Plant Archives Vol. 17 No. 2, 2017 pp. 929-934 *ISSN 0972-5210*

ISOLATION AND ENZYMATIC CHARACTERIZATION OF *STREPTOMYCES* ISOLATES FROM WESTERN RAJASTHAN

G. K. Meghwanshi[1], S. Kumar[2], D. S. Solanki[2], K. Parihar[2], K. Sharma[2], P. Gehlot[2*], S.K. Singh[3] and R. Pathak[3]

[1]Department of Microbiology, M.G.S. University, Bikaner- 334004, India
[2*]Department of Botany, J.N.V. University, Jodhpur- 342001, India
[3]Central Arid Zone Research Institute, Jodhpur- 342003, India

Abstract

Twenty-one *Streptomyces* isolates were isolated and purified from soils of Jodhpur district. These molecularly well characterized isolates were tested for their biocatalytic potential of industrial important enzymes i.e. amylase, protease and lipase. The Qualitative and qualitative screening revealed that 8 isolates for amylase, 3 isolates for protease and 6 isolates for lipase are hitherto new producers of these enzymes. *S. albogriseolus* ITD-12 exhibited the maximum amylase enzyme potential while *S. rochei* ITD-5 exhibited the maximum protease and lipase production potential. *S. rochei* ITD-5 is recorded the best candidate for industrial application having the maximum potential of producing all the three enzymes tested. These *Streptomyces* isolates can be potentially used in fermentation, textile and paper industries.

Keywords: Enzyme, isolation, *Streptomyces,* Amylase, Protease, Lipase

Introduction

The *Streptomyces* is an important genus of Actinobacteria and have been extensively exploited for the production of secondary metabolites and enzymes of commercial significance (Narayana and Vijayalakshmi, 2009; Maleki *et al.* 2013). It represents one of the largest taxonomic units of identified *Actinomycetes* with more than 550 species (Euzeby, 2008; Kampfer, 2012) and characterized by aerobic, gram positive, chaemo-organotrophic, non-acid fast, with of aerial, substrate mycelia bearing short chains of spores (Willey *et al*. 2008; Kekuda *et al.* 2010; Naine *et al*. 2011) with higher GC content (>70%) in their DNA (Ventura *et al*. 2007). Streptomyc*es* are widely distributed in different nutritionally, biologically and physically complex soils and perform a broad range of metabolic processes and to produce an immense diversity of bioactive secondary metabolites including antimicrobial, anticancer, immunosuppressant and industrially important enzymes (Willey *et al*. 2008; Kekuda *et al*. 2010; Naine *et al*. 2011; Sajid *et al*. 2011).

***Author for correspondence :** e-mail : drpg73@rediffmail.com

Industrial enzymes are used in various detergents, textile, pulp and paper industries. Microbial enzymes are getting preference over the chemical catalysts in manufacturing chemicals, food, leather and pharmaceuticals in recent years. Microbial fermentation may encounter the market demand for industrial enzyme due to quick doubling potential of microbes (Kumar and Takagi, 1999). Amylase and protease play a vital role in various industrial applications and the demand of these enzymes is increasing day by day (Uyar and Baysal, 2004).

Lipases have wide range of enzymatic properties and are used in the processing of fat and oils, additives, detergents, cosmetics, paper manufacturing and pharmaceuticals (Hasan *et al*. 2006). Amylase is the most significant industrial enzymes (Cowan, 1996; Kirk *et al*. 2002) and has been used in many industries including food and beverages, textile, pharmaceutical, detergent and also in waste management (Aiyer, 2005). Protease is used in baking bread and manufacturing of crackers (Godfrey and West, 1996; Gupta *et al*. 2002; Norus, 2006; Binod *et al*. 2008). Cereal foods are treated with proteolytic enzymes to modify their proteins for better

processing, handling and increasing drying capacity (Leisola *et al*. 2002; Schafer *et al*. 2006; Poulsen and Buchholz, 2003). Similarly, lipase has emerged as the most significant industrial enzyme with vast array of applications in food and dairy, detergents, oleo-chemicals, agro-chemicals, pharmaceuticals, textiles, leather, tea processing, pulp, paper, polymer synthesis, biosensors, waste management and many others industries (Saxena *et al*. 1999, Saxena *et al*. 2005, Meghwanshi and Vashishtha, 2012).

Amylase, protease and lipases have been reported from different *Streptomyces* species harboring in different tropical and temperate regions (Niehaus *et al*. 1999; Kim *et al*. 2000b; Lee *et al*. 2001) but scanty literature sis available from hot arid and semi-arid regions of the western Rajasthan. The climate of arid zones is often characterized as hot and dry summers, sub-humid monsoon and cold dry winters. The high temperature, low relative humidity, high evaporation rate and scanty rainfall are major features of arid regions. The soils of these regions are generally deficient in nitrogen and organic matter (Rajasekar, 2015). These ecosystems are characterized by lack of moisture and nutrition and affect the survival and growth of microorganisms (Fita, 2015; Rajasekar, 2015). However, this region harbours a plethora of *Streptomyces* diversity ranging from halo-tolerant to thermo-tolerant species but their exploitation is limited to assess their true potential (Gaur *et al*. 2012; Sharma *et al*. 2013a & b; Tiwari *et al*. 2015). Therefore, the present study was undertaken to investigate the *Streptomyces* species of the western Rajasthan to assess the production potential of enzymes viz., amylase, protease and lipase.

Materials and Methods

Survey and Collection of Soil Samples

The soil samples were collected during year 2014 from different locations of Jodhpur district, Rajasthan, India (table 1) at 10-15 cm depth. The soil samples were collected from 10 randomly selected points from each location and mixed thoroughly to form a composite sample, brought to laboratory, sieved to get rid of large debris and kept in refrigerator at 4°C until analyzed.

Isolation of *Streptomyces*

One gram of each composite and sieved soil sample were suspended in 100 ml sterile distilled water and incubated in an orbital shaker at 28°C with shaking at 180 rpm for 1 hr. The mixtures were allowed to settle and then serial dilutions of the soil suspensions were prepared up to 10^{-4}. From each dilution an aliquot of 0.5 ml was inoculated in a Petri plate containing Actinomyces Isolates Agar (AIA) media in three replications for the isolation of *Streptomyces* by the dilution plate technique (Seong *et al*. 2005). The inoculated Petri plates were incubated at 37°C in a BOD incubator for 7 days. The *Streptomyces* colony forming units (CFU's) were recorded and purified in Starch Casein Agar (SCA) medium and maintained 4°C in a refrigerator until used.

Separation of *Streptomyces* isolates

The morphological characteristics of spore bearing hyphae and spore chains of the isolates were examined using coverslip culture technique as described by Arifuzzaman *et al*. (2010). These characteristics were then compared with that of *Streptomyces* description from Bergay's manual of Determinative Bacteriology (Holt *et al*. 1994). The isolates showing non-filamentous bacterial characteristics were separated from the true cultures on SCA medium and typical characteristics of *Streptomyces* validated.

Authenticity of *Streptomyces* isolates

Twenty one test isolates exhibiting typical characteristics of Streptomyces were previously characterized in our laboratory (Kumar *et al*. 2016) using PCR amplification and sequencing of 16S rRNA gene and GenBank accession numbers KJ438290-KJ438291 (ITD-5 and ITD-6) and KM215719- KM215737 (ITD-7 to ITD-25). These molecularly well authenticated isolates were used under present study for enzymatic profiling.

Screening for enzymes production

1. Qualitative Screening

The *Streptomyces* isolates were screened on the basis of their growth phase for the production of amylase, protease and lipase by inoculating them on Tributyrin agar (TbA) [composition per liter - Beef extract 3g, peptone 5g, tributyrin 15 ml, agar 20g, pH 7.2 ± 0.2],(Skimmed milk agar (SkMA) [composition per liter - Skim milk powder 20g casein enzyme hydrolysate 5g, yeast extract 2.5g, dextrose 1g agar 15g, pH 7.0 ± 0.2] and Starch agar (StA) [composition per liter - Beef extract 3g, peptic digest of animal tissue 5g, soluble starch 2g, agar 15g , pH 7.2 ± 0.1], respectively on to culture plates. Himedia brand readymade culture media were used for the enzymatic assays. The inoculated plates were incubated at 37ºC for 24 h and were observed for the production of the enzymes in the form of clear halo zone around the bacterial growth.

2. Quantitative Screening

The microbial isolates found positive for test enzyme production were subjected to qualitative screening of the

three enzymes amylase, protease and lipase. The microbial isolates positive for enzyme production were inoculated onto nutrient broth (NB) [Composition composition per liter- Peptone 10g meat extract, 10g, NaCl 5g, pH 7.3±0.1) tubes and were incubated at 37ºC for 12 h under shaking conditions.

Two per cent of 12 h old seed culture of *Streptomyces* isolate was inoculated in 250 ml Erlenmeyer flasks containing 50 ml of amylase (composition per liter : Beef extract 3g, peptic digest of animal tissue 5g, soluble starch 2g, pH 7.2 ± 0.1) or protease (composition per liter- Skim milk powder 20g, casein enzyme hydrolysate 5g, yeast extract 2.5g, dextrose 1g, pH 7.0 ± 0.2)or lipase (composition per liter:Peptone 10g, yeast extract 5g, sodium sulphate 2g, K_2HPO_4 3g, $KH_2PO_4$1g, $MgSO_4.7H_2O$ 0.1g, glucose 2g, olive oil 10 ml, pH 7.0 ± 0.2) production medium. The inoculated flasks were incubated at 37ºC and shaking of150 rpm for 48 h.

The cells of each culture were removed from respective fermentation broth by centrifugation at 10,000 rpm for 10 minutes after 48 h of incubation and supernatants were collected and examined for enzyme activities. The enzyme activities were determined using two approaches *viz.*, Gel diffusion assay and Quantitative enzyme assay.

Table 1: *Streptomyces* isolates collected from different locations of Jodhpur district, Rajasthan.

S. No.	Isolate	Name of isolate	Collection site	Soil type
1	ITD-5	*Streptomyces rochei*	University campus	Sandy Loam
2	ITD-6	*S. rochei*	AFRI campus	Sandy Loam
3	ITD-7	*S. espinosus*	AFRI campus	Sandy Loam
4	ITD-8	*S. gancidicus*	CAZRI campus	Sandy Loam
5	ITD-9	*S. gancidicus*	CAZRI campus	Sandy Loam
6	ITD-10	*S. werraensis*	CAZRI campus	Sandy Loam
7	ITD-11	*Streptomyces sp.*	CAZRI campus	Sandy Loam
8	ITD-12	*S. albogriseolus*	Soorsagar	Sandy Loam
9	ITD-13	*S. variabilis*	Soorsagar	Sandy Loam
10	ITD-14	*S. enissocaesilis*	Mandore	Sandy Loam
11	ITD-15	*S. gancidicus*	Mandore	Sandy Loam
12	ITD-16	*S. griseorubens*	Mandore	Sandy Loam
13	ITD-17	*S. coelicolor*	Kailana	Sandy Loam
14	ITD-18	*S. werraensis*	Kailana	Sandy Loam
15	ITD-19	*S. cyaneus*	Machiya Safari Park	Sandy Loam
16	ITD-20	*S. cyaneus*	Mathania	Sandy Loam
17	ITD-21	*S. flavomacrosporus*	Tiwri	Sandy Loam
18	ITD-22	*S. griseorubens*	Tiwri	Sandy Loam
19	ITD-23	*Streptomyces sp.*	Tiwri	Sandy Loam
20	ITD-24	*S. rubrogriseus*	Osian	Sandy
21	ITD-25	*S. albogriseolus*	Osian	Sandy

Gel diffusion assay

The supernatants of each culture were inoculated in the wells bored in TbA (Tributyrin agar), SkMA (Skimmed milk agar) or StA (Starch agar), depending on the expected enzyme produced by a particular culture based on the result of the qualitative screening.

Quantitative assay

The enzymatic activities of the test isolates for amylase, protease and lipase were determined by the methods suggested by Sudharhsan *et al.* (2007), Meyers and Ahearn (1977) and Winkler and Stuckmann (1979), respectively.

Results and Discussion

In the present study, 21 *Streptomyces* isolates isolated from the soil samples collected from Jodhpur district were assigned culture codes (ITD-5 to ITD-25). The results of quantitative screening shown in the form of gel diffusion assay clearly indicated the production of the three enzyme *viz*. amylase, protease and lipase by different *Streptomyces* strains. The sizes of hydrolytic zones are approximately in accordance with the quantitative production of these enzymes. Out of these 21 *Streptomyces* isolates, 10 isolates (ITD-5, 6,7, 11, 16, 17, 20, 22, 24 and 25) produced the all three enzyme *i.e.* amylase, protease and lipase, whereas nine isolates (ITD-8, 9, 10, 12, 13, 15, 19, 21 and 23) produced amylase and protease, one isolate (ITD-14) showed only amylase activity while isolate ITD-18 had lipase activity (table 2).

Different strains belonging to the same *Streptomyces* species (ITD-10 & 18, ITD-12 & 25, 19 & 20) produced different enzymes while same enzymes produced by different strains of taxonomically identical species (ITD-5 & 6, ITD-8, 9 & 15, ITD-16 & 22).This inconsistency of biochemical enzymes assays indicates the strain specific enzyme production ability within *Streptomyces* species. Strain specific biochemical potential of metabolites production has been reported by other researchers (Krieg, 2005; Larsen *et al*. 2005; Mangamuri *et al*. 2016).

The 16S rRNA sequences polymorphism might occur at isolates level making it useful for phylogeny, evolution and biogeographically diversity studies (Rajwar and

Table 2: Quantitative screening of *Streptomyces* isolates for amylase, protease and lipase enzymes.

S. No.	Microorganism	Enzyme activity (U/ml)		
		Amylase	Protease	Lipase
1.	*Streptomyces rochei ITD-5*	112	155.4	10.1
2.	*S. rochei ITD-6*	39	24.8	6.9
3.	*S. esespinosus ITD-7*	26	20.7	8.6
4.	*S. gancidicus ITD-8*	42	36.3	-
5.	*S. gancidicus ITD-9*	57	32.5	-
6.	*S. werraensis ITD-10*	32	16.5	-
7.	*Streptomyces* sp. *ITD-11*	36	20.4	6.5
8.	*S. albogriseolus ITD-12*	208	28.9	-
9.	*S. variabilis ITD-13*	116	35.7	-
10.	*S. enissocaesilis ITD-14*	54	-	-
11.	*S. gancidicus ITD-15*	34	42.4	-
12.	*S. griseorubens ITD-16*	49	28.2	5.9
13.	*S. coelicolor ITD-17*	22	15.2	5.0
14.	*S. werraensis ITD-18*	-	-	6.8
15.	*S. cyaneus ITD-19*	132	42.5	-
16.	*S. cyaneus ITD-20*	127	37.5	3.7
17.	*S. flavomacrosporus ITD-21*	196	135.3	-
18.	*S. griseorubens ITD-22*	148	29.6	4.3
19.	*Streptomyces* sp. *ITD-23*	134	32.9	-
20.	*S. rubrogriseus ITD-24*	122	12.4	7.7
21.	*S. albogriseolus ITD-25*	97	17.6	7.0

Sahgal, 2016). The amylase production activity was detected in the all the *Streptomyces* isolates tested except *S. werraensis* (ITD-18) similarly, protease production was observed with all the isolates except *S. enissocaesilis* (ITD-14) and *S. werraensis* (ITD-18) showing the prevalence of amylase and protease enzyme within the *Streptomyces* isolates of desert ecosystem of Rajasthan. The lipase production was observed in lesser isolates as compared to amylase and protease. It was present in ten out of 21 *Streptomyces* isolates (table 2). Among these *S. espinosus, S. werraensis, S. albogriseolus, S. variabilis, S. enissocaesilis, S. griseorubens, S. flavomacrosporus* and *S. rubrogriseus* have not been reported for amylase production earlier. The highest amount (208 U/ml) of amylase was produced by *S. albogriseolus* ITD-12 followed by *S. flavomacrosporus* ITD-21(196 U/ml) and *S. griseorubens* ITD-22 (148 U/ml). These enzyme titer values are significant and show that they are potential candidates for industrial production of amylase. Out of 19 protease producers, three isolates *S.espinosus, S. flavomacrosporus* and *S. rubrogriseus* are new reports. Among these new producers the highest (135.3 U/ml) titer value for protease is from *S. flavomacrosporus* ITD-21, followed by *S. espinosus* ITD-5 (20.7 U/ml), *S. rubrogriseus* ITD-24(12.4 U/ml).The highest protease production was observed from *S. rochei* ITD-5 (155.4 U/ml).There are six new producers of lipase *viz. S.espinosus* ITD-7, *S. griseorubens* ITD-22, *S. cyaneus* ITD-20, *S. rubrogriseus* ITD-24 and *S. albogriseolus* ITD-25. Among these the highest (8.6 U/ml) titre value for lipase is from *S.espinosus* ITD-7. However, among all the lipase producers, the highest (10.1 U/ml) titre value for lipase is recorded from *S. rochei* ITD5.

Conclusion

In all, 21 molecularly characterized *Streptomyces* isolates were tested for their biocatalytic potential of industrial important enzymes *viz.*, amylase, protease and lipase. Several *Streptomyces* isolates are hithereto unknown for production of these important enzymes and are being reported for the first time for their industrial potential. The findings are of significance to microbial biocatalytic potential.

References

Aiyer, P.V. (2005). Amylases and their applications. *African J. Biotechnol*. **4 (13)**:1525-1529.

Arifuzzaman, M., M.R. Khatun and H. Rahman (2010). Isolation and screening of actinomycetes from Sundarbans soil for antibacterial activity. *African J. Biotechnol*. **9**: 4615-4619.

Binod, P., R.R. inghania, C.R. Soccol and A. Pandey (2008). Industrial enzymes. In: Pandey, A., C. Larroche, C. R. Soccol and C. G. Dussap (Eds.) Advances in Fermentation Technology, Asiatech Publishers, New Delhi, India, pp 291–320.

Cowan, D. (1996). Industrial enzyme technology. *Trends Biotechnol*, **14**: 177-178.

Euzeby, J.P. (2008). Genus *Streptomyces*. List of prokaryotic names with standing in nomenclature.http:// www. bacterio.cict.fr/s/streptomyces.html.

Fita, A., A. Rodríguez-Burruezo, M. Boscaiu, J. Prohens and O.Vicente (2015). Breeding and domesticating crops adapted to drought and salinity: a new paradigm for increasing food production. *Front. Plant Sci*., **6**: 978 doi:10.3389/fpls.2015.00978.

Gaur, D., P.K. Jain and V. Bajpai (2012). Production of Extracellular á-Amylase by Thermophilic *Bacillus* sp. Isolated from Arid and Semi-arid Region of Rajasthan, *Indian J. Microbiol.Biotech. Res*., **2(5)**: 675-684.

Godfrey, T. and S.West (1996). Introduction to industrial enzymology. In: Godfrey, T. and S.West (Eds.), Industrial enzymology, 2nd edn. Macmillan Press, London, pp 1–8

Gupta, R., Q.K. Beg and P. Lorenz (2002). Bacterial alkaline proteases: molecular approaches and industrial applications. *Appl. Microbiol. Biotechnol,* **59**: 15–32.

Hasan, F., A.A. Shah and A. Hameed (2006). Industrial applications of microbial lipases. *Enzyme Microb. Technol*, **39**: 235–251.

Holt, J.G., H.R. Krieg, P.H.A. Sneath, J.T. Stacey and S.T. Williams (1994). Beygey's manual of determinative bacteriology, 9thEdn. Baltimore: Williams and Wilkins.

Kampfer, P. (2012). Genus *Streptomyces*. In: Goodfellow, M., P. Kampfer, H. J. Busse, M. Trujillo, K. I.Suzuki, W.Ludwig and W. B.Whitman (Eds.) Bergey's Manual of Systematic Bacteriology, 2nd edn, vol. 5, The *Actinobacteria*, Springer, New York, pp 1455–1804.

Kekuda, T.R.P., K.S.Shobha and R. Onkarappa (2010). Fascinating diversity and potent biological activities of actinomycetes metabolites. *J. Pharm. Res*., **3**: 250-256.

Kim, M.H., H.K. Kim, J.K. Lee, S.Y. Park and T.K. Oh (2000). Thermostable lipase of *Bacillus stearothermophilus*: high-level production, purification and calcium-dependent thermostability. *Biosci. Biotechnol. Biochem*, **64**: 280-286.

Kirk, O., T.V. Borchert and C.C. Fuglsang (2002). Industrial enzyme applications. *Curr. Opini. Biotechnol,* **13**: 345-351.

Krieg, N.R. (2005). Identification of prokaryotes. In: Brennner D.J., Krieg N.R., Staley J.T. (eds), Bergey's manual of Systematic Bacteriology second edition vol. 2 part: A Introductory essays, Springer.

Kumar, C.G. and H.Takagi (1999). Microbial alkaline protease from a bio industrial view point. *Biotechnol. Adv*., **17**: 561-94.

Kumar, S., E. Priya, D.S. Solanki, R. Sharma, P. Gehlot, R. Pathak and S.K. Singh (2016). Occurrence and characterization of hitherto unknown *Streptomyces* species in semi-arid soils. *J. Environ. Biol*., **37(5)**: 927-936.

Larsen, T.O., J. Smedsgaard, K.F. Nielsen, M.E. Hansen and J.C. Frisvad (2005). Phenotypic taxonomy and metabolite profiling in microbial drug discovery. *Natural Products Rep*., **22**: 672-695.

Lee, D.W., H.K. Kim, K.W. Lee, B.C. Kim, A.C. Choe and H.S. Lee (2001). Purification and characterization of two distinct thermostable lipases from the gram-positive thermophilic bacterium *Bacillus thermoleovorans* ID-1. *Enz. Microbiol.Technol*, **29:** 363-371.

Leisola, M., J. Jokela, O. Pastinen, O. Turunen and H. Schoemaker (2002). Industrial use of enzymes. In: Hanninen, O. O. P. and M. Atalay (Eds.), Encyclopedia of Life Support Systems (EOLSS), EOLSS, Oxford, UK, pp 1–25.

Maleki, H., A. Dehnad, S. Hanifian and S. Khani (2013). Isolation and molecular identification of *Streptomyces* spp. with antibacterial activity from Northwest of Iran. *BioImpacts,* **3(3)**: 129-134.

Mangamuri, U., V. Muvva, S. Poda, K. Naragani, R.K. Munaganti, B. Chitturi and V. Yenamandra (2016). Bioactive metabolites produced by *Streptomyces Cheonanensis* VUK-A from Coringa mangrove sediments: isolation, structure elucidation and bioactivity. 3 Biotech.6:63 DOI 10.1007/s13205-016-0398-6.

Meghwanshi, G. K. and A. Vashistha (2012). Microbial enzymes: production and applications. In: Kapoor, B.B.S. and A. Arora (Eds.), Recent Trends in Microbiology, Madhu Publication, Bikaner. ISBN: 81-86644-23-7.

Meyers, S.P. and D.G. Ahearn (1977). Extracellular proteolysis by Candida lipolytica. *Mycologia,* **69**: 646651.

Naine, J., M.V. Srinivasan and S.C. Devi (2011). Novel anticancer compounds from marine actinomycetes: a review. *J. Pharm. Res*., **4** :1285-1287.

Narayana, K.J.P. and M. Vijayalakshmi (2009). Chitinase production by *Streptomyces* sp. ANU 6277. *Brazilian J. Microbiol,* **4**: 725-33.

Niehaus, F., C. Bertoldo, M. Kähler and G. Antranikian (1999). Extremophiles as a source of novel enzymes for industrial application. *Appl. Microbiol. Biotechnol,* **51(6)**: 711-29.

Norus, I. (2006). Building sustainable competitive advantage from knowledge in the region: the industrial enzymes industry. *Eur. planning Studies,* **14(5)**: 681-696.

Poulsen, P.B. and H.K. Buchholz (2003). History of enzymology with emphasis on food production. In: Whitaker, J.R., A.G.J. Voragen and D.W.S. Wong (Eds.), Handbook of Food Enzymology, Marcel Dekker, New York, NY, USA pp. 11–20

Rajasekar, M., G.A. Rabert and P. Manivannan (2015). Triazole induced changes on biochemical and antioxidant metabolism of *Zea mays* L. (Maize) under drought stress. *J. Plant Stress Physiol,* **1(1)**: 35-42.

Rajwar, A. and M. Sahgal (2016). Phylogenetic relationships of fluorescent pseudomonads deduced from the sequence analysis of 16S rRNA, *Pseudomonas*-specific and *rpo* D genes. *3 Biotech,* **6(1)**: 80.doi: 10.1007/s13205-016-0386-x.

Sajid, I., K.A. Shaaban and S. Hasnain (2011). Identification, isolation and optimization of antifungal metabolites from the *Streptomyces Malachitofuscusctf 9*. *Brazilian J. Microbiol*, **2**: 592-604.

Saxena, R.K., L. Agarwal and G.K. Meghwanshi (2005). In: Satyanaryana T, Johri BN (eds), Diversity of fungal and yeast lipases: present in future scenario for the 21st century, I.K. International Pvt. Ltd, New Delhi, pp 796-814.

Saxena, R.K., P.K. Ghosh, R. Gupta, W.S. Davidson, S. Bradoo and R. Gulati (1999). Microbial lipases: potential biocatalyst for the future industry. *Curr. Sci.,* **77**: 101-115.

Schafer, T., T.V. Borchert and V.S. Nielsen (2006). Industrial eznzymes. *Adv. Biochem. Eng/Biotechnol,* **105**: 59-131.

Seong, C.N., J.H. Choi and K.S. Baik (2005). An improved selective isolation of rare actinomucetes from forest soil. *J. Microbiol,* **39**: 17-23.

Sharma, N., G. Singh and Y. Sudarsan (2013a). Assessment of microbial diversity under arid plants by culture-dependent and culture-independent approaches. *African J. Biotechnol*, **12**: 5860-5868.

Sharma, R., R. Manda, S.Gupta, S. Kumar and V. Kumar (2013b). Isolation and characterization of osmotolerant bacteria from Thar Desert of Western Rajasthan (India). *Rev. Biol. Trop*. (*Int. J. Trop. Biol*.), **61(4)**: 1551-1562.

Sudharhsan, S., Senthil Kumar, S. and K. Ranjith (2007). Physical and nutritional factors affecting the production of amylase from species of *Bacillus* isolated from spoiled food waste. *Afr. J. Biotechnol*, **6:** 430-435.

Tiwari, K., D.J. Upadhyay, E. Mösker, R. Süssmuth and R.K. Gupta (2015).Culturable bioactive actinomycetes from the Great Indian Thar Desert. *Ann. Microbiol,* **65**: 1901-1914.

Uyar, F. and Z. Baysal (2004). Production and optimization of process parameters for alkaline protease production by a newly isolated *Bacillus* sp. under solid-state fermentation. *Process Biochem,* **39**: 1893-1898.

Ventura, M., C. Canchaya, A.Tauch, G. Chandra, G.F. Fitzgerald, K.F. Chater and D. Van Sinderen (2007). Genomics of Actinobacteria: Tracing the evolutionary history of an ancient phylum. *Microbiol. Mol. Biol. Rev*., **71(3)**: 495-548.

Willey, J.M., L.M. Sherwood and C.J. Woolverton (2008). Prescott, Harley and Klein's Microbiology: An introduction, 7th Edn. USA: McGraw Hill Higher Education.

Winkler, U.K. and M. Stuckman (1979). Glycogen, hyaluronate, and some other polysaccharides greatly enhance the formation of exolipase by *Serratia marcescens*. *J. Bacteriol,* **138**: 663-679.