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In-vitro Screening of Actinobacteria Isolated from Natural Ecosystem for Production of Hydrolytic Enzymes

C. Bindushree, M. K. Shivaprakash and L. Krishna Naik
Department of Agricultural Microbiology, College of Agriculture, UAS, GKVK, Bengaluru - 560 065
e-Mail : shreebindu12.bs@gmail.com

Authors Contribution

C. Bindushree :
Design of work, data collection, data analysis, interpretation and Drafting the article;
M. K. Shivaprakash :
Design of the work and critical revision;
L. Krishna Naik :
Design of the work and final approval

Corresponding Author :
C. Bindushree
Department of Agricultural Microbiology, College of Agriculture, UAS, GKVK, Bengaluru

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Abstract

Actinobacteria are considered as a promising source for novel bioactive compounds and hydrolytic enzymes with a broad range of biological activities. Isolation and screening of actinobacteria for hydrolytic enzyme production was carried out and a total of one hundred and twenty isolates were obtained from different ecosystem like forest soil, compost and rhizosperic soils of vegetable crops. Out of 120 isolates, 39 showed amylase activity with dissolution factor varied from 0.10 - 3.50. Twenty five isolates exhibited cellulase activity with the dissolution factor ranged from 0.10-3.50. The actinobacteria isolate CA32 from compost showed maximum cellulolytic activity with dissolution factor of 3.50. Twenty one actino bacterial isolates showed chitinolytic activity ranging from 0.10-2.00 and maximum dissolution factor was recorded by isolate of rhizosperic soil SA12 (2.00) followed by the isolate of compost CA4 (1.43). Twenty six isolates exhibited lipolytic activity with the dissolution factor ranging from lowest 0.11 in SA22 to highest 1.64 in FA12. The hydrolysis of complex organic compounds by actinobacterial isolates in-vitro showed that the actinobacteria are good degraders of organic matter in the soil.

Keywords : Hydrolytic enzymes, Actinobacteria, Amylase, Cellulase, Chitinase, Dissolution factor

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Enzymes produced by microoganisms are considered as potential biocatalysts for a large number of reactions. Enzymes derived from microbial source are generally regarded as safe. Theses enzymes also remain functional at wide range of temperature, pH, salinity and other extreme conditions. Actinobacteria are one of the most diverse groups of microorganisms that are well characterized and recognized for their metabolic versatility. They play a vital role in decomposition of organic matter like, cellulose, chitin and pectin. Therefore they play an important part in carbon cycle and also to maintain the soil structure (Fodil *et al*., 2011). Majority of actinobacteria are saprophytic in nature. Decomposition by actinobacteria is aided by the synthesis of various classes of extracellular enzymes like nucleases, lipases, glucanases, xylanases, amylases, proteinases, peptidases, peroxidases, chitinases, cellulases, ligninases and keratinases. All these enzymes together also contribute for biocontrol potential against a wide range of phytopathogens, because the cell wall of most fungal and bacterial pathogens consists of polymers such as chitin, glucan, cellulose, proteins and lipids (Lim and Cha, 2000).

Actinobacteria represent a high proportion of the soil microbial biomass and have the capacity to produce a wide variety of antibiotics and of extracellular enzymes (Doumbou *et al*., 2001). Among the enzymes produced by actinobacterial isolates, chitinases are of great importance and many *Streptomyces* spp. are observed to inhibit both fungal pathogens and insect

pests (Tahmasebpour *et al*., 2014). While chitinolytic enzymes are distributed in all kingdoms of life, actinobacteria are recognized as particularly good decomposers of chitinous material and several members of this taxon carry impressive sets of genes dedicated to chitin and chitosan degradation. Degradation of these polymers in actinobacteria is dependent on endo and exo-acting hydrolases as well as lytic polysaccharide mono oxygenases.

Actinobacteria can metabolize many different compounds including sugars, alcohols and amino acids. Additionally, many of them (e.g. *Streptomyces* and *Rhodococcus*) produce extracellular hydrolytic enzymes to obtain nutrients from cellulose, hemicellulose, proteins and fats (Trujillo *et al*., 2008). Many previous research works revealed the ability of actinobacteria isolated from various habitats to produce cellulase. Reports have shown cellulase production by different actinobacteria belonging to *Cellulomonas*, *Streptomyces*, *Micromonospora*, *Actinopolyspora*, *Actinoplanes*, *Microbispora*, *Thermomonospora*, *Rhodococcus*, *Nocardia* and *Thermoactinomyces* genera (Nguyen *et al*., 2020).

Material and Methods

Isolation of Actinobacteria from the Natural Ecosystem

Collection of Samples from the Natural Ecosystem

Samples of forest soils were collected from the Biodiversity hotspot of GKVK, UAS, Bangalore, Chamarajanagar and BR (Biligiri ranagana) Hills. Different types of compost like, spent mushroom compost, coir pith compost, vermi-compost, FYM were collected and rhizospheric soils of solanaceous crops like tomato, brinjal, capsicum, and chilly were used for isolation of actinobacteria.

Isolation and Purification of Actinobacteria

Isolation of actinobacteria from samples was done by serial dilution followed by pour plate method. Ten gram of sample was suspended in to 90 ml sterile water blank and shaken on a mechanical shaker for 15 minutes. Further, a ten-fold dilution series was prepared by transferring 10 ml of aliquots of suspension each time to 90 ml sterile water blanks till 10^{-5} dilutions were obtained. The contents in the flasks were shaken between each transfer to ensure uniform suspension. One ml aliquot from the desired dilution was transferred to sterile Petri-plates and starch casein agar (SCA) was poured into plates and was incubated for seven to ten days. The isolated colonies of the actinobacteria in dilutions of 10^{-3} to 10^{-4} were picked and streaked on freshly prepared starch casein agar medium and then individual colonies were sub-cultured on SCA slants and stored at 4 °C. Cultures were maintained by sub-culturing after every 15 days (Tian *et al*., 2004).

Screening of Actinobacteria for Hydrolytic Enzyme Production

Amylase Production

One gram of potato starch suspended in 10 ml of distilled water was added to 90 ml of nutrient agar, autoclaved at 121 °C for 15 minutes and poured into Petri plates. The actinobacterial isolates streaked on nutrient agar containing starch and kept for incubation for 4 days. Then plates containing streaked colonies were flooded with Gram's iodine solution. A clear zone surrounding the colony indicates the hydrolysis of starch (Jones *et al*., 1979).

Cellulase Production

Actinobacteria was tested for the presence of cellulase enzyme on cellulose agar minimal medium. The media was used according to Samanta *et al*. (1989). The actinobacterial isolates were streaked on cellulose agar minimal medium containing cellulose and incubated at 30 °C for 2 to5 days. After incubation plates were flooded with 0.2 per cent aqueous Congo red and de-stained with 1M NaCl for 15 minutes. The plates were observed for the presence of clear zones surrounding the colonies after 30 minutes. The clear zone surrounding the colony indicated cellulose activity.

Chitinase Production

The actinobacterial isolates were spot inoculated on the colloidal chitin minimal salt agar medium

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supplemented with 1 per cent chitin and incubated at 28 °C for 7 to 14 days. The plates were flooded with 0.5 per cent Congo red solution. The plates were observed for the presence of clear zones surrounding the colonies after 30 minutes. A clear zone surrounding the colony indicated chitinase activity (Taechowisan *et al*., 2003).

Lipase Production

Hydrolysis of tween 80 by actinobacteria was done by supplementing nutrient agar with tween-80 (1% w/v) and $CaCl_2$ (0.01% w/v) and autoclaved at 121 °C for 15 minutes and poured into Petri plates. Browning of the medium around the colony is an indication of lipase production and utilization of tween-80 as carbon source by actinobacteria (Jones *et al*., 1979).

Results and Discussion

Isolation of Actinobacteria from Natural Ecosystem

A total of one hundred and twenty actinobacterial isolates were obtained from different natural ecosystem (Table1). The most abundantly occurring actinobacterial genus in soil is *Streptomyces*. The genera, *Nocardia*, *Micromonospora* and *Streptosporangium* are less abundant. Earlier the actinobacterial isolates were isolated from different ecosystem ranging from rhizospheric soil of vegetable crops such as tomato, brinjal, chilli and capsicum (Dias *et al*., 2017) and from mangrove soil collected from Nellore region of Andhra Pradesh, India (Janardhan *et al*., 2013).

Table 1

Isolation of actinobacterial from natural ecosystem

Source	Location	Total isolates
Soil		
	GKVK	10
	Mandya	9
	Raichur	6
	Kolar	8
	Hassan	8
Forest soil		
	BR Hills	11
	Chamarajanagar	13
	GKVK Biodiversity hotspot	15
Compost		
Spent mushroom compost	IIHR	11
Vermicompost	IIHR GKVK	10
FYM	GKVK	8
Coir pith compost	IIHR	11
	Total	120

Screening of Actinobacteria for Hydrolytic Enzyme Production

Amylase Production

Alpha-amylase, starch degrading amylolytic enzymes are of great significance in biotechnological applications ranging food, fermentation, textile to paper industries (Janaki, 2017). Out of 120 isolates, 39 showed the amylase activity with dissolution factor for amylolytic activity varied from 0.10-3.50. Maximum amylolytic activity was displayed by actinobacteria SA21 and CA35 (DF = 3.50), followed by FA33 (DF = 2.50) (Table 2). The results of the present study are in accordance with Gulve and Deshmukh (2011) who have isolated 90 actinobacteria, among which 65 (72.22%) exhibited amylase activity. Jain and Chen (2003) have evaluated seventeen antagonistic strains of actinobacteria for amylase producing potential and found *Streptomyces somaliensis* GS 1242 and *Streptomyces sampsonii* GS 1322 to have with highest amylase producing potentials. Likewise, twenty five actinobacteria obtained from rhizospheric soil of *Capsicum annum* L. showed ability to degrade starch (Ashokvardhan *et al*., 2014 and Kesarwani and Sharanappa, 2009). Sixty seven *Streptomyces* sp. were identified by Jaralla *et al*. (2014), from which forty isolates (SA2, SK5, SM42 and SI63) were able to produce amylase.

TABLE 2
Screening of actinobacteria for amylase production

Isolates	Zone diameter (cm)	Colony diameter (cm)	Dissolution factor
SA 1	2.90	2.00	0.45
SA 2	3.20	2.90	0.10
SA 5	2.20	1.80	0.22
SA 6	1.60	1.00	0.60
SA 10	2.50	2.00	0.25
SA 11	2.40	2.00	0.20
SA 12	1.70	0.50	2.40
SA20	1.10	0.70	0.57
SA21	0.90	0.20	3.50
SA32	3.50	2.80	0.25
FA4	3.00	2.00	0.50
FA5	3.40	2.60	0.31
FA13	0.90	0.30	2.00
FA15	1.00	0.50	1.00
FA17	1.10	0.70	0.57
FA18	1.00	0.75	0.33
FA22	1.00	0.50	1.00
FA23	3.00	2.20	0.36
FA28	3.30	2.40	0.38
FA32	1.80	0.90	1.00
FA33	0.70	0.20	2.50
FA34	2.10	0.80	1.63
FA36	2.80	2.10	0.33
FA38	1.30	0.90	0.44
CA1	0.70	0.50	0.40
CA3	3.10	2.50	0.24
CA4	1.10	0.90	0.22
CA6	2.90	2.20	0.32
CA7	4.75	3.20	0.48
CA13	1.40	1.00	0.40
CA15	2.30	1.70	0.35
CA18	1.00	0.40	1.50
CA25	2.30	1.30	0.77
CA29	2.90	1.60	0.81
CA34	3.30	2.60	0.27
CA35	0.90	0.20	3.50
CA37	1.10	0.80	0.38
CA38	2.00	1.00	1.00

Cellulase Production

Cellulose is the major component of plant biomass and potentially utilizable source of glucose, therefore, the process of microbial degradation of cellulose can be considered as economically viable. Cellulose can be degraded by microbial enzymes such as cellulase (Lynd *et. al*., 2002). Actinobacterial cellulases are inducible extracellular enzymes that can be produced during their growth on cellulosic materials. Twenty five isolates exhibited cellulase activity on nutrient agar medium supplemented with 1 per cent of Carboxyl methyl cellulose (CMC). The value of dissolution factor for cellulolytic activity ranged from 0.10-3.50 (Table 3). The isolate CA32 showed maximum cellulolytic activity with dissolution factor of 3.50, followed by CA18 having dissolution factor (2.6). Minimum cellulolytic activity was shown by SA2, CA28 and CA39 (0.10). The results are in concordance with Jog *et. al*. (2012) who screened actinobacteria for enzyme production, isolate *Streptomyces rochei* possessed highest cellulase activity (7.4 Umg^{-1}).

The results are also in line with Bui (2014) who has obtained 20 actinobacterial isolates from 15 coffee exocarp samples, from which 10 actinobacteria showed enzyme degradation of cellulose. Forty-two actinobacteria isolated from soil and compost samples were tested for the production of cellulolytic enzyme, from which 12 isolates produced clear zones (Prasad *et. al*., 2013). Mohanta (2014) found nine isolates of cellulose-degrading actinobacteria from different sediments samples of Mangrove forest. Cellulose production was reported in 18 actinobacterial isolates and the dissolution factor for cellulolytic activity ranged from 0.24 to 5.89 where, maximum cellulase production was showed by isolate WS-35 (DF=5.89) (Kamara, 2015). Eighty-five actinobacteria were isolated from vermicompost, out of which 12 isolates possessed the ability to degrade carboxy methyl cellulose (Sreevidya *et al*., 2016 and Yogananda *et al*., 2010). Similar studies on actinobacteria cellulases were also conducted by Janaki (2017).

TABLE 3

Screening of actinobacteria for cellulase production

Isolates	Zone diameter (cm)	Colony diameter (cm)	Dissolution factor
SA 2	2.30	2.10	0.10
SA 5	2.00	1.70	0.18
SA 13	1.40	1.00	0.40
SA 22	0.70	0.50	0.40
FA 1	1.40	1.00	0.40
FA 9	1.00	0.80	0.25
FA 12	2.80	2.20	0.27
FA17	1.14	0.90	0.27
FA19	2.60	2.20	0.18
FA26	2.00	1.60	0.25
FA32	2.00	1.80	0.11
FA33	0.80	0.50	0.60
CA1	1.50	1.20	0.25
CA5	2.60	2.20	0.18
CA7	2.60	2.40	0.08
CA12	1.00	0.50	1.00
CA18	1.80	0.50	2.60
CA23	2.10	1.80	0.17
CA28	2.20	2.00	0.10
CA32	1.80	0.40	3.50
CA33	1.20	0.50	1.40
CA35	2.40	2.00	0.20
CA37	1.00	0.60	0.67
CA38	2.00	1.70	0.18
CA39	2.20	2.00	0.10

Chitinase Production

Chitinases are industrially important enzymes belonging to glycosyl hydrolase group which can degrade chitin, an insoluble linear β-1, 4-linked polymer of N-acetylglucosamine into its end products *i.e.* N-acetylglucosamine, glucosamine and chitobiose. Among all microorganisms, about 90-99 per cent of chitinolytic organisms are actinobacteria (Jha *et al*., 2016). Twenty one actinobacterial isolates showed the chitinolytic activity ranging from 0.10-2.00 (Table 4). Maximum dissolution factor was recorded by isolate SA12 (2.00) followed by CA4 (1.43).

TABLE 4

Screening of actinobacteria for chitinase production

Isolates	Zone diameter (cm)	Colony diameter (cm)	Dissolution factor
SA 8	1.70	1.40	0.21
SA 12	1.80	0.60	2.00
SA 22	1.70	1.50	0.13
SA 24	1.00	0.50	1.00
SA 36	1.80	1.00	0.80
FA 10	3.00	2.80	0.07
FA 13	2.20	1.00	1.20
FA15	2.20	1.20	0.83
FA18	1.10	1.00	0.10
FA26	3.00	2.70	0.11
FA32	2.60	2.40	0.08
FA33	3.00	2.80	0.07
CA4	1.70	0.70	1.43
CA5	1.70	1.20	0.42
CA18	2.40	1.80	0.33
CA20	2.20	2.00	0.10
CA28	3.20	2.60	0.23
CA33	3.00	2.50	0.20
CA37	1.50	1.00	0.50
CA38	1.10	1.00	0.10
CA39	3.20	2.80	0.14

The present findings were in corroboration with Narayana and Vijayalakshmi (2009) who have studied actinobacteria, *Streptomyces viridificans* strain ANU 6277 producing maximum chitinase in medium amended with 1 per cent chitin. Thirty actinobacterial strains from agricultural fields of Egypt were evaluated for chitinase activity whereas only 5 per cent of isolates exhibited formation of clear zones. Two isolates named S1 and S2 showed highest zones of 1.1 and 0.9 cm, respectively (Goma, 2012). Nineteen isolates were found positive for chitinase production by Passari *et al*. (2017). Among 19 isolates, chitin degrading activity was found to be high in *Leifsonia xyli* 24 and *Microbacterium* sp. 21 which exhibited a degradation zone of 15 and 17mm, respectively. Kamboj *et al*. (2017) also evaluated

actinobacterial isolates for chitinolytic activity, where two isolates produced distinct zone of more >5 mm diameter.

Lipase Production

Among the 120 isolates, 26 actinobacterial isolates exhibited lipolytic activity with the dissolution factor ranging from lowest 0.11 in SA22 to highest 1.64 in FA12 (Table 5). Our results are in conformity with Selvam *et. al*. (2011) who have isolated fifty-six actinobacteria from marine sediments of India and among these only 3 strains exhibited lipolytic activity.

TABLE 5

Screening of actinobacteria for lipase production

Isolates	Zone diameter (cm)	Colony diameter (cm)	Dissolution factor
SA 1	2.50	1.60	0.56
SA 8	3.00	2.20	0.36
SA 12	0.90	0.40	1.25
SA 22	3.00	2.70	0.11
FA 1	2.60	2.00	0.30
FA 8	1.40	0.90	0.56
FA 12	4.75	1.80	1.64
F17	2.00	1.40	0.43
A23	1.70	1.00	0.70
FA26	1.90	1.00	0.90
FA32	3.00	2.50	0.20
FA33	3.00	2.00	0.50
CA2	2.80	2.20	0.27
CA5	1.00	0.50	1.00
CA7	1.10	0.60	0.83
CA12	3.50	2.20	0.59
CA18	3.50	2.30	0.52
CA20	3.80	2.20	0.73
CA22	1.00	0.40	1.50
CA26	2.20	1.20	0.83
CA27	3.00	2.50	0.20
CA29	5.20	3.00	0.73
CA32	3.40	2.50	0.36
CA34	1.40	0.80	0.75
CA37	1.00	0.40	1.50
CA 39	1.00	0.60	0.67

Twenty, out of 33 actinobacterial isolates were also reported to produce lipase enzymes on tributyrin agar and Tween 80 agar media (Aly *et. al*., 2012). Gopalakrishnan *et. al*. (2013) found all the five strains of *Streptomyces* obtained from herbal vermicompost to exhibit lipolytic activity. Similar studies were conducted by Singh and Padmavathy (2014) who have reported that 60 per cent lipase activity by occurrence of hydrolytic zone around the colonies. A total of 22 isolates out of 35 were found to produce lipase enzyme with the dissolution factor (DF) ranging from lowest 0.33 in CR-4 (DF=4.80) and minimum was obtained from CR-35 (DF=0.33) (Kamara, 2015). Sreevidya *et. al*. (2016) reported four actinobacterial strains with ability to produce cellulase, lipase, protease, β-1, 3- glucanase and chitinase enzymes. Of the four isolates, SAI-13 produced highest amounts of lipase.

Microbial enzymes play a key role as metabolic catalysts, leading to their diverse applications and use in various industries. The constant search for novel microbial enzymes has led to improvisations in the industrial processes which is the key for profit growth. Actinobacteria form a significant group of microbial populations in soil, plant tissues and marine ecosystems. Actinobacteria produce many valuable extracellular enzymes like cellulases, proteases, amylases, lipases, chitinases and pectinases which can decompose a variety of organic materials.

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