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Potential of soil fungi to produce penicillin V acylase and its optimization conditions

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Fifty fungal isolates representing 31 species, related to 8 genera, were isolated from soil and screened for their abilities to produce endo- and exocellular penicillin V acylase enzymes. For endocellular enzyme, eleven isolates (represent 22% of total isolates) exhibited high enzyme activity and 9 isolates (18%) had moderate ability. However, twenty isolates (40%) were low producers and ten isolates (20%) had no ability to produce the enzyme. For exocellular enzyme, four isolates only (representing 8% of total isolates) exhibited high enzyme activity, and fifteen isolates (30%) were found to be moderate. However, twenty two isolates (44%) were low producers and nine isolates (18%) had no activity. The most active fungal isolates were *Aspergillus terreus* and *Penicillium chrysogenum*, producing 325 and 280 U/ml, respectively. Maximum activity of penicillin V acylase produced by *A. terreus* and *P. chrysogenum* were obtained after 7 and 6 days of incubation, respectively at 35°C and initial pH 6. Dextrin or glucose was the best carbon source for penicillin V acylase produced by *A. terreus* and *P. chrysogenum*, respectively. However, peptone was the best nitrogen source. Inoculation of cultures by 3 discs of fungi and incubation of cultures at 180 rpm shaking condition improved the enzyme production.

Key words: Penicillin V acylase, soil fungi, *Aspergillus terreus*, *Penicillium chrysogenum*.

INTRODUCTION

Penicillin acylase is one of the most important enzymes applied in the pharmaceutical industry for large scale production of 6-aminopenicillanic acid. This enzyme is the starting material for the manufacture of penicillin derivatives. β -lactam antibiotics, like penicillins and cephalosporins represent one of the major world's biotechnology markets, with annual sales of 15 billion Dollars and about 65% of the total antibiotic market (Chou et al., 2000; Parmar et al., 2000; Elander, 2003; Chandel et al., 2008).

Due to the worldwide demand for semisynthetic penicillins, production of 6-aminopenicillanic acid has been increased up to 7000 tons (Javadpour et al., 2002).

Penicillin G acylase is estimated to be in the range of 10 to 30 million tons (Chandel et al., 2008). Penicillin acylases are microbial enzymes and are found in a wide range of bacteria, actinomycetes, yeasts and filamentous fungi (Suddhakaran and Borkar, 1985a, b). Based on their substrate specificities, they have been classified into three groups: Penicillin V acylase, penicillin G acylase and ampicillin acylase. Penicillin G acylase is present in several bacteria (Arshad and Ahmad, 2000; Abedi et al., 2004; Souza et al., 2005; Arshad et al., 2006; Cheng et al., 2006; Yang et al., 2006; Zuza et al., 2007; Chandel et al., 2008; El Enshasy et al., 2009; Kafshnochi et al., 2010).

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Recently, penicillin G acylase DNA of *Shigella boydii* was isolated, amplified and sequenced. The sequencing gene composed a polypeptide of 846 amino acid residues, and the highest homology was about 96% (Hassan et al., 2009). Penicillin V acylase is produced mainly by moulds and actinomycetes. They are mainly intracellular enzymes and also could produce from genus *Fusarium* (Lowe et al., 1986; Sudhakaran and Shewale, 1993) and actinomycetes *Streptomyces lavendulae* (Torres-Bacete et al., 2000) as extracellular acylases.

The enzymes which hydrolysis the peptide linkage are named penicillin acylase. Acylases are differing in their properties according to the source they come from such as bacteria, yeasts or filamentous fungi (Claridge et al., 1963; Spence and Ramsden, 2007). Fungal acylase hydrolyze phenoxymethyl penicillin more rapidly than benzylpenicillin. This enzyme has been described in many species of actinomycetes, yeasts and filamentous fungi, including genera *Alternaria*, *Aspergillus*, *Botrytis*, *Cephalosporium*, *Cryptococcus*, *Emicellopsis*, *Epicoccum*, *Epidermophyton*, *Fusarium*, *Mucor*, *Penicillium*, *Phoma*, *Trichoderma*, *Trichophyton* and *Trichosporon* (Erickson and Bennett, 1965; Cole, 1966; Hamilton-Miller, 1966, 2008; Lowe et al., 1986; Sudhakaran and Shewale, 1993; Jose et al., 2003; Bashir et al., 2008).

The *Fusarium* sp. SKF 235 produced penicillin V acylase intracellularly and extracellularly. The maximum yield of penicillin V acylase was 430 IU/g dry cell wet. This maximum activity was achieved at pH 6.5. Penicillin V acylase from *Fusarium moniliforme* and *Fusarium avenaceum* showed maximum activity at pH values of 8.5 and 7.5, respectively (Vanderhaeghe et al., 1968; Vandamme and Voets, 1972). The optimum pH values for the penicillin V acylase from *Fusarium* sp. SKF 235 is therefore lower than that from other *Fusarium* isolates. This low optimum pH value (6.5) is advantageous for the commercial production of 6-aminopenicillanic acid from penicillin V since the stability of penicillin V in solution is maximum at pH 5.7. On the other hand, pharmacological studies of some plant extracts showed various antibacterial and antifungal activities such as *Peganum harmala* (Asgarpanah and Ramezanloo, 2012), *Viburnum grandiflorum* (Alam et al., 2012) and *Allium cepa* (Nasri et al., 2012). This research was carried out to investigate the incidence of penicillin V acylase enzyme in different genera and species of soil fungi as well as the optimum conditions for the production of this enzyme by the most active fungal isolates.

MATERIALS AND METHODS

Screening of fungi for production of penicillin V acylase enzyme

Fifty fungal isolates representing 31 species related to 8 genera were recovered from soil and screened for their abilities to produce intracellular and extracellular penicillin V acylase enzymes. These

isolates belong to several genera including *Aspergillus* (10 species), *Emicella* (1 species), *Fusarium* (7 species), *Gibberella* (3 species), *Nectria* (1 species), *Paecilomyces* (1 species), *Penicillium* (7 species) and *Trichoderma* (1 species).

Production medium

Penicillin V acylase was tested by using the following standard medium which contained the following constituents (g/L): Glucose, 20; peptone 5; yeast extract, 5; K_2HPO_4 , 1; $MgSO_4 \cdot 7H_2O$, 0.2 and penicillin V as inducer 0.3. The pH was adjusted to 5.8 (Bashir et al., 2008).

Cultivation and culture conditions

The medium was distributed in Erlenmeyer flasks (250 ml), each flask contained 100 ml of the medium. The flasks were sterilized at 121°C for 20 min. Each flask was inoculated with 10 mm mycelial disc cut out from 5 days fungal colony grown on glucose-Czapek's agar medium. The inoculated flasks were incubated under shaking condition (180 rpm) at 30°C for 7 days. At the end of the incubation period, the content of each flask was filtrated.

Determination the activity of penicillin V acylase

Three grams of wet biomass was weighed and suspended in a 6 ml mixture of 0.1 M phosphate buffer (pH = 7), 20% sucrose and 1 mM EDTA (ethylene diamine tetraacetic acid). 0.5 g of glass beads were added and the microtubes were placed in a bead mill at 4°C for 25 min, followed by centrifugation at 13,000 rpm for 5 min in case of intracellular enzyme but in case of extracellular enzyme, the supernatant was used directly after filtration and centrifugation of samples. 6-aminopenicillanic acid was determined by colorimetric method as described by Balasingham et al. (1972). The mixture of 0.25 ml of 0.1 M phosphate buffer (pH = 7) containing 2.5 mg of penicillin V, 0.25 ml of cell suspension (intracellular enzyme) or supernatant (extracellular enzyme) was incubated at 37°C for 30 min, 3 ml of solution made by mixing 2 ml of glacial acetic acid with 1 ml of 0.05 M NaOH was added to the reaction mixture and centrifuged. 0.5 ml of 0.5% P-dimethylaminobenzaldehyde in methanol was added to the supernatant, and the ultra violet (UV)-absorbance was measured at 415 nm. One unit of enzyme activity was defined as the quantity of the enzyme which produced 1 µmol of 6-aminopenicillanic acid per 1 min.

Effect of incubation periods on penicillin V acylase production by *Aspergillus terreus* and *Penicillium chrysogenum*

A. terreus and *P. chrysogenum* were found to be the most active penicillin V acylase (endoenzyme) producers. So these fungi were used for further studies. The medium previously described by Bashir et al. (2008) was employed.

Cultivation and culture conditions

The influence of different incubation periods (2, 4, 6, 7, 8, 10 and 12 days) on the production of penicillin V acylase and fungal dry weight were measured. The flasks were sterilized at 121°C for 20 min, inoculated with 10 mm disc cut out from 5 days fungal colony grown on glucose-Czapek's agar medium. Two replicates were used for each treatment. Inoculated flasks were incubated under shaking condition (180 rpm) at 30°C. At the end of different incubation periods, fungal mycelium was separated from the growth

medium by suction filtration through pre-weighed Whatman #1 filter paper placed in a buchner funnel. The filter paper with mycelium was dried in an oven at 85°C for 24 h. The mycelial dry weight was recorded and also the activity of the enzyme was determined according to the method described by Balasingham et al. (1972).

Effect of temperature on penicillin V acylase production by *Aspergillus terreus* and *Penicillium chrysogenum*

The influence of various temperature values (15, 20, 25, 30, 35 and 40°C) on the activity of penicillin V acylase and fungal dry weight were tested by incubating the tested fungi at different temperatures in liquid synthetic medium for 7 days. After the incubation period, fungal dry weight and penicillin V acylase activity in two replicates were measured according to method described by Balasingham et al. (1972).

Effect of pH on penicillin V acylase production by *Aspergillus terreus* and *Penicillium chrysogenum*

The influence of different pH values (2, 4, 6, 7, 8, 10 and 12) on penicillin V acylase production as well as fungal dry weight of tested fungi was measured by incubating *A. terreus* and *P. chrysogenum* at 30°C in liquid synthetic medium for 7 days. After the incubation period, fungal dry weight and penicillin V acylase activity in two replicates were measured according to the method described by Balasingham et al. (1972).

Effect of different carbon sources on penicillin V acylase production by *Aspergillus terreus* and *Penicillium chrysogenum*

A. terreus and *P. chrysogenum* were grown in Erlenmeyer flasks (250 ml) containing 100 ml liquid medium. Nine carbon sources (glucose, fructose, maltose, lactose, sucrose, cellulose, starch, dextrin and glycerol) were added individually to the basal medium by 20 g/L. Flasks were sterilized at 121°C for 20 min, inoculated with 10 mm disc cut out from 5 days fungal colony grown on glucose-Czapek's agar medium. The inoculated flasks were incubated under shaking condition (180 rpm) at 30°C for 7 days. At the end of the incubation period, the dry weight of mycelium and the activity of penicillin V acylase enzyme were determined according to method described by Balasingham et al. (1972).

Effect of different nitrogen sources on penicillin V acylase production by *Aspergillus terreus* and *Penicillium chrysogenum*

A. terreus and *P. chrysogenum* were grown in Erlenmeyer flasks (250 ml) containing 100 ml liquid medium. Nine nitrogen sources (Peptone, NH_4Cl , $(\text{NH}_4)_2\text{SO}_4$, NH_4NO_3 , $(\text{NH}_4)_2\text{HPO}_4$, NaNO_3 , KNO_3 , $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ and $\text{CH}_3\text{COONH}_4$) were added individually to the basal medium by 5 g/L. The flasks were sterilized at 121°C for 20 min, inoculated with 10 mm disc cut out from 5 days fungal colony grown on glucose-Czapek's agar medium. Two replicates were used for each nitrogen source. Inoculated flasks were incubated under shaking condition (180 rpm) at 30°C for 7 days. At the end of the incubation period, the mycelial dry weight was recorded and also the activity of the enzyme was determined according to method described by Balasingham et al. (1972).

Effect of inoculum size on penicillin V acylase production by *Aspergillus terreus* and *Penicillium chrysogenum*

The influence of inoculum size on the production of penicillin V

acylase was tested by inoculating of culture medium with 1 disc, 2 discs and 3 discs (10 mm in diameter) cut out from 5 days colony of the tested fungi grown on glucose-Czapek's agar medium. The flasks were incubated under shaking condition (180 rpm) at 30°C for 7 days. After the incubation period, penicillin V acylase activity was measured in two replicates according to method described by Balasingham et al. (1972). Fungal dry weight was also determined.

Effect of shaker speed on penicillin V acylase production by *Aspergillus terreus* and *Penicillium chrysogenum*

The production of penicillin V acylase enzyme by tested fungi was estimated by using different random speed of rotary shaker (160, 180 and 200 rpm). The two fungi were grown in Erlenmeyer flasks (250 ml) containing 100 ml liquid medium. The flasks were divided into 3 groups: The first group placed on rotary shaker at 160 rpm, the second placed on rotary shaker at 180 rpm and the third group placed on rotary shaker at 200 rpm for 7 days at 30°C. After the incubation period, the activity of the enzyme was determined according to method described by Balasingham et al. (1972). Fungal dry weight was also determined.

Effect of medium volume on penicillin V acylase production by *Aspergillus terreus* and *Penicillium chrysogenum*

This experiment occurred by using 2 volumes of liquid synthetic medium (50 and 100 ml) in 250 ml Erlenmeyer flasks. The flasks were inoculated with tested fungi and incubated under shaking condition (180 rpm) at 30°C for 7 days. After the incubation period, penicillin V acylase was measured in two replicates according to the method described by Balasingham et al. (1972). Fungal dry weight was also determined.

RESULTS AND DISCUSSION

Screening of fungi for their abilities to produce penicillin V acylase enzyme

Screening of fifty fungal isolates for production of endocellular penicillin V acylase showed that eleven isolates represented 22% of total isolates which showed high enzyme activity. Nine isolates contributed 18% of total isolates found to be moderate activity. Twenty isolates represented 40% of total isolates which were low producers of penicillin V acylase enzyme. Ten isolates represented 20% of total isolates which had no enzyme activity (Table 1).

For exocellular penicillin V acylase, four isolates (represent 8% of total isolates) only showed high enzyme activity and fifteen isolates (30%) were moderate. However, twenty two isolates (44%) were low producers of the enzyme. Nine isolates (18%) had no activity. *A. terreus* and *P. chrysogenum* were the most active producer isolates of fungi for penicillin V acylase. These fungi produced 325 and 280 U/ml, respectively (Table 1). Bashir et al. (2008) screened 12 different strains of fungi for their abilities to produce penicillin G acylase enzyme under submerged condition. They showed that *A. niger* had the highest enzyme activity.

Table 1. Screening of fungi for their abilities to produce endo- and exoenzyme penicillin V acylase.

Fungal species	Enzyme concentration		Enzyme activity	
	Endoenzyme	Exoenzyme	Endoenzyme	Exoenzyme
<i>Aspergillus flavus</i>	22.85 L	32.85 L	658.87	947.21
	-	6.42 L	-	185.11
<i>A. fumigatus</i>	-	3.57 L	-	102.93
	-	8.57 L	-	247.11
<i>A. glaucus</i>	180.00 H	-	5190.24	-
<i>A. niger</i>	11.42 L	127.14 M	329.29	3666.03
	2.85 L	42.85 L	82.17	1235.56
<i>A. ochraceus</i>	7.14 L	115.71 M	205.87	3336.45
<i>A. sydowii</i>	141.42 M	-	4077.79	-
<i>A. tamarii</i>	214.28 H	-	6178.69	-
	-	20.71 L	-	597.16
<i>A. terreus</i>	325.00 H	3.57 L	9371.26	102.93
	84.28 M	114.28 M	2430.18	3295.22
<i>A. ustus</i>	24.28 L	5.71 L	700.10	164.64
<i>A. versicolor</i>	73.57 M	57.85 M	2121.36	1668.08
	180.00 H	-	5190.24	-
<i>Emericella nidulans</i>	179.20 H	14.28 L	5167.17	411.75
	252.14 H	42.85 L	7270.37	1235.56
	90.71 M	152.85 H	2615.59	4407.37
<i>Fusarium chlamydosporum</i>	-	-	-	-
<i>F. compactum</i>	5.71 L	14.28 L	164.64	411.75
<i>F. heterosporum</i>	84.28 M	114.20 M	2430.18	3292.91
<i>F. mersmipoides</i>	197.14 H	45.71 L	5684.46	1318.03
<i>F. oxysporum</i>	7.14 L	37.85 L	205.87	1091.39
	13.57 L	-	391.28	-
<i>F. sambucinum</i>	-	-	-	-
<i>F. tricinctum</i>	15.71 L	51.42 M	452.99	1482.67
<i>Gibberella avenacea</i>	-	-	-	-
<i>G. fujikuroi</i>	-	37.14 L	-	1070.91
	2.85 L	29.28 L	82.17	844.27
<i>G. intricans</i>	7.85 L	83.57 M	226.35	2409.71
	4.28 L	80.00 M	123.41	2306.77
<i>Nectria haematococca</i>	-	26.42 L	-	761.81
	8.57 L	91.42 M	247.11	2636.06
	13.57 L	65.71 M	391.28	1894.72
<i>Paecilomyces lilacinus</i>	10.00 L	107.14 M	288.34	3089.34
<i>Penicillium aurantiogriseum</i>	152.85 H	5.00 L	4407.37	144.17
<i>P. brevicompactum</i>	126.42 M	70.71 M	3657.67	2038.89
	152.85 H	236.42 H	4407.37	6811.90
	88.57 M	225.00 H	2553.88	6487.80
	280.00 H	22.14 L	8073.70	638.39
<i>P. chrysogenum</i>	24.28 L	250.71 H	700.10	7229.13
	100.71 M	113.57 M	2903.93	3274.75
	234.28 H	58.57 M	6755.38	1688.84
<i>P. duclauxii</i>	30.00 L	-	865.04	-
<i>P. funiculosum</i>	42.85 L	65.71 M	1235.56	1894.72
	28.57 L	44.28 L	823.80	1276.79

Table 1. Contd.

<i>P. purpurogenum</i>	101.42 M	35.71 L	2924.41	1029.68
<i>P. roqueforti</i>	25.00 L	2.85 L	720.86	82.17
<i>Trichoderma harzianum</i>	-	2.14 L	-	61.70

High (H) > 150, Moderate (M) = 50 to 150, Low (L) < 50, - = no enzyme detected.

Table 2. Effect of incubation periods on growth and penicillin V acylase production by *Aspergillus terreus* and *Penicillium chrysogenum*.

Incubation period (days)	<i>Aspergillus terreus</i>		<i>Penicillium chrysogenum</i>	
	Enzyme activity	Dry weight (mg/100 ml)	Enzyme activity	Dry weight (mg/100 ml)
2	-	-	-	-
4	2883.46	383.44	3295.22	343.91
6	8650.40	403.21	7826.30	372.10
7	9062.15	432.10	7002.49	342.11
8	7826.30	391.20	6178.69	301.15
10	4119.03	382.80	4531.07	371.30
12	2471.41	371.70	3707.27	369.52

Effect of incubation periods on growth and penicillin V acylase by *Aspergillus terreus* and *Penicillium chrysogenum*

A. terreus and *P. chrysogenum* exhibited maximum penicillin V acylase production at 7 and 6 days of incubation, respectively. These fungi had enzyme activity of 9062.15 and 7826.3 U/ml, respectively. At 8 days of incubation, the two tested fungi produced relatively considerable amounts of penicillin V acylase enzyme, however decreasing of incubation period to 2 or 4 days had negative effect on the enzyme production by the two tested fungi. Increasing of incubation period to 10 or 12 days decreased the production of penicillin V acylase enzyme by *A. terreus* and *P. chrysogenum*. The results recorded for the effect of incubation time on mycelial growth of *A. terreus* and *P. chrysogenum* was nearly similar to those reported for penicillin V acylase enzyme (Table 2). In this respect, maximum production of penicillin G acylase of *A. niger* was recorded between 20 to 24 h of incubation under shaking conditions (Bashir et al., 2008).

Effect of temperatures on fungal growth and penicillin V acylase production by *A. terreus* and *P. chrysogenum*

Temperature greatly influenced the production of penicillin V acylase by *A. terreus* and *P. chrysogenum*. Maximum production of the enzyme was recorded at 35°C by the two tested fungi producing 9062.15 and 7785.36 U/ml, respectively. Decreasing of incubation

temperature below 35°C decreased the enzyme production. Decreasing of the incubation temperature to 15°C prevented the secretion of the enzyme by the two fungi. Also increasing of incubation temperature to 40°C decreased the production of penicillin V acylase. The results obtained for the effect of incubation temperature on mycelial growth of *A. terreus* and *P. chrysogenum* was variable with different incubation temperatures, with maximum growth at 25 and 35°C, respectively (Table 3). The optimum temperature for maximal penicillin G acylase enzyme of *A. fumigatus* and *Mucor gryseoclanum* was at 40°C (Jose et al., 2003). Abedi et al. (2004) showed that the optimum temperature for penicillin G acylase synthesis activity was at 25°C. Souza et al. (2005) reported that penicillin G acylase produced by *Bacillus megaterium* showed maximum hydrolysis rate at 37°C. Zuza et al. (2007) found that the optimal temperature of *Escherichia coli* penicillin acylase activity were 27.5 and 31.5°C for free and immobilized enzyme, respectively.

Effect of pH values on growth and penicillin V acylase production by *A. terreus* and *P. chrysogenum*

The pH value was greatly affected the production of penicillin V acylase by *A. terreus* and *P. chrysogenum*. Maximum production of the enzyme was recorded at pH 6 by the two fungi, producing 9062.15 and 7002.49 U/ml, respectively. Decreasing of pH value to pH 4 exhibited an inhibitive effect on penicillin V acylase production. Also, increasing of pH value to 7, 8 or 10 decreased the

Table 3. Effect of temperatures on growth and penicillin V acylase production by *Aspergillus terreus* and *Penicillium chrysogenum*.

Temperature	<i>Aspergillus terreus</i>		<i>Penicillium chrysogenum</i>	
	Enzyme activity	Dry weight (mg/100 ml)	Enzyme activity	Dry weight (mg/100 ml)
15	-	-	-	-
20	4984.07	402.03	3892.68	367.84
25	7950.00	419.09	4757.72	371.50
30	8609.16	413.35	6487.80	361.06
35	9062.15	390.67	7785.36	414.34
40	3624.80	302.15	7249.61	392.08

Table 4. Effect of pH values on growth and penicillin V acylase production by *Aspergillus terreus* and *Penicillium chrysogenum*.

pH value	<i>Aspergillus terreus</i>		<i>Penicillium chrysogenum</i>	
	Enzyme activity	Dry weight (mg/100 ml)	Enzyme activity	Dry weight (mg/100 ml)
2	-	-	-	-
4	4119.03	301.50	3707.27	398.99
6	9062.15	493.51	7002.49	364.07
7	8238.35	482.10	6178.69	369.26
8	7414.54	372.10	4942.83	363.46
10	2471.41	294.31	2059.37	302.20
12	-	-	-	-

enzyme production by the two tested fungi. No enzyme production was recorded at pH 2 and 12 by the two fungal species. Data recorded for the effect of pH value on mycelial growth of *A. terreus* and *P. chrysogenum* was nearly similar to those reported for penicillin V acylase enzyme (Table 4). Javadpour et al. (2002) reported that the optimal pH of penicillin acylase produced by *E. coli* was determined to be 8. The optimum pH rang for dialyzed preparations of penicillin G acylase produced by *A. fumigatus* and *M. gryseoclanum* was 7 to 8 and 7.5 to 8.5, respectively (Jose et al., 2003). Abedi et al. 2004 showed that the optimum pH for penicillin G acylase synthesis activity was 6. Souza et al. (2005) reported that penicillin G acylase produced by *Bacillus megaterium* showed maximum hydrolysis rate at pH 8. Zuza et al. (2007) reported that the optimal pH of *E. coli* penicillin acylase activity was 8.5 for both free and immobilized enzyme.

Effect of different carbon sources on growth and Penicillin V acylase production by *A. terreus* and *P. chrysogenum*

Carbon source is one of the most essential components in the microbial medium. The effect of different carbon sources on growth and penicillin V acylase production by *A. terreus* and *P. chrysogenum* was variable. Glucose

and dextrin were the most favorable carbon sources for penicillin V acylase production by *P. chrysogenum* and *A. terreus*, respectively. Maximum production of the enzyme was achieved by incorporation of glucose and dextrin in the culture medium for enzyme production, producing 8897.51 and 7702.89 U/ml for *A. terreus* and *P. chrysogenum*, respectively. Addition of other carbon sources in the fungal cultures decreased the enzyme production by the two fungal species. Mycelial growth of *A. terreus* and *P. chrysogenum* was not greatly affected by the addition of different carbon sources to the culture medium for fungi (Table 5). Among 7 carbon sources tested for penicillin G acylase, 0.4% lactose was the best for mycelial growth and enzyme production of *A. niger* (Bashir et al., 2008). Other carbon sources such as glucose, sorbitol glycerol, sucrose, PAA (Phenyl Acetic Acid) and tryptone increased the activity of Penicillin G acylase by *E. coli* and *Bacillus megaterium* (Chandel et al., 2008).

Effect of different nitrogen sources on growth and penicillin V acylase production by *A. terreus* and *P. chrysogenum*

Nitrogen source is one of the most essential components in the microbial medium because the nitrogen element

Table 5. Effect of different carbon sources on growth and penicillin V acylase production by *Aspergillus terreus* and *Penicillium chrysogenum*.

Carbon sources	<i>Aspergillus terreus</i>		<i>Penicillium chrysogenum</i>	
	Enzyme activity	Dry weight (mg/100 ml)	Enzyme activity	Dry weight (mg/100 ml)
Glucose	5931.57	384.00	7702.89	392.14
Fructose	2965.64	395.21	3624.80	373.27
Maltose	6590.73	394.47	7244.42	372.66
Dextrin	8897.51	381.45	7244.42	370.72
Lactose	2965.64	394.25	4531.12	370.43
Sucrose	3295.22	387.50	6343.62	360.48
Cellulose	1647.61	380.57	1812.25	301.24
Starch	1977.19	385.90	2533.12	352.14
Glycerol	2636.06	393.73	4077.79	372.10

Table 6. Effect of different nitrogen sources on growth and penicillin V acylase production by *Aspergillus terreus* and *Penicillium chrysogenum*.

Nitrogen sources	<i>Aspergillus terreus</i>		<i>Penicillium chrysogenum</i>	
	Enzyme activity	Dry weight (mg/100 ml)	Enzyme activity	Dry weight (mg/100 ml)
(NH ₄) ₂ SO ₄	8238.35	357.91	7435.01	392.08
(NH ₄) ₂ HPO ₄	4942.83	361.37	4695.72	377.24
KNO ₃	5931.57	369.32	5087.01	366.11
CH ₃ COONH ₄	5602.03	364.46	5766.93	372.11
Ca(NO ₃) ₂	3954.38	373.53	3913.15	374.11
NH ₄ NO ₃	6261.15	359.01	5478.58	373.28
NaNO ₃	5931.57	366.03	5087.01	366.22
NH ₄ Cl	7249.69	357.77	6261.15	389.53
Peptone	9103.39	360.51	7826.30	401.24

participates in the formation of amino and nucleic acids and protein in microbial cells. The effect of different nitrogen sources on fungal growth and penicillin V acylase production by *A. terreus* and *P. chrysogenum* varied. Among nine nitrogen sources studied, peptone was the most suitable nitrogen source for penicillin V acylase production.

Maximum production of the enzyme was recorded by the incorporation of peptone in the culture medium for enzyme production, producing 9103.39 and 7826.30 U/ml by *A. terreus* and *P. chrysogenum*, respectively. Addition of ammonium sulphate and ammonium chloride to the fungal cultures exhibited considerable amount of penicillin V acylase.

However, the other nitrogen sources decreased the enzyme production. Mycelial growth of *A. terreus* and *P. chrysogenum* was not greatly affected by the addition of different nitrogen sources to the culture medium for fungi (Table 6). Chandel et al. (2008) reported that maximum Penicillin G acylase activity was obtained using casein

hydrolysate supplemented with 0.6 L of alcalase and cheese whey.

Effect of inoculum size on growth and penicillin V acylase production by *A. terreus* and *P. chrysogenum*

The inoculum size influenced the production of penicillin V acylase by *A. terreus* and *P. chrysogenum*. Maximum yield of penicillin V acylase was achieved by inoculating of liquid synthetic medium with 3 discs (10 mm diameter) of fungi. *A. terreus* produced 9759.38, corresponding to 7785.36 U/ml for *P. chrysogenum*. Decreasing of the inoculum size to 2 or 1 disc decreased the production of penicillin V acylase enzyme by the two fungal species. Mycelial growth of *A. terreus* and *P. chrysogenum* did was not greatly affected by inoculation size of fungi (Table 7). Spore concentration 1.5×10^7 spores/ml and germination during 24 and 72 h showed maximum

Table 7. Effect of inoculum size on growth and penicillin V acylase production by *Aspergillus terreus* and *Penicillium chrysogenum*.

Inoculum size	<i>Aspergillus terreus</i>		<i>Penicillium chrysogenum</i>	
	Enzyme activity	Dry weight (mg/100 ml)	Enzyme activity	Dry weight (mg/100 ml)
1 disc	6653.88	384.43	6014.04	373.11
2 discs	6243.57	389.04	6920.32	384.10
3 discs	9759.38	391.70	7785.36	364.91

Table 8. Effect of medium volume on growth and penicillin V acylase production by *Aspergillus terreus* and *Penicillium chrysogenum*.

medium volume (ml)	<i>Aspergillus terreus</i>		<i>Penicillium chrysogenum</i>	
	Enzyme activity	Dry weight	Enzyme activity	Dry weight
100	9981.11	360.40	8672.31	365.14
50	4077.79	356.04	4736.95	366.10

Table 9. Effect of shaker speed on growth and penicillin V acylase production by *Aspergillus terreus* and *Penicillium chrysogenum*.

Shaker speed (rpm)	<i>Aspergillus terreus</i>		<i>Penicillium chrysogenum</i>	
	Enzyme activity	Dry weight (mg/100 ml)	Enzyme activity	Dry weight (mg/100 ml)
160	8032.47	351.55	7599.95	369.43
180	9479.39	388.10	7723.36	377.15
200	8856.27	379.15	7620.42	376.20

penicillin G acylase activity by *B. megaterium* (Chandel et al., 2008).

Effect of shaker speed on growth and penicillin V acylase production by *A. terreus* and *P. chrysogenum*

Speed of shaker had an effect on growth and penicillin V acylase production by *A. terreus* and *P. chrysogenum*. *A. terreus* and *P. chrysogenum* showed maximum penicillin V acylase production (9479.39 and 7723.36 U/ml, respectively) at 180 rpm. Mycelial growth of *A. terreus* and *P. chrysogenum* was not greatly affected by incubation shaking of fungal cultures (Table 8). Recently, Saleem et al. (2012) reported that the endocellular D-amino acid oxidase enzyme produced by *Fusarium heterosporum* and *Nectria haematococca* showed maximum activity in cultures incubated at 160 rpm shaking condition.

Effect of medium volume on growth and penicillin V acylase production by *A. terreus* and *P. chrysogenum*

The effect of medium volume on growth and penicillin V

acylase production by *A. terreus* and *P. chrysogenum* was studied by using different volumes of cultivation medium. *A. terreus* and *P. chrysogenum* produced higher amounts of penicillin V acylase (9981.11 and 8672.31 U/ml, respectively) in flask containing 100 ml of medium. Decreasing of the amount of medium to 50 ml decreased the enzyme production by nearly half amount (4077.79 and 4736.95 U/ml, respectively). Mycelial growth of *A. terreus* and *P. chrysogenum* was not greatly affected by the amount of medium (Table 9). Saleem et al. (2012) found that D-amino acid oxidase enzyme and mycelial growth of *F. heterosporum* and *Nectria haematococca* were higher in flasks containing 100 ml of medium than those containing 50 ml of medium by nearly half amount.

Conclusion

This article would contribute to finding new sources of penicillin acylases by fungi and increase the production of these enzymes by optimizing cultural conditions which will lead to the production of some antibiotics on a large scale and development of some antibiotic manufacture.

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