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Research Article

APPLICATION OF ENZYME EXTRACTS OF BACTERIAL PECTINASE AND MANNANASE IN FABRIC STAIN REMOVAL

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ABSTRACT

Enzymes are capable of degradation of many substrates and can be used in many industrial applications. Degradative enzymes from bacterial origin are numerous and are employed in many industrial applications commercially. Detergent formulations need enzymes that can degrade stains containing substrates like protein, pectin, sticky gum, etc. Present study describes the application of pectinase (Pectin degrading enzymes) and mannanase (mannans or gum degrading enzymes) enzyme purified from bacterial species of hot spring origin. Enzymes purified to homogeneity from bacterial grown culture and found to have activity 79.37 U/ml of mannanase and 299 U/ml of pectinase. Both enzymes were highly stable in tested inactivated commercial detergent solution retaining considerable catalytic activity. Their cleaning action was effective in getting rid of stains with and without detergents. Morphological and cultural studies have identified the mannanase producing bacteria as *Bacillus licheniformis* and pectinase producing species belonging to *Anoxybacillus* genus.

Keywords: Pectinase, Mannanase, Detergent.

1. INTRODUCTION

Industrial applications are heavily dependent on different types of catalysts to increase the rate of reactions leading to product formation. Although these reactions were mostly carried out by chemical catalysts, in recent times several of those processes are carried out with enzymes of biological origin. However, to use enzymes as biocatalysts one needs to develop green industrial processes. It requires robust and efficient enzymes which resist the various harsh conditions under which industrial processes take place, including temperature stress, acidic or basic pH, presence of denaturing agents and solvents. This is one of the challenging aspects of developing biocatalyst for industrial processes and requires enzymes capable of withstanding stress conditions of temperature and pH [1-3]. It's very well-known fact that the enzyme based detergent formulations have better cleaning and distaining abilities compared to non-enzyme-based formulations of chemical and synthetic origin. Enzymes not only actively destains fabrics but also maintain the brightness and quality of fabric after washing [4, 5]. Enzymes used in detergents are protease, mannanases, pectinase, amylase, etc. These are mainly microbial origin enzymes from bacteria and fungi [6]. Bacterial enzymes majorly from *Bacillus* genus are preferred due to alkaline stability they possess [7].

Mannanases are group of enzymes that can degrade mannans, a plant-based gum that's being used increasingly as thickening agents in ice-creams, sauces, hair gels, shampoos, conditioners, tooth-pastes etc. these make stains on fabric sticky and difficult to remove by routine detergents [8]. Alkaline mannanases compatible with detergent formulations can be of use for removing stains and have found way in laundry segments. Today, mannanase containing detergents introduced by Novozymes (Mannaway®) and by DuPont (Effectenz™) are commercially available in the market [9, 10].

Similarly, pectinases are group of enzymes that can degrade pectic substances poly galacturonic acid into mono-galacturonic acid by opening glycosidic linkages, which are present in many fruits and are used extensively in beverage industry for juice extraction and clarification, degumming of fibers, vegetable oil extraction. Therefore, pectinase is used in various industries as pulp industry, textile industry, food industry, etc. In order to remove pectin-based stains, detergent containing pectinase (XPect®) was introduced by Novozymes company [11-14].

In applications where substrates are poorly soluble at ambient temperature, thermostable enzymes have proved more useful and for usage in detergents activity in alkaline pH is desired. Microorganisms and enzymes to be used in industrial applications can be isolated from hot spring niche are preferred since they are stable under high temperature conditions [15, 16].

In the present study attempts were made to extract enzyme pectinase and mannanase from bacterial isolates of Ganeshpuri hot spring origin and to test detergent application of these enzymes by checking their detergent compatibility and destaining potential.

2. MATERIALS AND METHODS

2.1. Collection of water from hot spring

The selected hot spring is present at Ganeshpuri/ Vajreshwari town near Thane, Maharashtra. Water from spring was collected in new, unused plastic bottles which were rinsed with hot spring water two- three times before water collection. Capped bottles were transported to lab and stored at 4°C in refrigerator until further microbiological analysis [17].

2.2. Isolation of bacteria

Nutrient agar medium plates containing (g/L) peptone 5, beef extract 3, NaCl 5, and agar 27 were prepared aseptically and 100µl water sample was spread onto it. Incubation of the plates was done for 24 hours at 55°C and morphologically different colonies isolated on the medium were selected for enzyme screening. Their morphological and microscopical characters were recorded for the identification purpose. These colonies or isolates were cultivated on nutrient agar slants and glycerol stocks were made for maintaining culture [18-21].

2.3. Qualitative screening for mannanase and pectinase production

The bacterial isolates were spot inoculated onto two types of agar media, pectin nutrient agar for pectinase production and locust bean gum (LBG) nutrient agar for mannanase production. The basal nutrient media was same for both containing (g/L), 5.0 peptones, 5.0 Nacl, 1.5Beef extract, 1.5yeast extract and, 27 agar. 5.0 g/L apple pectin was added to this media for pectinase and LBG 5.0 g/L for mannanase screening purpose. The inoculated plates were incubated at 55°C for 24-48 hours and grams iodine solution was flooded onto the plates to check degradation of substrates by enzymes. Isolates showing maximum zone of clearance with respect to colony size were selected for further analysis [23, 24, 26].

2.4. Quantitative assay by DNS method

For quantitative assay of enzymes media composition used was same, the one used for screening purpose except agar for solidification was not added. The selected isolate was inoculated into liquid media and incubated at 55°C for 24 hours in shaking incubator. Grown cultures were centrifuged at 10,000 rpm for 10 minutes at 4°C, supernatant was collected and filtered. This was used as enzyme source for reaction using Di-nitro salicylic acid (DNS) method for determination of both enzyme activities. It is used to determine the amount of reducing sugars (galacturonic acid & mannose sugars) released from the degradation of poly galacturonic acid substrate i.e. pectin and LBG respectively. Apple pectin substrate at 0.5% in 50mM phosphate buffer (pH 8.0) was prepared as substrate for the pectinase assay and 0.5% LBG in50mM phosphate buffer (pH 8.0) for mannanase assay. Assay reaction mixture was prepared by adding 200 ul of enzyme to 500ul of buffer and 300ul of substrate, it was incubated at 60°C for 30 min. After incubation reaction was terminated by addition of 1.0 ml DNS solution followed by keeping it in boiling water bath for 5 min. Absorption of each sample was recorded at 540 nm and reducing sugar liberated was quantified by using standard curve of glucose as reducing sugar. One-unit activity was defined as the amount of enzyme forming 1 µmol of galacturonic acid per minute for pectinase and 1µmol of mannose for mannanase activity. Total protein content was determined by biuret method using bovine serum albumin as standard.

Enzyme assays were performed in triplicate for maximum enzyme activity and mean value was taken for analysis purpose [23, 24, 25].

2.5. Effect of detergents on enzyme activity

Commercial detergents Rin, Wheel, Tide, Ariel, Surf and SDS was prepared 8.0% w/v in distilled water, inactivated for 40min at 90°C and used for reaction. The enzymes were pre-incubated with detergents solution (1:1) for 30 minutes at 60°C, and the effect on enzyme activity was determined. The residual activity of enzyme without any detergent under same conditions was taken as control with 100% activity [26].

2.6. Detergency activity

This experiment was performed to test the detergency of the enzymes isolated substrate-based stains from

cotton fabrics. LBG and apple pectin was mixed with some mud, gravy separately for mannanase and pectinase test to create a harsh stain that would stick to the fabric and is easily visible. Fabric pieces were artificially stained and ironed to fix the stain. After drying the cloth pieces were dipped in flasks, each containing: a) 20 mL of tap water (control) + stained cloth, b) 15 mL of tap water with 5 mL enzyme + stained cloth, b) 15 mL of tap water with 5mL of heat inactivated detergent + stained cloth and c) 15 mL of tap water with 2.5 mL of heat inactivated detergent and 2.5mL of enzyme + stained cloth. All flasks were kept inside rotary shaker, containing different test samples set at room temperature. After 20 min, cloth pieces were rinsed with water, dried and observed for the detergency action [23, 26].

3. RESULTS AND DISCUSSION

3.1. Selection of the isolates

Different types of colonies were observed on nutrient agar plate after incubation. Out of them morphologically different colonies were selected for pectinase and mannanase enzyme screening. The isolates showing greater substrate clearance zone were selected for further analysis. The isolates with hydrolysis zone ≥1.0 cm is considered significant or isolates having ratio of hydrolysis zone to colony diameter ≥2.0 were subjected to enzyme activity determination. For mannanase the selected isolate had 2.8 cm hydrolysis zone to colony diameter ratio and for pectinase it was 2.3.

3.2. Identification of the isolates

Based on colony morphology, microbiological analysis of the selected isolates, the bacterial species were found to be *Bacillus licheniformis* for mannanase enzyme and *Anoxybacillus* genus species for pectinase [20].

3.3. Enzyme activity determination

In liquid media mannanase activity of extracted enzyme was 79.37 U/ml and of pectinase enzyme 299 U/ml at 60°C and pH 8.0. The assay conditions indicate that enzymes were active at moderately high temperature and alkaline pH. Pectinase purification reports from *Anoxybacillus* genus are almost none but compared to other *Bacillus* species it's considerably high [25, 26].

3.4. Detergent compatibility of enzymes

3.4.1. Mannanase compatibility

Relative effect of inactivated detergents on mannanase enzyme activity is shown in fig.1. The graph and values clearly show that detergents had very minimal or no reduction in enzyme activity. Tide detergent had no effect on enzyme activity, slight activity reduction was observed in presence of SDS followed by Rin& Ariel, Surf and wheel. Enzyme additives in detergents need to be compatible and these results show that mannanase enzyme can be used along with detergents for cleaning and laundry applications.

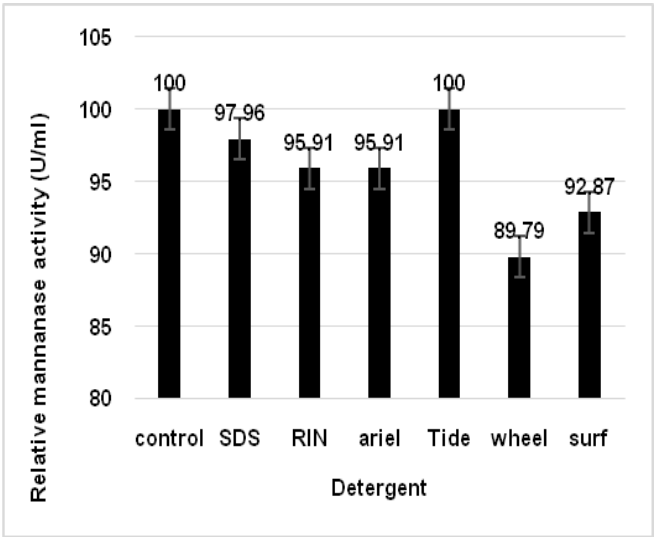


Fig. 1: Effect of detergents on mannanase activity

3.4.2. Pectinase compatibility

Relative effect of inactivated detergents on pectinase activity is shown in fig.2. These values are either comparable with or slightly more than control values.

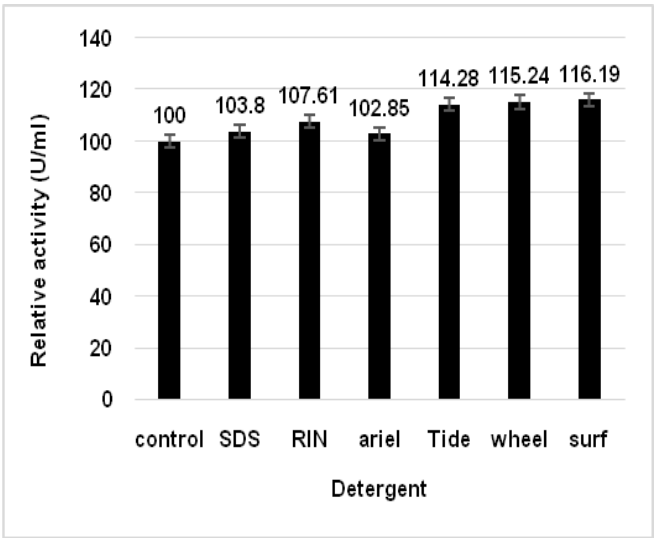


Fig. 2: Effect of detergents on pectinase activity

Slight increase in pectinase activity was seen in presence of Ariel followed by SDS, Rin, Tide, Wheel and Surf. These could be due to favorable effect of detergents on hydrophobic interactions of enzyme protein resulting in its solubilization and increase in activity. Similar reports of enhanced or no effect of detergents on pectinase activity are reported earlier [25-27]. Since Both enzymes were found to be detergent compatible, their destaining potential in laundry application was designed and performed.

3.5. Destaining activity of enzymes

3.5.1. Destaining activity of mannanase

Detergent action of enzyme was tested by washing stained cloth with water, with enzyme, with inactivated detergent solution and enzyme-detergent solution separately. Results of these washes can be seen in fig.3 there is gradual disappearance of mannan stain from tap water wash to enzyme wash. Enzyme along with detergent solution wash was found to be most successful in stain removal compared to other washes. Enzyme based detergent formulations require enzyme to be compatible with detergents and effective in detergency action. Both this could be observed with mannanase enzyme extract.

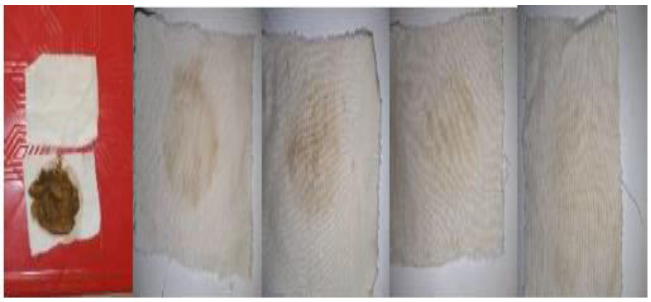
a b c d e

a-Plain & stained cloth, b-washed with water, c-washed with inactivated detergent, d- washed with enzyme, e- washed with enzyme & detergent

Fig. 3: Destaining activity of mannanase enzyme

3.5.2. Destaining activity of pectinase

Detergent action of pectinase was tested in the similar procedure as used for mannanase. Washes of stained cotton fabric with water, with enzyme, with inactivated detergent solution and enzyme-detergent solution was performed. Results of these washes can be seen in fig.4 Stain removal with consecutive washes was observed and enzyme-detergent solution was most effective of all the washes.



a b c d e

a-Plain & stained cloth, b- washed with water, c- washed with inactivated detergent, d- washed with enzyme, e- washed with enzyme & detergent

Fig. 4: Destaining activity of pectinase enzyme

4. CONCLUSION

Commercial detergents need improvement in formulations to increase its washing potential. This can be achieved by addition of new, effective enzymes with better stability with detergents and satisfactory detergency action. The present study has given a new candidate enzyme of pectinase and mannanase with good activity, detergent compatibility and washing ability. These enzymes can find potential application in laundry detergents as they could destain the substrate-based stains effectively. Enzyme consortium of these two enzymes can work more effectively and evaluation of such consortia needs further investigation.

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Conflicts of Interest

The authors with listed names declare no conflict of interest to disclose.

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