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Enzyme profiling of selected chitinase producing *Actinomycetes*

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Abstract
Twenty five *Actinomycetal* isolates, obtained during the screening of Chitinase production were plate-screened for their ability to produce eight extracellular enzymes activities to evaluate their other potential industrial application. Isolates were assessed for Amylase, Cellulase, Xylanase, Lipase, Caseinase, β-Mannanase, Chitinase, and Caffeinase enzyme activities by determining their enzymatic index. In our results, limited number of isolates present in lipase followed by for xylanase and caseinase activities. An average number of isolates present Amylase, Cellulase and β-Mannanase activities. Isolates number SP3, SP7, SP14 and SP25 showed diversified enzymatic competence, among them SP25 showed high level of extracellular enzyme activities particularly for Amylase, Chitinase and for Caseinase activities.

Keywords: *actinomycetes*, enzyme profiling, chitinase, β-mannanase

1. Introduction
Actinomycetes are widely present in nature and are aerobic, Gram positive group of filamentous bacteria with a high G+C content in their DNA [1, 2]. This group accounts for degradation of organic molecules and synthesis of biological active compounds as well [3]. They have their own economic importance in industries as producers of bioactive substances, such as antibiotics, vitamins and enzymes [4]. In last 55 years around 12000 antibiotics have been discovered in which *Actinomycetes* alone contributes 70% in production. The uses of *Actinomycetes* in terms of metabolites production is not overcome and the predictive model from the researcher suggest that over 150,000 bioactive metabolites are still waiting to be discovered from the member of *Streptomyces* genus alone [5, 6]. Various genera of *Actinomycetes* has been reported for enzymes and their products applied in biotechnological industries and biomedical field [7]. The source of commercially available enzyme are plants, animals and microorganisms. The major fraction of commercially available enzymes are produced from the microorganisms. The production of enzyme using microorganism is the most obvious choice of researcher interest due to ease of nutritional requirement, rapid growth rate and downstream processing [8].

Table 1: Industrial importance of some enzymes.

Enzyme	Industrial applications	References
Amylase	Paper, textile, alcohol (from starch), nutritional and detergents industries	[9, 10]
Cellulase	Pulp, biofertilizer, biofuel and textile industries	[11]
Xylanase	Pulp, paper, bakery, feed, juice, and beer industries	[12]
Lipases	Broad range biocatalyst	[13]
	Detergent formulations, Dairy industry, Chemical and Agrochemical industry, Paper manufacture, Nutrition, Cosmetics, and Pharmaceutical processing.	[14, 15, 16]
Caseinase	To prepare characteristic coagulant in cheese making	[17]
β-Mannanase	Poultry diets, Pulp industries, Oil industries, Copra waste treatment	[18, 19, 20, 21]
Chitinase	Control of Phytopathogens, production of chito-oligosaccharides, Degradation of chitinous waste	[22]
Caffeine degrading enzymes	Caffeine degradation	[23, 24, 25]

The present paper deals with the enzymatic profiling of selected chitinase producing *Actinomycetes* species at the level of 1% substrate concentration.

2. Materials and Methods
2.1 *Actinomycetes* isolates
A total of twenty five isolates were selected, which were preliminary obtained after the primary screening of chitinase producing *Actinomycetes*.

2.2 Screening steps for enzymatic profiling
A total of twenty five isolates were evaluated for extracellular enzyme production on solid culture media containing specific substrate as a sole source of utilization. The Isolates were investigated for the production of Amylase, Cellulase, Xylanase, Lipase, β-Mannanase, Caseinase, Caffeinase and Chitinase enzymes as described follows:

2.3 Media Preparation
Amylase activity
To determine the amylase activity, media was prepared using 1% starch solution incorporated with 0.3% NaCl, 0.1 % KH_2PO_4 and 2.8 % agar. Isolates were transferred to starch agar media and incubated at 28 °C for 7 days. After the

incubation period, lugol solution was used to measure the diameter of halo zone by adding it into the plate. Amylase activity was determined using EI.

Cellulase activity
Cellulase production was assessed using medium supplemented with 1.0% carboxymethylcellulose (CMC), 0.3% NaCl, 0.1 % KH_2PO_4 and 2.0 % agar. Spot inoculation were done for each isolates and incubated at 28 °C for 7 days. Cellulase production was measured by adding a 0.1% solution of Congo red onto the colonies. After 10 min the solution was drained and plate was washed using the 1 molar NaCl solution. Kept it for 20 to 30 min, halo zones around the colony indicate cellulolytic activity and EI was determined.

Xylanase activity
Xylanase activity of the selected isolates was measured using culture media containing 1% Xylan, 0.3% NaCl, 0.1 % KH_2PO_4 and 2.0 % agar. Spot inoculation of the isolate was done on petriplates and incubated at 28 °C for 7 days. After incubation period, transparent halo around the colony showed xylan degradation and EI was determined.

Lipase activity
The lipase activity of the isolates was assessed using the medium containing 1.0% tributrin oil, 0.3% NaCl, 0.1 % KH_2PO_4 and 2.0 % agar. Isolates were inoculated by spot inoculation technique and plates were incubated at 28 °C for 7 days. After incubation plates were stored in refrigerator for better visualization for 24 hours. The production of lipase was determined by observation of white halo contrasting with the medium and EI was determined.

Caseinase activity
The ability of actinomycetes to produce casienase was assessed using 1% casein as a sole source of substrate in the medium along with 0.3% NaCl, 0.1 % KH_2PO_4 and 2.8 % agar. Spot inoculation plates were incubated at 28 °C for 7 days. After incubation, hydrolysis zones showed enzyme activity and EI was determined.

β-Mannanase activity
To determine the β-Mannanase activity, medium was prepared using 1% guargum, 0.3% NaCl, 0.1 % KH_2PO_4 and 2.0% agar, spot inoculation was done for all the isolates and plates were incubated at 28 °C for 7 Days. After incubation, congo red dye was poured on to the plates and clear halo zones showed the β-Mannanase activity and EI was determined.

Chitinase activity
For the determination of chitinase activity, media were prepared by using 1% colloidal chitin, incorporated with 0.3% NaCl, 0.1 % KH_2PO_4 and 2.0% agar. Spot inoculation was done for all the isolates and plates were incubated at 28 °C for 7 Days. After incubation transparent clear zone around the colony showed chitinase activity and EI was determined. During the media preparation colloidal chitin was prepared from chitin flakes [26].

Caffein degrading activity
Caffein degrading activity was determined by preparing medium containing 1% caffeine, 0.3% NaCl, 0.1 % KH_2PO_4 and 2.8% agar. Spot inoculation was done for each isolate and plates were incubated at 28 °C for 7 days. After incubation, clearing halo zones around the colony confirmed the production of caffien degrading enzyme and EI was determined.

2.4 Enzymatic Index (EI)
The production and semi-quantitative measurement activity was analyzed. For analyzing the potency of each isolate towards the enzyme profiling, the growth of *Actinomycetes* were analyzed using Enzymatic Index (EI). The Enzymatic Index (EI) was expressed by the relationship between the average diameter of the degradation halo and the average diameter of the colony growth [27, 28].

$$EI = \frac{\text{diameter of hydrolysis zone}}{\text{diameter of colony}}$$

2.5 Statistical analysis
The data of EI obtained during the study were analyzed for knowing the significant difference within the particular enzyme. The experiment was performed using the four replicates and results were analyzed by performing analysis of variance test followed by tukey post-hoc test using software IBM SPSS 23.0 trial version [29].

3. Results & Discussions
Extracellular enzyme production by selected isolates of *Actinomycetes* in solid media for prepared specific substrates was measured by enzymatic index. The obtained result are shown in Table 2.

Enzymatic activity
Amylase activity
Eleven isolate were able to degrade starch by showing a clear zone on the plate. Isolate SP23 shows the highest starch degrading EI of 3.33, on the other hand SP7 isolate shows least starch degrading EI. Earlier thermophilic and acidophilic amylases have been studied from *Streptomyces erumpens* [30] as well as the cold-active α-amylases production was reported using *Actinomycetes* [31].

Cellulase activity
Thirteen isolates were found positive for the degradation of cellulose. The highest EI was observed with SP6 of 2.22 and lowest EI 1.06 for SP20. Alkalo-stable and thermo-stable cellulase is reported from *Streptomyces genus* [32].

Table 2: Enzymatic index (EI) for various enzymes by selected isolates.

	Enzymatic Index							
Isolate Code	**Amylase**	**Cellulase**	**Xylanase**	**Lipase**	**Caseinase**	**β-Mannanase**	**Chitinase**	**Caffeine Degrading**
SP1	ND	ND	ND	ND	ND	ND	2.09^{e}	ND
SP2	2.05^{h}	ND	1.65^{g}	ND	ND	1.37^{d}	1.52^{i}	ND
SP3	ND	2.98^{b}	2.42^{d}	2.58^{c}	1.33^{i}	1.44^{c}	$1.66^{g,h}$	ND
SP4	2.09^{h}	1.11^{i}	1.47^{h}	ND	ND	1.08^{h}	1.84^{f}	ND

SP5	ND	ND	ND	1.49^{h}	ND	ND	1.36^{k}	ND
SP6	ND	2.19^{c}	ND	3.76^{a}	ND	ND	3.16^{b}	ND
SP7	1.72**i**	1.98^{e}	2.98^{b}	ND	1.72^{g}	1.29^{f}	1.74^{g}	ND
SP8	ND	ND	ND	ND	ND	ND	1.19^{m}	ND
SP9	2.74^{e}	3.04^{a}	ND	ND	2.83^{e}	ND	1.35^{k,l}	ND
SP10	2.93^{d}	ND	ND	2.02^{e}	4.06^{b}	ND	2.24^{d}	ND
SP11	3.02^{c,d}	ND	3.75^{a}	ND	3.23^{d}	2.15^{a}	1.47^{i,j}	ND
SP12	2.38^{f}	1.51^{g}	ND	2.24^{d}	ND	ND	1.74^{g}	ND
SP13	ND	2.02^{d}	ND	ND	ND	ND	1.62^{h}	ND
SP14	ND	1.13^{i}	2.33^{e}	1.62^{g}	2.68^{f}	1.39^{d}	2.19^{d}	ND
SP15	ND	ND	2.04^{f}	ND	2.66^{f}	1.73^{b}	1.19^{m}	ND
SP16	ND	ND	ND	ND	ND	1.18^{g}	1.09^{n}	ND
SP17	ND	1.39^{h}	2.51^{c}	ND	ND	ND	2.09^{e}	ND
SP18	ND	1.41^{h}	ND	ND	ND	ND	1.49^{i,j}	ND
SP19	3.08^{b,c}	ND	ND	ND	ND	1.09^{h}	1.43^{j,k}	ND
SP20	ND	1.03^{j}	ND	2.82^{b}	ND	2.15^{a}	1.27^{l,m}	ND
SP21	ND	ND	ND	ND	ND	ND	1.34^{j,k,l}	ND
SP22	2.21^{g}	ND	ND	ND	1.48^{h}	1.09^{h}	2.51^{c}	ND
SP23	3.18^{a,b}	1.74^{f}	1.67^{g}	ND	ND	ND	2.05^{e}	ND
SP24	ND	ND	ND	ND	3.43^{c}	1.33^{e}	1.22^{m}	ND
SP25	3.27^{a}	1.50^{g}	ND	1.76^{f}	5.19^{a}	1.16^{g}	3.64^{a}	ND

ND: Not Detected, Values with the same letters within the column are not significantly different according to tukey test.

Xylanase activity

Many species of *Streptomyces* are reported to produce multiple xylanases [33]. Among Twenty five isolates only nine isolates were able to produce xylanase enzyme in solid plate media with highest EI of 3.75 for isolate SP11 and five isolates having EI above 2.00 which is higher compared to the other hemicellulolytic enzymes in the study.

Lipase activity

Isolate SP6 showed highest EI of 3.75 followed by SP20 with 2.83. Only eight isolates showed positive for lipase activity. The production of lipase enzymes from *Actinomycetes* were earlier reported. [34, 35]

Caseinase activity

Ten isolates were found positive for caseinase activity, among them isolate SP25 was showing highest EI of 5.2. The lowest EI was observed with SP22. Earlier casein hydrolysis activity was reported with *Streptomyces sp.* [36].

β-Mannanase activity

Earlier β-Mannanase produced using *Streptomyces sp. Bf3.1* and *Streptomyces glabus* respectively were reported in the breakdown of copra waste [37] and pulp industry [38]. In our study, thirteen isolates showed clear halos on solid agar medium containing guar gum as carbon source, from these isolates SP11 and SP20 showed highest EI of 2.15.

Chitinase activity

All the isolates showed chitinase production except isolates SP16 and SP20. Highest activity was observed for SP25 with EI of 3.6. Earlier various species of *Streptomyces sp.* Including *S. antibioticus, S. griseus, S. plicatus, S. lividans, S. aureofaciens and S. halstedii* have been known for the chitinolytic enzyme production [39].

Caffeine degrading activity

In earlier reports, nature caffeine is identified as antimicrobial substance at concentration above 2000 µg/ml which can inhibit the bacterial growth and above 5000 µg/ml was found to inhibit mold growth [40]. In this study, the concentration of caffeine was used 10000 µg/ml in Solid agar plate. So, none of the isolates were able to utilize caffeine.

4. Conclusion

In the study, twenty five isolates were analyzed in relation to their ability to produce extracellular enzymes. There is enormous importance of *Actinomycetes* for the production of diverse extracellular enzymes. Selected Chitinase producers have also found to produce other industrially important enzymes.

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6. References

1. Kuster E. The actinomycetes. In: Soil Biology, eds. Burges (A.) & Raw (F.), Academic Press, London. 1968, 111-124.
2. Crawford DL, Sutherland JB. The role of actinomycetes in the decomposition of lignocelluloses, Dev Ind Microbiol. 1979; 20:143-151.Institute
3. Naikpatil SV, Rathod JL. Selective isolation and antimicrobial activity of rare actinomycetes from mangrove sediment of Karwar. J. Ecobiotechnol. 2011; 3(10):48-53.
4. DeBoer W, Folman LB, Summerbell RC, Boddy L. Living in an fungal world: impact of fungi on soil bacterial niche developement. FEMS Microbial Rev. 2005; 29:795-811.
5. Baltz RH. Antibiotic discovery from actinomycetes: Will a renaissance follow the decline and fall? SIM News. 2005; 55:186-196.
6. Watve MG, Tickoo R, Jog MM, Bhole BD. How many antibiotics are produced by the genus Streptomyces? Arch. Microbiol. 2001; 176:386-390.

7. Divya Prakash, Neelu Nawani, Mansi Prakash, Manish Bodas, Abul Mandal, Madhukar Khetmalas *et al.* Actinomycetes: A Repertory of Green Catalysts with a Potential Revenue Resource, Hindawi Publishing Corporation Bio Med Research International. 2013, 1-8.
8. Leisola M, Jokela J, Pastinen O, Turunen O, Schoemaker HE. Industrial use of enzymes," in In Encyclopedia of Life Support Systems (EOLSS), UNESCO, Eolss Publishers, Oxford, UK. 2004, 2.
9. Upadek H, Kottwitz B. Application of amylases in detergents. In: Van Ee, J.H., Misset, O., Baas, E.J. (Eds.), Enzymes in Detergency. Marcel Dekker, Inc, New York. 1997, 203-212.
10. Van der Laan, May JMM. Novel amylolytic enzymes derived from the Bacillus licheniformis, having improved characteristics. International patent. 1995. WO 95/35382.
11. Reyad MA. Diverse of enzymatically active actinomycetes associated with mangrove rhizosphere in Janzan coast. Ann. Biol. Res. 2013; 4(4):100-108.
12. Mokni-Tlili S, Abdelmalek IB, Jedidi N, Belghith H, Gargouri A, Abdennaceur H *et al.* Exploitation of biological wastes for the production of value-added hydrolases by Streptomyces sp. MSWC1 isolated from municipal solid waste compost. Waste Manage Res. 2010; 28:828-837.
13. Sharon CM, Nakazato HI, Ogawa Y, Kato. Bioreactor operated production of lipase: Castor oil hydrolysis using partially purified lipase. Indian J. Exp. Biol. 1999; 37:481-486.
14. Akram Kashmiri M, Ahmad Adnan, Beenish Waseem Butt-production, purification and partial characterization of lipase from Trichoderma Viride, African Journal of Biotechnology. 2006; 5:878-882.
15. Fariha Hasan, Aamer Ali Shah, Abdul Hameed, Industrial applications of microbial lipases, Enzyme and Microbial Technology. 2006; 39:235-251.
16. Haki GD, Rakshit SK. Developments in industrially important thermostable enzymes: a review, Bioresource Technology. 2003; 89:17-34.
17. Aehle Wolfgang. Enzyme in industry production and application. Edn 2, Weinheim: wiley-vch verlag GmbH & Co. 2004.
18. Jackson ME, Fodge DW, Hsiao HY. Effects of β-mannanase in corn-soybean meal diets on laying hen performance. Poult. Sci. 1999: 78(12):1737-1741.
19. Montiel MD, Hernandez M, Rodriguez J, Arias ME. Evaluation of an endo-β-mannanase produced by Streptomyces ipomoea CECT 3341 for the biobleaching of pine kraft pulps. Appl.Microbiol. Biotechnol. 2002; 58(1):67-72.
20. McCutchen CM, Duffaud GD, Leduc P, Petersen ARH, Tayal A, Khan SA *et al.* Characterization of extremely thermostable enzymic breakers (α-1,6-galactosidase and β-1,4-mannanase) from the hyperthermophilic bacterium Thermotoga neapolitana 5068 for hydrolysis of guar gum. Biotechnol. Bioeng. 1996; 52(2):332-339.
21. Regalado C, Garcia-Almendarez BE, Venegas-Barrera LM, Tellez-Jurado A, Rodriguez-Serrano G, Huerta-Ochoa S *et al.* Production, partial purification and properties of β- mannanases obtained by solid substrate fermentation of spent soluble coffee wastes and copra paste using Aspergillus oryzae and Aspergillus niger. J. Sci. Food Agric. 2000; 80(9):1343-1350.
22. Karthik N, Akanksha K, Binod P, Pandey A. Production, purification and properties of fungal chitinases--a review. Indian J Exp Biol. 2014; 52(11):1025-35.
23. WOOLFOLK CA. Metabolism of N-methylpurines by a Pseudomonas putida strain isolated by enrichment on caffeine as the sole source of carbon and nitrogen. Journal of Bacteriology. 1975; 123:1088-1106.
24. Madyastha KM, Sridhar GR. A novel pathway for the metabolism of caffeine by a mixed culture consortium. Biochem. Biophys. Res. Commun. 1998; 249:178-181.
25. Hakil M, Denis S, Gonz´alez GV, Augur C. Degradation and product analysis of caffeine and related dimethyl xanthines by filamentous fungi. Enzyme Microb. Technol. 1998; 22:355-359.
26. Hsu SC, Lockwood JL. Powdered chitin agar as a selective medium for enumeration of actinomycetes in water and soil. Applied Microbiology. 1975; 29:422-426.
27. Hankin L, Zucker M, Sands DC. Improved solid medium for the detection and enumeration of pectolytic bacteria. Appl Microbiol. 1971; 22:205-209.
28. Hankin L, Anagnostakis SL. The use of solid media for detection of enzyme production by fungi. Mycologia. 1975; 67:597-607.
29. IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.
30. Kar S, Ray RC. Statistical optimization of *α*-amylase production by *Streptomyces erumpens* MTCC 7317 cells in calcium alginate beads using response surface methodology. Polish Journal of Microbiology. 2008; 57(1):49-57.
31. Kuddus M, Roohi JM, Arif JM, Ramteke PW. An overview of cold-active microbial alpha-amylase: adaptation strategies and biotechnological potentials. Biotechnology. 2011; 10:246-258.
32. Jang HD, Chang KS. Thermostable cellulases from Streptomyces sp.: scale-up production in a 50-l fermenter. Biotechnology Letters. 2005; 27:239-242.
33. Beg QK, Bhushan B, Kapoor M. Production and characterization of thermostable xylanase and pectinase from *Streptomyces sp. QG-11-3*. J. Ind. Microbiol. Biotech. 2000; 24:396-402.
34. Vishnupriya B, Sundaramoorthi C, Kalaivani M, Selvam K. Production of lipase from *Streptomyces griseus* and evaluation of Bioparameters. International Journal of Chem Tech Research. 2010; 2:1380-1383.
35. Magda M, Aly, Sanaa Tork, Salel M. Al-Garni, Lubna Nawar. Production of lipase from genetically improved *Streptomyces* exfoliates LP10 isolated from oilcontaminated soil African Journal of Microbiology Research. 2012; 6:1125-1137.
36. Mohamed Sonya H, Altalhi AD, El-Zahrani Gh, Badria S, Sadik AS. Enzymatic Activities of some *Streptomycetes* Isolated from Soil at Taif Region. Int. J. Curr. Microbiol. App. Sci. 2015; 4:19-32
37. Ariandi, Yopi, Anja Meriyandini. Enzymatic Hydrolysis of Copra Meal by Mannanase from *Streptomyces sp. BF3.1* for The Production of Mannooligosaccharides. HAYATI Journal of Biosciences. 2015; 22:79-86.

38. Kansoh AL, Nagieb ZA. Xylanase and mannanase enzymes from *Streptomyces galbus* NR and their use in biobleaching of softwood kraft pulp. Anton van Leeuwonhoek. 2004; 85:103-114.
39. Narayana KJP, Vijayalakshmi M. Chitinase production by *Streptomyces sp*. ANU 627, Braz J Microbiol. 2009; 40:725.
40. Nonthakaew A, Matan Na, Aewsiri T, Matan Ni. Caffeine in foods and its antimicrobial activity. International Food Research Journal. 2015; 22(1):9-14.