Morphological Characteristics and Taxonomic Position of α-Amylase Producing *Penicillium* Species Isolated from Soil

Gowhar Hamid Dar*[1, 2], Azra N. Kamili[2], Ruqeya Nazir[2] and Suhaib A. Bandh[2]

[1]Department of Environmental Science, University of Kashmir, Srinagar-190006, J & K, India
[2]Microbiology and Pathology Research Laboratory, Centre of Research for Development, University of Kashmir, Srinagar-190006, J & K, India
***Corresponding author: dargowharhamid@gmail.com**

Abstract
This study reports the morphology, colony forming unit (CFU) and taxonomy of the α-amylase producing *Penicillium* species isolated from soil. Soil samples were collected from the study sites under sterile conditions and processed in the laboratory for isolation of fungal isolates. The soil samples were diluted by serial dilution method and 0.1 ml innoculum was inoculated onto the Potato Dextrose Agar (PDA) plates and incubated for 3-7 days in an incubator at 37º C to develop the fungal colonies. *Penicillium* species isolated from the soil were screened for α-amylase activity on starch agar medium through iodine test. *P. chrysogenum, P. purpurogenum, P. caesicolum, P. funiculosum* showed positive α-amylase activity and were further selected for production. During the study the morphological characteristics like appearance, colour, elevation and margins were observed for *Penicillium* species and it was found that *Penicillium* species showed a varied morphology, some were circular, rhizoidal and some were filamentous in appearance. The taxonomic position of four isolated *Penicillium* species indicated that they all belong to division Ascomycota and class Trichocomaceae of kingdom Fungi. Among all the isolates, P. chrysogenum showed the colony forming units of 2.8×103 at site I in the month of June and colony forming units of 3.5×103 at site II in the month of August.
Keywords: Soil, morphology, *Penicillium*, α-amylase, colony forming units (CFU)

Introduction

Soil is the best medium not only for the growth of plants but also for the micro-organisms. It is defined as a natural body consisting of layers (soil horizons) of mineral constituents of variable thicknesses, which differ from the parent materials in their morphological, physical, chemical and mineralogical characteristics. Soil microbial population is the key element in the bio-geochemical cycling of nutrients in nature (Pelczar *et al.*, 1993). The role of fungi in the soil is extremely complex and is fundamental to the soil ecosystem. They perform ecological services that strongly impact the quality of human life and have enormous potential for providing economic benefits. It is estimated that there are 1.5 million fungal species on earth, of which only about 70,000 have been described till recent (Hawksworth and Rossman, 1997). Different species of genus *Aspergillus* and *Penicillium* serve in the production of a number of biotechnologically produced enzymes and other macromolecules, such as gluconic, citric, and tartaric acids, as well as several pectinases, lipase, amylases, cellulases, and proteases (Akpan *et al*., 1999). Cellulases and amylase have been obtained both in the wild and mutant strains of *Aspergillus* sp such as *Aspergillus fumigatus*, *Aspergillus versicolor*, *Aspergillus nidulans* and *Aspergillus niger* (Dar *et al*., 2014). Amylases are the hydrolytic enzymes, widespread in nature with potential application in a number of industrial processes constitute a class of industrial enzymes representing approximately 25-33% of the world

enzyme market (Nguyen *et al.*, 2002; Van der Maarel *et al.*, 2002) can be obtained in bulk from different species of this genus. Evaluation of morphological characters and population of different species of genus *Penicillium* for the production of α-amylases will help in the proper channelization of the agro industry based wastes into the world enzyme market in addition to the solution of the solid waste generation and disposal problems in state like that of ours (Dar *et al*., 2014) and furthermore it will help us in the optimization of the conditions for the production of these hydrolytic enzymes from these species. In recent years, the potential of using micro organisms as biotechnological sources of industrially relevant enzymes has stimulated renewed interest in the exploration of extra cellular enzymatic activity in several microorganisms and in this regards, the present study dealing with the Morphological characteristics and Taxonomic position of α-amylase producing *Penicillium* species isolated from soil was taken up.

Material and Methods

Location and site description

Harwan area of the Srinagar city was choosen for the collection of soil samples. This place is situated in Srinagar, Jammu and Kashmir, India. Its geographical coordinates are 34º 10′ 0′′ North, 74º 54′ 0′′ East. Harwan-situated at an altitude of about 2743m asl, lying in the Srinagar District of Jammu and Kashmir state, is a small village set in the heart of mountains to the South East of Srinagar. Two study sites were selected, Site I was Orchard land and Site II was Fallow land area

Collection of Samples

Composite samples of soil from the sites were collected during the study period, from a depth of 5 inches. Samples were collected in sterile polythene bags and carried to laboratory for analysis (Dar *et al.*, 2013). The samples were processed using the soil plate method (Warcup, 1950) and Soil dilution plate Method (Waksman, 1922). The pure cultures of *Penicillium* isolates were prepared and stored

Soil plate method

About 1g of soil was scattered on the bottom of a sterile Petri dish and molten cooled (40-45^{o}C) Rose Bengal Agar medium (RBA) was added, which was then rotated gently to disperse the soil particles in the medium. The plates were then incubated at 35±2^{o}C for 3-7 days.

Soil dilution plate method

The soil samples were mixed with sterile distilled water and a series of dilutions were made. From the dilutions, 0.1ml inoculum was poured onto Potato Dextrose Agar and incubated at 35±2^{o}C for 3-7 days. The number of colonies counted was expressed as cfu/g and were calculated by using the formula:

$$Cfu/g = n \times d$$

Where n= number of colonies; d = dilution factor = 1/dilution (10^{-1}, 10^{-2} etc.)

Identification of *Penicillium* species

The identification of *Penicillium* isolates was done on the basis of the micro and macro morphological features, reverse and surface coloration of colonies grown on Czapek's dox Agar (CZ), Malt Extract Agar (MEA), Czapek's Yeast Agar (CYA) and Potato Dextrose Agar (PDA) media and the Fungal morphology was studied macroscopically by observing colony features (Colour and Texture) and microscopically by staining with lacto

phenol cotton blue and observed under microscope for the conidia, conidiophores and arrangement of spores. They were further identified from Agharkar Research Institute Pune, India

Screening and Bioprospecting of *Penicillium* Isolates

Screening of α-amylase producing fungi

The isolated strains of *Aspergillus* were streaked onto the starch agar plate and incubated at room temperature for 72 hours. After incubation 1% of iodine solution was layered on the agar plates and zone of clearance was observed for screening the fungi (Pandey *et al*., 2006).

Submerged fermentation of Amylase

Submerged fermentation was carried out in the Ehlenmeyer flasks by taking 100 ml of amylase production medium (Bernfed, 1951); containing Peptone (6.0g/L), MgSO4 (0.5g/L), KCl (0.5g/L), Starch (1g/L). In addition to this certain agricultural waste products like Cocos nut meal (Cocos nut oil cake) will be used as a submerged fermentation medium. The medium was maintained at a pH range of 3, 6 and 9, at 30°C on a shaker with 120rpm for 6 to 18 days (Pandey *et al*., 1999).

Enzyme extraction

Crude enzyme was extracted by mixing a known quantity of fermented substrate with distilled water containing 0.1%, tween 80 on rotator shaker at 180 rpm/1 hr. The suspension was centrifuged at 7000xg at 4°C and the supernatant used for enzyme assay (Pandey *et al*., 2006).

α- amylase assay

α-amylase activity was determined (Pandey *et al*., 1999). Then reaction mixture containing 1.25 ml of 1% soluble starch, 0.25 ml of 0.1 mM acetate buffer (pH 5.0) and 0.25 ml of crude enzyme extract will be incubated for 10 minutes at 50°C. After incubation the reducing sugar will be estimated by Dinitrosalicylic acid (DNS) method (Miller, 1959).

Results and Discussion

Among all the isolates, *Penicillium chrysogenum* showed the colony forming units of 2.8×10^3 at site I in the month of June and colony forming units of 3.5×10^3 at site II in the month of August. The two sites were having a marked difference in various biotic and abiotic factors. *Pencillium* species were isolated and selected for further study. The 4 isolates which showed positive amylase activity were present at both the sites. During the study the morphological characteristics like appearance, colour, elevation and margins were observed for *Penicillium* species and it was found that *Penicillium* species showed a varied morphology, some were circular, rhizoidal and some were filamentous in appearance. The taxonomic position of four isolated *Penicillium* species indicated that they all belong to division Ascomycota and class Trichocomaceae of kingdom Fungi (Table 1), and the colony forming units (cfu/g) of *Penicillium* species obtained during the study are shown in the Tables 2-5 and Fig 1. Fungal cultures were isolated from soil sample by serial dilution on Potato Dextrose Agar medium (PDA). Four cultures of *Penicillium* species isolated from soil at the two sites showed positive amylase activity. All the *Penicillium* isolates were tested for positive amylase production by starch hydrolysis through iodine test. On the basis of the area of clearance, all the four *Penicillium* isolates were selected for further studies on amylase production. The monthly population (cfu/gm) was found highest for *P. chrysogenum* as 2.8×10^3 and 3.5×10^3 in the months of June and August respectively at the two sites under consideration and the diversity of *Penicillium* species was highest at Site II. This may be attributed to the difference in various biotic and abiotic factors like pH and temperature that have been found to influence the composition and diversity of soil microbial communities and the results obtained are in consonance with the findings of Piao *et al*., (2000), Fierer and Jackson, (2006), who worked on various biotic and abiotic factors influencing the microbial communities. Since the temperature is found to be highest in the months of June and August in Kashmir valley, which might have increased the

reproductive rate of microbial communities. These results are in accordance with the studies of Murphy (2000) and Dar *et al.,* (2013), who evaluated a correlation between temperature changes and microbial communities. The total count (cfu/g) was highest at site II which was dominated by cattle activities. The cattle activities thus might have induced changes in the microbial community structure which is in concurrence with the studies carried out by Kohler *et al.,* (2005) and Dar *et al.,* 2013 in which they have reported the effects of cattle grazing on microbial communities in pastures and has shown that microbial community changes due to simulated effects of cattle grazing. *P. funiculosum* and *P. chrysogenum* were obtained in the present study which were also reported by Bandh *et al.,* (2011), from Dal lake and reported that *P. funiculosum* was the most abundant (28.7%) followed by *P. chrysogenum* (27.04%), which also concurs with the present study. The highest colony forming units (cfu/g) was observed for *P. chrysogenum* in the month of August at site II while as no colony forming unit (cfu/g) was recorded in the months of Feb for *P. chrysogenum* and *P. purpurogenum* where as *P. caseicolum* and *P. funiculosum* showed no cfu/g in the months of Dec, Apr and Oct. at site I respectively, but all these species were present at site II. A comparison graph shown in Fig. 1 indicates that the colony forming unit (cfu/g) was highest at site II which was a fallow land area.

Table 1. Morphological characteristics and taxonomic position of isolated *Penicillium* species

S. No.	Isolated species	Taxonomic Position				Morphological Characteristics			
		Kingdom	Division	Family	Genus	Appearance	Margin	Elevation	Colour
1.	*Penicillium chrysogenum* Thom	Fungi	Ascomycota	Trichocomaceae	*Penicillium*	Circular	Entire	Flat	Green
2.	*Penicillium purpurogenum* Stoll					Filamentous	Filamentous	Convex	White
3.	*Penicillium caseicolum* Bain					Circular	Filamentous	Convex	Cream
4.	*Penicillium funiculosum* Thom					Rhizoidal	Filamentous	Flat	Green

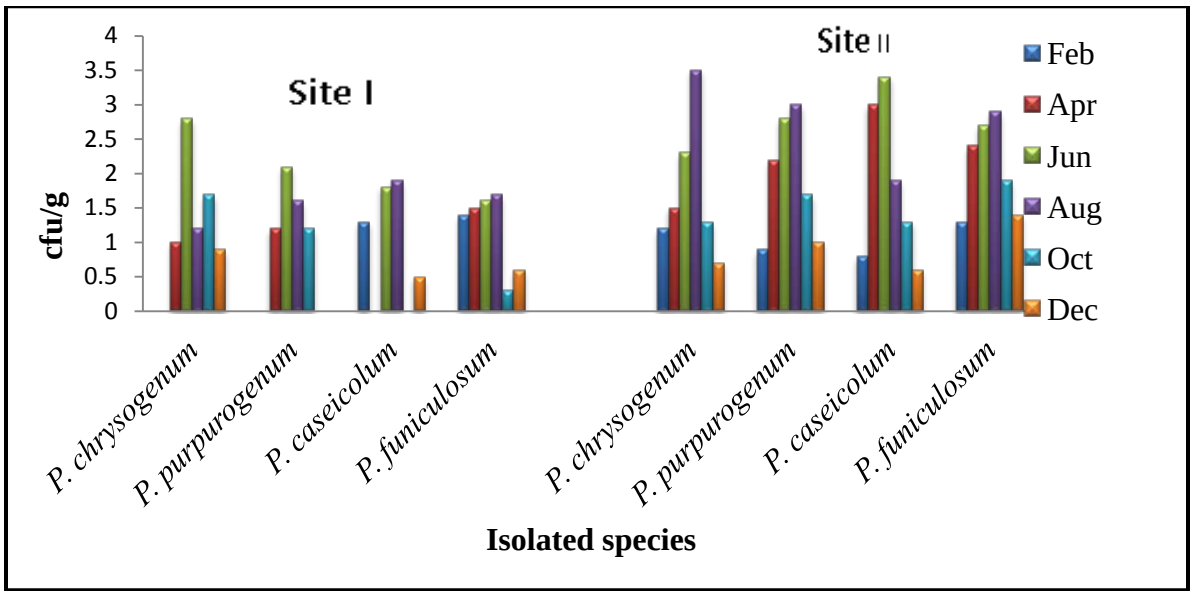

Fig. 1. Comparison of colony forming units (cfu/gm) of *Penicillium* species at different sites

Table 2. Colony count of isolated *Penicillium* species at Site I

S. No.	Isolated species	Feb.	Apr.	Jun.	Aug.	Oct.	Dec.
1.	*P. chrysogenum*	0	10	28	12	17	9
2.	*P. purpurogenum*	0	12	21	16	12	0
3.	*P. caseicolum*	13	0	18	19	0	5
4.	*P. funiculosum*	14	15	16	17	3	6

Table 3. Colony forming units (cfu/g) of isolated *Penicillium* species at Site I

S. No.	Isolated species	Feb.	Apr.	Jun.	Aug.	Oct.	Dec.
1.	*P. chrysogenum*	0	1×10^3	2.8×10^3	1.2×10^3	1.7×10^3	0.9×10^3
2.	*P. purpurogenum*	0	1.2×10^3	2.1×10^3	1.6×10^3	1.2×10^3	0
3.	*P. caseicolum*	1.3×10^3	0	1.8×10^3	1.9×10^3	0	0.5×10^3
4.	*P. funiculosum*	1.4×10^3	1.5×10^3	1.6×10^3	1.7×10^3	0.3×10^3	0.6×10^3

Table 4. Colony count of isolated *Penicillium* species at Site II

S. No.	Isolated species	Feb.	Apr.	Jun.	Aug.	Oct.	Dec.
1.	*P. chrysogenum*	12	15	23	35	13	7
2.	*P. purpurogenum*	9	22	28	30	17	10
3.	*P. caseicolum*	8	30	34	19	13	6
4.	*P. funiculosum*	13	24	27	29	19	14

Table 5. Colony forming units (cfu/g) of isolated *Penicillium* species at Site II

S. No.	Isolated species	Feb.	Apr.	Jun.	Aug.	Oct.	Dec.
1.	*P. chrysogenum*	1.2×10^3	1.5×10^3	2.3×10^3	3.5×10^3	1.3×10^3	0.7×10^3
2.	*P. purpurogenum*	0.9×10^3	2.2×10^3	2.8×10^3	3.0×10^3	1.7×10^3	1.0×10^3
3.	*P. caseicolum*	0.8×10^3	3.0×10^3	3.4×10^3	1.9×10^3	1.3×10^3	0.6×10^3
4.	*P. funiculosum*	1.3×10^3	2.4×10^3	2.7×10^3	2.9×10^3	1.9×10^3	1.4×10^3

Conclusion

The taxonomic position of four isolated *Penicillium* species indicated that they all belong to division Ascomycota and class Trichocomaceae of kingdom Fungi. Among all the isolates, *P. chrysogenum* showed the colony forming units of 2.8×10^3 at site I in the month of June and colony forming units of 3.5×10^3 at site II in the month of August.

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