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Isolation of Actinomycetes for Chitinase Production

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Abstract

One hundred and three isolates of actinomycetes were isolated from ten soil samples by serial dilution technique and spread plate technique on starch casein agar. The samples were collected from five soil underneath animal dung and five different rhizosphere soils. Soil samples were screened for actinomycetes by air dry treatment at room temperature (37±2^{O}C) for 2-3 days, dry heat treatment at 110 ^{O}C for 1 hour and moist heat treatment at 50 ^{O}C for 15 minute. The largest number of actinomycetes were found by moist heat treatment (18.76x10^{6}CFU/ml), dry heat and air dry respectively. The identification of isolates of actinomycetes was done following Bergey's Manual of Determinative Bacteriology (1994). Isolates of actinomycetes were identified as a species of the genus Streptomyces (97 isolates) and genus Microbispora (6 isolates). Chitinase production from isolates of actinomycetes by submerged fermentation 1% colloidal chitin was used in the liquid medium. Amongst the isolates of actinomycetes, the isolate Ba2-3 showed highest chitinase activity (0.387 unit) and specific chitinase activity (9.675 unit/mg protein).

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Keywords: actinomycetes; chitinase; chitin

1. Introduction

Actinomycetes are grame positive bacteria, in the shape of single cell till the spread branch (branched mycelium) and there small cells are broken into short pieces which one symbol like the fungi fibers but are smaller as they are 0.5-12 micrometer in diameter, apart than that actinomycetes reproduction by asexual spore called as conidia[1]. Actinomycetes can spread in the soil, compost, or those decay things etc. Actinomycetes is aprophyte microorganism which is found much in the upper layer and the lower layer of the soil, even at the very deep soil Actinomycetes can be found. Actinomycetes are very important in ecosystems as digestion of carcass that made soil fertile [2]. Actinomycetes are important for industry of manufacturing such as antibiotics and enzyme etc. Actinomycetes can produce many enzyme such as xylanase, cellulase, amylase and chitinase especially enzyme chitinase can digest chitin. Most of Actinomycetes in the group of Streptomyces produce enzyme chitines [3].

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Chitinase is enzyme to digest chitin at the obligation area ß-(1,4) glycoside of N-acetylglucosamine (GlcNAc) results as oligomor of GlcNAc. Chitin is the essential structural component of fungal and insect pathogens of vascular plants. As it is absent from the vascular plants themselves, chitin could be used as a target molecule for fungicidal and insecticidal agents [4]. The cell wall of many phytopathogens contains chitin; hence, chitinases are exploited for their use as biocontrol agent [5]. Chitinase application increases the population of chitinolytic acinomycetes, fungi and bacteria. The increase is shown to be correlated with the reduction in pathogenic fungi, nematodes and insects and are important for reduction of infection and, hence, crop damage [6]. A biological control agent of fungal pathogen should exert a sufficient amount of antagonistic activity [2,7,8].

Accordingly, the major objectives of the present study were to, i) isolation actinomycetes from soil; ii) screening actinomycetes for production of chitinase; and iii) identification the isolates of actinomycetes.

2. Materials and Methods

2.1 Isolation actinomycetes from soil samples

The soils collected from underneath animal dung and rhizosphere soil. Soil samples were pretreated by 1) air dry for 1 to 3 days, 2) dry heat at 120 ºC for one hours and 3) moist heat at 50 ºC for 15 minute. Isolation of the actinomycetes from the soil samples was initially carried out by using serial dilution and spread plate technique on starch casein agar plate (SCA). The plates were incubated at 37 ºC for 5 to 7 days. The colonies of the actinomycetes developed on the agar surface had a firm consistency and adhered tenaciously to the solidified substratum. The surface of the colonies was powdery and often became pigmented when the spores were produced.

2.2 Screening actinomycetes for production of chitinase in colloidal chitin liquid medium

Chitinase production depended on the amount of chitinase produced under submerged fermentation. The isolates of the actinomycetes were grown on a starch casein agar plate at 30±2°C for 7 days until they produced mycelium with conidia. The mycelium with conidia incubated in a cork borer (5mm in dia) which was used as inoculum. Five discs of mycelium were inoculated into a 125-ml Erlenmeyer flask containing 80 ml of an enzyme production medium, which consisted of 1% chitin precipitated. After static incubation at 30±2°C for 7 days, the crude enzyme was obtained by centrifugation at 3,000 rpm/min for 10 min at 4°C and then was measured for chitinase activity by Miller method [9] and protein concentration by Lowry method [10].

2.3 Identification the isolates of actinomycetes

Morphological and culture properties of the isolates of the actinomycete were studied on starch casein agar, oat meal agar and peptone yeast extract iron agar medium. Characterization of the isolates was done with the help of standard literature [11,12,13,14,15]. Morphological observations were made visually for colony growth characteristic. Mycelial and sporulation characteristics were studied under Olympus BX 60 microscope. Purified isolates of the actinomycetes were identified up to the genera level by comparing their morphology of spore-bearing hyphae with entire spore chain as described in Martin (1977).

3. Results

3.1 Isolation actinomycetes from soil

Soil samples were collected from underneath animal dung and rhizosphere soil. The color of the samples was brown to black and type of soil was loamy and loam-sandy. One hundred and three isolates of actinomycetes were isolated from ten soil samples, 41 isolates from soil of rhizosphere soil, 62 isolates from soil underneath

animal dung. Number of the actinomycetes presented in soil pretreated by moist heat more than dry heat and air dry respectively; see Table 1. The colonies of actinomycetes developing on starch casein agar were powdery and often became pigmented when the spores were produced; see figure 1.

Table 1. Total number of the isolates cultured from the soil sample by pretreatment

Soil samples	Colony forming unit per ml x10^6			Total number of the isolates
	Pretreatment			
	Air dry	Dry heat	Moist heat	
rhizosphere soil A1	6.70	3.80	2.15	41
rhizosphere soil A2	1.26	1.60	1.28	
rhizosphere soil A3	1.06	4.30	1.70	
rhizosphere soil A4	7.20	3.20	7.80	
rhizosphere soil A5	4.70	<30	2.69	
soil underneath animal dung B1	2.54	2.96	2.35	62
soil underneath animal dung B2	1.02	>300	>300	
soil underneath animal dung B3	2.03	1.29	2.25	
soil underneath animal dung B4	2.52	3.50	7.60	
soil underneath animal dung B5	1.14	>300	1.40	
average	13.43	14.07	18.76	

<30, less than 30 colonies

>300, more than 300 colonies

Fig. 1. Characteristics of the colonies of the actinomycetes on starch casein agar

3.2 Screening actinomycetes for production of chitinase

One hundred and three isolates of actinomycetes were screened for production chitinase in enzyme production medium; 1% colloidal chitin. The total protein was contained maximum in isolate Aa1-1, 0.28 mg/ml protein which was followed by the isolate Ad1-2, Am1-3, Bd1-2, Bd1-3, Am3-3, Ba3-1, Bm4-2, Aa5-3, Ad5-1, Ba5-3, Bd5-4, Bd5-5, Bm5-1 and Bm5-3 respectively. The chitinase activity was also maximum in case of Aa1-1, 0.58 unit. The specific chitinase activity was maximum in case of Ba2-3, 9.68 unit/mg protein; see Table 2.

Table 2. Maximum total protein, chitinase activity and specific chitinase activity of 16 isolates of actinomycetes cultured under ubmerged fermentation

Isolate	total protein (mg/ml protein)	chitinase activity (unit)	specific chitinase activity (unit/mg protein)
Aa1-1	0.16	0.58	3.69
Ad1-2	0.16	0.58	3.69
Am1-3	0.16	0.58	3.72
Bd1-2	0.16	0.58	3.53
Bd1-3	0.16	0.58	3.69
Am3-3	0.16	0.26	1.58
Ba3-1	0.16	0.58	3.62
Bm4-2	0.16	0.58	3.51
Aa5-3	0.20	0.58	2.87
Ad5-1	0.16	0.58	3.65
Ba5-3	0.16	0.58	3.67
Bd5-4	0.16	0.58	3.62
Bd5-5	0.16	0.58	3.72
Bm5-1	0.16	0.58	3.65
Bm5-3	0.16	0.58	3.62
Ba2-3	0.04	0.39	9.68

3.3 Identification of actinomycetes

Ninety seven isolates of actinomycetes were found to have morphology similar to the genus Streptomyces, the spores were spiral produced in long chain. Aerial mycelium was formed abundantly on various agar media and mycelia were not fragmented. Six isolates of actinomycetes were found to have morphology similar to the genus Microbispora, they produced longitudinally paired spores on the tips of short sporophores alternately branched from aerial hyphae; see figure 2.

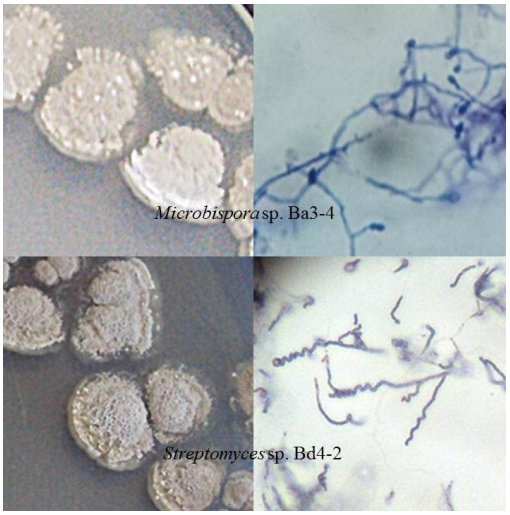

Fig. 2. Morphology of the isolates of actinomycetes

4. Discussion

Soil samples were collected from underneath animal dung and rhizosphere soil. The colory of the samples was brown to black and type of soil was loamy and loam-sandy. Soil samples were pretreated by i) air dry for 1 to 3 days; ii) dry heat at 120 ºC for one hours; and iii) moist heat at 50 ºC for 15 minute. Isolation of the actinomycetes from the soil samples was initially carried out by using serial dilution and spread plate method on starch casein agar (SCA). The colonies of the actinomycetes developed on the agar surface had a firm consistency and adhered tenaciously to the solidified substratum. The surface of the colonies was powdery and often became pigmented when the spores were produced. The most number of actinomycetes were found in the soils pretreated by moist heat; 18.76x106CFU/ml, dry heat; 14.07x106CFU/ml and air dry; 13.43x106CFU/ml respectively. While the isolation of chitinase actinomycetes in soil by selected pretreatment method was found air dry soil gave the maximum number of actinomycetes. Whereas, the lowest number of actinomycetes were obtained from dry heat soil. There is some slightly difference in number of the actinomycetes between the two techniques, soil treated with phenol and soil treated with calcium carbonate [16].

Chitinase production from isolates of actinomycetes by submerged fermentation, 1% colloidal chitin in the liquid medium. The total protein and chitinase activity was contained maximum in isolate Aa1-1, 0.28 mg/ml protein and 0.58 unit. In case of isolate Ba2-3 showed maximum specific chitianse activity, 9.68 unit/mg protein. Endophytic actinomycetes were screened extracellular chitinase production. Endophytic actinomycetes were grown on basal medium containing colloidal chitin. After 7 days the chitinolytic zone around the actinomycete colonies were observed and measured. Fourteen isolates produced a chitinolytic zone more than 5mm dia [17].

Identification isolates of actinomycetes, Ninety seven isolates of actinomycetes were found to have morphology similar to the genus *Streptomyces.* Six isolates of actinomycetes were found to have morphology similar to the genus *Microbispora.* Actinomycetes were identified physiological characteristics by micromorphological, biochemical tests and chemotaxonomic characters. A total of 159 actionomycete strains were isolated, studied that were assigned to various generic group viz. *Micromonospora, Micropolyspora, Nocardia, Streptomyces* and *Streptosporangium* [18].

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