth	Moist	Transparent	Cream
PB12	Puntiform	Round	Entire	Convex	Smooth	Moist	Transparent	Yellow

3.4 Morphological characterization

The isolates were morphologically characterized and results are shown in Table No. 2. The results show that out of 12 isolates, 2 were Gram positive and 10 were Gram negative by using Gram staining method while in capsule staining not a single bacterium was found to have capsule.

3.5 Morphological Characterization of Isolates

Table 2. Morphology characteristics of isolates

Bacteria	Size	Shape	Arrangement	Gram reaction	Capsule staining
PB1	Big	Big rod	Single, pair, in chain	Gram Negative	Negative
PB2	Big	Big rod	Single, pair, in chain	Gram Negative	Positive
PB3	Small	Short rod	Single, pair, in cluster	Gram Positive	Negative
PB4	Small	Short rod	Single, pair, in cluster	Gram Negative	Positive
PB5	Small	Short rod	Single, double	Gram Negative	Negative
PB6	Small	Short rod	Single, pair	Gram Negative	Negative
PB7	Small	Short rod	Single, pair, in group	Gram Negative	Positive
PB8	Small	Short rod	Single, pair, in group	Gram Positive	Negative
PB9	Big	Short rod	Single, pair, in cluster	Gram Negative	Positive
PB10	Big	Short rod	Single, pair	Gram Negative	Negative
PB11	Small	Short rod	Single	Gram Positive	Negative
PB12	Small	Short rod	Single, pair, in group	Gram Negative	Positive

3.6 Biochemical Characterization

Isolates were identified by performing various biochemical tests such as MR–VP test, Urea hydrolysis, H_2S production test while sugar fermentation with acid and gas was positive for almost all isolates using Bergey's Manual of Systemic Bacteriology and results are tabulated in Table No 3.

3.7 Biochemical Characterization of Isolates

Table 3. Biochemical characteristics of isolates

Sr.no	Name of test	PB1	PB2	PB3	PB4	PB5	PB6	PB7	PB8	PB9	PB10	PB11	PB12
1	Methyl red test	-	-	-	-	-	-	-	-	-	-	-	-
2	Voges – Prauskar test	-	-	-	-	-	-	-	-	-	-	-	-
3	Indole production test	-	-	-	-	-	-	+	-	-	+	-	-
4	H_2S gas production test	-	-	-	-	-	-	-	-	-	-	-	
5	Nitrate Reduction test	+	+	-	-	-	-	-	+	-	-	-	+
6	Urea hydrolysis test	-	-	-	-	-	-	-	-	-	-	-	-
7	Phenyl alanine de amines	-	-	-	-	-	-	-	-	-	-	-	-
8	Starch hydrolysis test	-	-	+	-	+	+	-	-	-	+	-	-
9	Fermentation test												
10	Glucose	+	+	+	+	+	+	+	+	+	+	+	+
11	Mannitol	-	-	-	-	-	-	-	-	-	-	-	-
12	Fructose	-	+	+	+	-	-	+	-	-	-	-	-
13	Maltose	-	-	+	+	-	-	-	-	-	-	-	-
14	Sucrose	-	+	+	+	-	-	-	+	-	+	-	-

(+) indicates positive results (–) indicates negative results

3.8 Optimization of Growth of Isolates

It was observed that almost all isolates showed optimum growth between pH6.5 -7.5 while in case of temperature, 36°C to 38 °C was found as optimum for best growth of Isolates. All isolates were screened for production of various enzymes and results obtained are shown in Figure–2. From the 12 isolates, total 6 isolates produced amylase, 5 were showing lipase activity and 1 was capable of producing protease. From these PB-1, PB-2, PB-6, PB-9, PB-10, PB-11 give positive result for amylase activity but PB-2 showed highest zone of hydrolysis for amylase activity and for lipase PB-1, PB-3, PB-5, PB7, PB10, give positive result for lipase activity but PB-10 shows high zone of hydrolysis of lipase activity and for protease PB-12 gives highest zone of hydrolysis of protease activity, while some isolates are multienzymes producers, PB-1 amylase and lipase producers, PB-5 amylase and lipase producers, PB-6 amylase and protease producers, PB-10 amylase and lipase producers.

3.9 Results of amylase producers

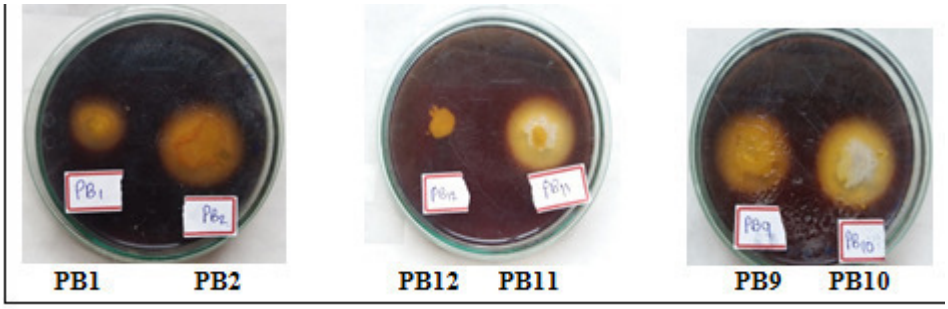

Fig 6. Amylase producers

3.10 Results of lipase producers

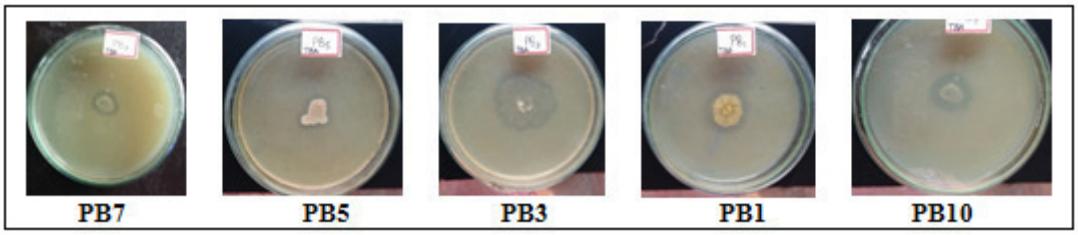

Fig 7. Lipase producers

3.11 Results of protease producers

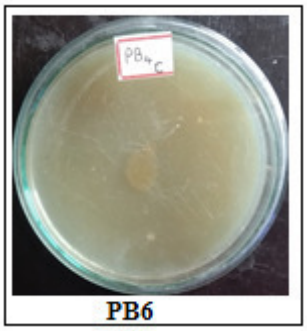

Fig 8. Protease producers

We have isolated cultivable bacteria from Banaskantha (Bhabhar), Rajkot (Saphar), Ahmedabad (Shilej) and Surat (Chikhali), Sabarkatha (Palanpur) Gujarat India shown in (Fig-5). The isolates were diversified in morphological, biochemical characteristics (Tab-2,3) as well as enzyme producing capacity. [6,20] This diversity based study of soil isolates is a vast research area with enormous potential to be exploited and manipulated for agriculture benefits. [9,15,26] The main objective is to study amylase, protease and lipase that are widely used in industrial applications from the isolates shown in (Fig-6,7,8). Graphs show enzyme activity which is affected by different physical parameter likes temperature, PH and Nacl (shown in Fig –2,3,4). PB10 and PB11 have highly amylase activity observed by zone of hydrolysis on starch agar plate. They are able to grow on 5%NaCl but PH requirement are different. 6.5PH is optimum for PB10 and 8PH is optimum for PB11 for enzyme activity. Both required 37°C for optimum enzyme activity. These results of amylase are compared with amylase producer Bacillus Cereus ATCC 11778. [2,29] PB10 and PB11 gave maximum activity of amylase production compare with the result of standard result of Bacillus Cereus ATCC 11778. An extracellular α-amylase

was purified from Thalassobacillus sp. one of the rich sources of microorganisms. In this starch degrading microorganism isolated sources plant. Biologically active enzymes may be extracted from any living organism. A very wide range of sources were used for commercial enzyme production from Actinoplanes to Zymomonas, from spinach to snake venom. Of the hundred or so enzymes being used industrially, over a half is from fungi and yeast and over a third are from bacteria with the remainder divided between animal (8%) and plant (4%) sources. [10] The present investigation dealt with isolation of a bacterial strain, from soil samples collected from college campus Gujarat, India and physiological and biochemical features were determined. Its amylolytic activity under different physiological conditions was correlated with the growth of three isolates. In the present study, we report the isolation and characterization of bacteria. [14,21] LY18. [9,15,25,] PB10 have high ability for protease activity and this activity observed by zone of hydrolysis on casein agar medium. PB10 has ability to produced enzyme with 5%NaCl, 6.5PH at 37°C and this result is compared with Bacillus Cereus ATCC 9634. On the other hand, the extremely halophilic bacterium, Salicola marasensis sp. IC10 showing lipase and protease activities, was studied for its ability to produce promising enzymes in terms of its resistance to temperature and salinity. [14,16,24] PB6 has high ability of lipase activity and this activity was observed on Tributyrin agar medium. PB6 has capacity to produced enzyme at 39°C, 1%NaCl at 6PH. This result is compared with Bacillus Cereus ATCC 6633. The intracellular lipolytic enzyme LipBL, produced by the moderately halophilic bacterium Marinobacter lipolyticus, showed advantages over other lipases, being an enzyme active over a wide range of pH values and temperatures.[4,10,13] The importance of soil enzymes in soil biogeochemical processes, soil productivity, pharmaceutical industries, leather industries and agriculture productivity is well. [29,30] The information will also help to design coping mechanisms to maintain soil enzyme activity to degrade various material under climate change conditions.

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5. CONCLUSION

In the present study, the bacteria were isolated from different area of sample. This area also consist of starch, protein and lipid materials we found starch, protein and lipid degrading bacteria have capacity to grow in different physical condition. PB8 isolate enzyme is used in detergent industry because they have optimum PH8 for enzyme activity. PB10 and PB11 gave highly amylase activity so they are used in fermentation industries.

6. CONFLICT OF INTEREST

Conflict of interest declared none

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