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HYDROLYTIC ENZYME PRODUCTION POTENTIAL OF BACTERIAL POPULATION FROM SAHASTRADHARA COLD SULFUR SPRING, UTTARAKHAND

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Abstract

Hydrolytic potential of bacterial isolates from Sahastradhara sulfur spring was explored using qualitative approach. A total of 14 isolates, obtained by serial dilution and spread plate method, were purified by quadrate streaking. The isolates were characterized morphologically and biochemically. Bacterial population was comprised of rod shaped members. All the recovered isolates were Gram positive in nature and were screened for production of amylase, cellulase, lipase and protease. All the isolates exhibited amylolytic activity. 71.4%, 85.7% and 64.2% were positive for cellulase, lipase and protease respectively. Majority of isolates were positive for more than one hydrolytic activity. The percentage of isolates exhibiting single, two, three and four hydrolytic activities was 7.1%, 14.2%, 14.2% and 64.2% respectively. Larger proportion of population had good amylolytic potential. Enzyme index greater than one was shown by 42.8%, 28.5%, 14.2% and 21.4% isolates for amylase, cellulase, lipase and protease respectively.

Key words: Sulfur spring, Amylase, Cellulase, Lipase, Protease.

Introduction

Microorganisms are absolutely indispensable for sustenance of life on earth and intricately involved in virtually every aspect of life. They are ubiquitous in nature, inhabiting almost every environment from favourable habitats to extreme environments like hydrothermal vents, where sustenance of life is really hard to even imagine. Microbes are very sturdy and adaptable to changes in environment and bedwell a wide range of temperature, pH, salinity and nutrient availability conditions. They maintain balance in ecosystem by involvement in biogeochemical cycling and interaction with other life forms. To thrive in and adapt to a variety of conditions, they have to produce a vast array of enzymes and other compounds. A number of microbial products produced for their sustenance are also commercially valuable to humans. Therefore microbial wealth has been exploited from long time and continues to be studied for availing novel products. Amylases, cellulases, lipases and proteases are extracellular hydrolytic enzymes with well established uses in a number of industries. Continuous investigations are being carried out to avail better variants of existing enzymes to make the processes safer for environment and lighter on pocket. Present study is an attempt to explore hydrolytic potential of an untouched site to reveal the hidden microwealth.

Amylases are used in starch processing industries, liquefaction, manufacturing of high fructose containing syrups, maltotetraose syrup (G4 syrup), maltose, oligosaccharide mixtures, high molecular weight dextrins, desizing process, direct fermentation of starch to ethanol and an array of other applications (Aiyer 2005; Karnwal & Nigam 2013). Cellulases have uses in paper and pulp, detergent, textile, bioethanol, wine and brewery, food processing, animal feed, olive oil extraction, carotenoid extraction industries and in waste management (Kuhad *et al.* 2011). Lipases have application in food, dairy, paper and pulp, textile and detergent industries (Hasan *et al.* 2006). Proteases have essential role in manufacturing of all types of detergents including regular detergents and the ones used for cleaning contact lenses and dentures. Use of proteases has made leather processing process safer for environment by replacing hazardous chemicals

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like sodium sulfide. Other uses include meat tenderization, cheese production, baking, manufacturing of protein hydrolysates used in dietary and health products, preparation of artificial sweetener aspartame, waste management and silver recovery. Owing to their selective bond cleavage property proteases are used in understanding the structure function relationship, synthesis of peptides and sequencing of proteins (Rao *et al.* 1998; Suganthi *et al.* 2013).

Due to great economical importance of microbial enzymes in a variety of areas from industries to research microbial wealth from various habitats has been investigated for its enzymatic potential. Different habitats investigated include soil (Singh & Kumari 2016) (Pokhrel *et al.* 2013; Masngut *et al.* 2017), hot springs (Adhikari *et al.* 2015; Daupan & Rivera 2015; Baltaci *et al.* 2016; Zahoor *et al.* 2016), mines (da Costaa *et al.* 2016) and saline habitats (Sa´nchez-Porro *et al.* 2003; Rohban *et al.* 2009; Kakhki *et al.* 2011; Kumar *et al.* 2012).

Materials and Method

i) Sample collection: Sahastradhara sulfur spring is situated 15 km northeast from Dehradun city in vicinity of Baldi river in Uttarakhand, India and is well known for its healing properties (Bhat & Mir 2015). Water and microbial mat samples were collected in the month of October. Temperature and pH of samples were recorded at the time of sampling. Water samples were collected in autoclaved bottles by immersing the bottles in water and microbial mat samples were collected using sterile swabs. Water samples were collected from two points i.e. from spring origin and spring exit, approximately 3ft away from spring. Samples from spring origin were coded SCWo, from exit were coded SCWe and microbial mat samples were coded as SCM followed by numbering.

ii) Isolation: Isolation was done using serial dilution and spread plate method. Nutrient broth and nutrient agar media were used for the procedure. Recovered isolates were morphologically characterized by recording colony characteristics and Gram staining. Biochemical characterization was done by performing various tests *viz*. indole, methyl red, Voges-proskauer, citrate utilization, triple sugar iron agar test, urease production, nitrate reduction test, catalase and oxidase tests. Biochemical tests were performed according to Cappucino and Sherman (1992).

iii) Qualitative Screening hydrolytic enzyme production: Recovered isolates were screened for amylase, cellulase, lipase and protease production. 24 hours old cultures were used for spot inoculation in the procedures followed for all the enzymes screened. Starch agar media was used for determining amylolytic activity. Starch agar plates were spot inoculated with active cultures and incubated at 30°C for 24 hours. After incubation plates were observed for clear zone around colonies (Chadda *et al*. 1997). Cellulose degrading capacity of the isolates was determined by screening them for production of cellulase enzyme. Carboxy methyl cellulose (CMC) was used as a substrate in agar media and active cultures of recovered isolates were spot inoculated on appropriately labelled plates. The plates were incubated at 30°C for 24 hrs. After incubation plates were flooded with 1% congo red solution and allowed to stand for 15 minutes followed by 1M NaCl solution for another 15 minutes and observed for orange colored zone around colonies (Teather & Wood 1982). Lipase producing ability of the isolates was determined by screening for tributyrin hydrolysis. Tributyrin agar media plates were appropriately labelled and spot inoculated with respective isolates. Plates were incubated for 24 hrs at appropriate temperatures. After incubation plates were observed for clear zone around colonies (Cappuccino & Sherman 2007). For determination of protease producing ability recovered isolates were screened for casein hydrolysis. Casein is the major milk protein and substrate used in the agar media for this test. Skim-milk agar media was used for screening. The plates were appropriately labelled and inoculated with respective isolates by spot inoculation. After incubation period of 24 hrs at appropriate temperature plates were observed for clear zone around the colonies (Ladd & Butler 1972).

Presence of clear zones around colonies in case of all enzymes other than cellulase and presence of orange colored zone in case of cellulase; was taken as positive indicator of hydrolytic activity. Colony diameter and combined diameter of colony and clear zone were measured to nearest cm. these measurements were implied in calculation of enzyme indices. Enzyme indices are measure of efficiency of particular isolate to produce an extracellular enzyme. Enzyme index was calculated using the formula (Florencio *et al.* 2012):

$$Enzyme\ Index = \frac{Hydrotysis\ zone\ diameter}{Colony\ diameter}$$

Results and Discussion

Temperature and pH of the spring was recorded 20.9°C and 7.0 respectively, at the time of sampling. A total of 14 isolates were recovered from different samples and all of them were gram positive bacilli.

Enzymatic potential of bacterial population from a variety of environmenta has been explored. Different sets of conditions are presented by different environments

for survival of inhabiting bacterial members hence bringing in the variation in the enzyme profile of bacterial population. Habitats investigated include soil, glacires, hot springs, magnesite soil etc. (Kuddus *et al*. 2012; Suseenthar *et al.* 2012; Baltaci *et al.* 2016; Gupta *et al.* 2016). Enzymatic potential of bacterial isolates from Sahastradhara cold sulfur spring was explored. Recovered isolates were screened for amylase, cellulase, lipase and protease production. Out of the isolates screened all of the isolates were positive for amylase, 71.42% showed cellulolytic activity, 85.7% exhibited lipolytic activity and 64.2% were protease positive. Variation was observed in terms of ability to produce hydrolytic enzymes and efficiency of production. Enzuyme indices were calculated for estimationof enzyme production efficiency. Enzyme indices for all of the four enzymes screened are represented in fig. 1. Majority of the population displayed good amylolytic and cellulolytic activity. Out of four enzymes least activity was observed for lipase and highest was observed for amylase.

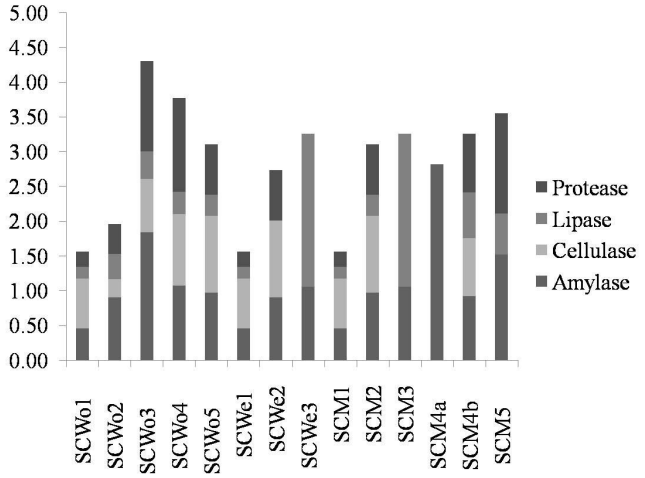

Fig.1: Enzyme indices for amylase, cellulase, lipase and protease

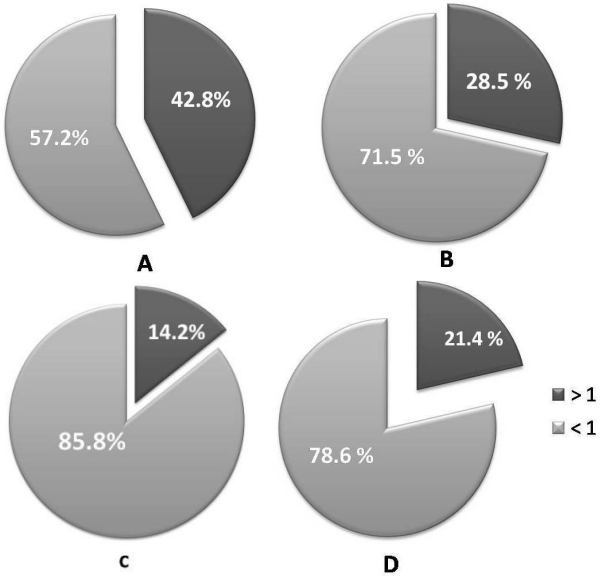

Fig. 2: Percent distribution of isolates exhibiting enzyme index more than one for **(A)** Amylase, **(B)** Lipase, **(C)** Cellulase and **(D)** Protease

Highest enzyme indices recorded were 2.82 for amylase (SCM4a), 1.1 for cellulase (SCM2), 2.2 for lipase (SCWe3, SCM3) and 1.44 for protease (SCM5) (fig. 1). Combined activity for more than one enzymes was also observed. 64.2% isolates exhibited all four hydroltic activitues, 14.2% were positive for three enzymes, 14.2% showed two hydrolytic activities and 7.1% were positive for one enzyme.

Enzyme indices higher than one for more than one enzyme were recorded for four isolates. Isolate SCWo3 had indices greater than one for amylase (1.84) and protease (1.3), Scwe3 for amylase (1.05) and lipase (2.2), SCM3 for amylase (1.05) and lipase (2.2), SCM5 for amylase (1.52) and protease (1.44), SCWo4 for amylase (1.07), cellulase (1.03) and protease (1.35).

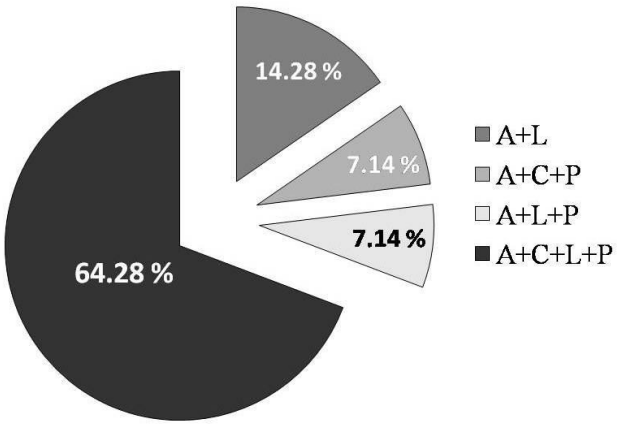

Fig. 3: Distribution of different hydrolytic activities for isolates from Sahastradhara, where A=amylase, C=cellulase, L= lipase and P= protease

Conclusion

Preliminary investigation of hydrolytic enzyme production potential of bacterial isolates from Sahastradhara cold sulfur spring was carried out. Qualitative estimation was performed by plate assay method. Majority of the isolates exhibited hydrolytic activities for all the four substrates tested. Results revealed that some of the recovered isolates hold good hydrolytic potential, which is represented by enzyme indices. These isolates can be exploited for use in industry. Isolates SCWo3, SCWo4, SCWe3, SCM3 and SCM5 exhibited good activity for more than one enzyme, giving them more weigh against others.

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