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Enzymatic Activity of Marine Lactobacillus Species from South East Coast of India.

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ABSTRACT

To study the impact on enzymic activity of five marine *Lactobacillus* species isolated from modified MRS medium was determined. *Lactobacillus* strains were grown in MRS medium at 37ºC for 16 h. At the end of incubation, bacterial population and production of enzymic in different pH and duration was determined by plating and enzymatic activity was determined spectrophotometricaly using the corresponding substrate. Marine *Lactobacillus* strains continue to enzyme activity of the strains chosen for study with respect to different pH ranges. Strain LA1 showed maximum activity at pH 9 (0.178) followed by strain LA2 at pH 10(0.157). Moderate activity was shown by strains LA3 and LA4 and least activity was shown by strain LA5. Lactobacillus (LA5) showed the highest enzymic activity 4.098, followed by strains LA4 (3.980) and strain LA2 (3.912) at 72 hours respectively.

INTRODUCTION

Microbial enzymes have several advantages over enzymes derived from plant or animal sources by virtue of their variety of catalytic activities, cheaper in cost, regular abundant supplies at even quantity and relatively more stable. Major targets of modern enzyme technology continue to be preservation of food and food components, efficient use of raw materials, improvement of food quality such as texture and taste, manufacture of dietic foods, eliminating anti nutritive substances from certain nutritional raw materials, utilization of raw materials for preparation of animal feed, and optimization process to reduce product costs. Enzymes are used as cost-effective and environmentally effective substitutes for chemical processing in several industries including pharmaceuticals, food, starch, laundry, detergents, for processing textiles, leather, wood pulp and paper, and for the production of fine and speciality chemicals and industrial catalysis organic synthesis and transformation of compounds and bioremediation. Several industrial applications demand that industrial enzymes must be stable at extremes of temperature,

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pH, salt concentration. For this reason, the isolation and characterization of enzymes from microorganisms known as extremophiles may yield useful enzymes for this purpose. Marine environment, which encompasses about 71% of the earth's surface, is not only rich with biodiversity but also a vast resource of potential microorganisms of useful application. Almost all of the microbes of the marine environment as potential sources of enzymes remains unexplored. Enzymes catalyze not only biochemical reactions in living cells but also mineralization and cycling of various elements in various environments. Hence every microorganism should be a potential source of various enzymes. Marine bacteria produce an enormous amount of enzymes. With the birth of biotechnology, enzyme engineering and other innovative technologies there is plenty of scope for efficient management of our rich marine microbial biodiversity towards deriving novel enzymes from marine microbes and efficiently exploited not only as cost effective biocatalyst but also as an ecofriendly reagent in the coming years. Various microbial enzymes are produced by high yielding Lactobacillus *sp*. Furthermore various studies performed on Lactobacillus sp show that nutritional factors, physical factors such as inoculums concentration (Kaur et al., 1998), temperature, pH (Tobe et al., 2005) and incubation time (Yossan et al., 2006) can significantly enhance enzyme production. Hence in the present study an attempt has been made to study the enzymes produced by *Lactobacillus sp.*

MATERIALS AND METHODS

Thirty five marine bacteria were isolated from the Pichavaram mangrove region. All the isolates were cultured on *Lactobacillus* (LA) in MRS Agar plates. Only those isolates which show clear zone on the plates, were cultivated in liquid production medium to test their ability to produce enzymes.

Production of cellulase

Enzyme production was carried out in the production media containing (g/l) CMC (20), peptone (20), NaCl (1), CaCl2 (0.005), MgSO4 (0.82), K2HPO4 (1.25), KH2PO4 (3), FeSO4 (0.01), ZnSO4 (0.005), MnCl2 (0.0001), and NH4Cl (1) (Wei et al. 2011). 10 ml of bacterial inoculum was added into 500 ml production medium and the flask was kept in a rotary shaker incubator at room temperature for 24 h. After incubation, the fermented broth was centrifuged at 10,000 rpm for 10 min in a cooling centrifuge. The supernatant was collected and subsequently used for the estimation of cellulase.

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Enzyme assay for cellulose

The enzyme activity of cellulase was studied according to the Denison and Koehn's method (1977). One ml of 1 % CMC was added to 1 ml of cellulase and the tubes were incubated at 55 C for 15 min in a water bath. Two ml of dinitrosalicyclic acid (DNS) was added so as to stop the reaction and the tubes were subsequently kept in a boiling water bath for 5 min. Thereafter, 1 ml of sodium potassium tartrate was added. Absorbance was recorded at 540 nm and after cooling the tubes.

RESULTS AND DISCUSSION

The temperature and pH were maintained at different time intervals and the corresponding enzyme activity of the five different strains were determined.

Table 1 shows the activity of the five different strains with respect to time. Among the five different strains chosen for study strain LA5 showed maximum activity at 48 hours (4.098) followed by strains LA4(3.980) and strain LA2(3.912) at 72 hours. Moderate activity was found in strain LA3.

Table 2 shows the enzyme activity of the strains chosen for study with respect to different pH ranges. Strain LA1 showed maximum activity at pH 9 (0.178) followed by strain LA2 at pH 10(0.157). Moderate activity was shown by strains LA3 and LA4 and least activity was shown by strain **LA**5. These results are in agreement with other studies indicating that the ability of *Lactobacillus* strains to produce α- or β-glucosidases is a strain dependent [Mahajan *et al.,* 2010; Otieno *et al.,* 2005; Pyo *et al.,* 2005 and Di Cagno et al., 2005]. On the other hand, *L. reuteri and L. delbruecki subsp. bulgaricus* SR35 should be given more attention with regard to having the highest α-glucosidase and β-glucosidase activity respectively. *L. delbruecki* was also reported in another study to have higher β-glucosidase activity compared to other lactobacilli species [Choi, 2002]. The high production of α-glucosidase by *L. reuteri* could be of special interest in probiotic applications since these strains were also reported to produce high quanties of α-galactosidase and β-galactosidase [Alazeh *et al.,* 2009]. These results clearly show the potential of the *Lactobacillus sp*. as sources of enzymes.

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Table 1: Shows the activity of *Lactobacillus sp.* with respect to time.

Time in hrs	LA1	LA2	LA3	LA4	LA5
2	0.015	0.080	0.004	0.088	0.098
4	0.128	0.195	0.026	0.124	0.890
6	0.279	0.292	0.081	0.199	1.238
8	0.353	0.385	0.142	0.285	1.983
10	0.461	0.509	0.191	0.300	2.546
12	0.507	0.964	1.009	0.589	2.999
24	1.236	1.702	1.498	1.898	3.456
48	2.043	3.067	1.973	2.387	4.098
72	3.912	0.934	2.507	3.980	3.245
96	1.051	0.356	2.003	2.004	2.980

Table 2: Shows the activity of *Lactobacillus sp.* with respect to *pH*.

pH	LA1	LA2	LA3	LA4	LA5
7	0.008	0.020	0.013	0.010	0.003
8	0.019	0.061	0.045	0.064	0.009
9	0.178	0.095	0.079	0.053	0.011
10	0.093	0.157	0.031	0.041	0.020
11	0.030	0.073	0.019	0.028	0.016

CONCLUSION

Cellulosic biomass is the most abundant and ubiquitous polysaccharide on earth. The ability of cellulases to saccharify lignocellulosic material makes them a valuable commodity in industry, agriculture and biofuels. Current processes of raw biomass degradation and fermentation to produce biofuels are far from being economically stable. Highly specific and stable biomass degrading enzymes are the need of today. Mangrove forests are untrapped resources of various enzymes. Hence the present study was made to find out the potential of *Lactobcillus* sp. to produce cellulose. The studies can further be extended by large scales production using different parameters which would minise the problems of high production cost and economy. Further studies would be undertaken in studying the genes responsible for the degradation of cellulose.

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