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Research Article

Soil fungi as a new source for production of cephalosporin C acylase and its optimization conditions

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Abstract

Fifty fungal isolates representing thirty one (31) species and eight (8) genera were isolated from soil and screened for their abilities to produce endocellular and exocellular cephalosporin C acylase enzymes. For endocellular enzyme, sixteen (16) isolates (represent 32% of total isolates) exhibited high enzyme production and nine (9) isolates (18%) had moderate ability. However, seventeen isolates (34%) were low producers and eight isolates (16%) had no ability to produce the enzyme. For exocellular enzyme, twenty seven isolates (represent 54% of total isolates) exhibited high enzyme production and six isolates (12%) were found to be moderate. However, nine isolates (18%) were low producers and eight isolates (16%) had no production. The most active fungal isolates for exocellular enzyme were *Gibberella intricans* and *Nectria haematococca.* They produced 998 and 1000 units/ml, respectively. Maximum activity of cephalosporin C acylase produced by these fungi was obtained after seven (7) incubation days at 30°C with initial pH 7 in culture medium containing glucose or maltose and ammonium sulphate or peptone as carbon and nitrogen sources, respectively. Inoculation of cultures by three (3) discs of fungi and incubation of cultures at 180 rpm shaking condition increased the enzyme production.

Keywords: Cephalosporin C acylase, *Gibberella intricans* and *Nectria haematococca*

INTRODUCTION

Cephalosporin acylase (CA) is a recently identified N-terminal hydrolase. It is also a commercially important enzyme in producing 7-aminocephalosporanic acid (7-ACA), a backbone chemical in synthesizing semi-synthetic cephalosporin antibiotics. CA is translated as an inactive single chain precursor, being post-translationally modified into an active enzyme. The post-translational modification takes place in two steps. The first intramolecular autocatalytic proteolysis takes place at one end of the spacer peptide by a nucleophilic Ser or Thr, which in turn becomes a new N-terminal Ser or Thr. The second intermolecular modification cleaves off the other end of the spacer peptide by another CA. Two binary structures in complex with glutaryl-7-ACA (the most favored substrate of CAs) and glutarate (side chain of glutaryl-7-ACA) were determined, and they revealed the detailed interactions of glutaryl-7-ACA with the active site residues (Kim and Hol, 2001). In other report they have mutated key active site residues into nonfunctional amino acids, and their roles in catalysis were further analyzed and performed mutagenesis studies indicating that secondary intermolecular modification is carried out in the same active site where deacylation reaction of CA occurs and the cleavage site of secondary intermolecular modification by another CA was identified in the spacer peptide using mutational analysis (Kim and Kim, 2001).

The effective production of 7-aminocephalosporanic acid (7-ACA) is a matter of concern in the pharmaceutical

industry because it is a starting material for the synthesis of semi synthetic cephalosporins, which are the best-sold antibiotics worldwide, with global sales of $8.3 billion of $466.3 billion of the total pharmaceutical market in 2003. Semi synthetic cephalosporins are made by the modification of the side chains of positions 3 and 7 of 7-ACA, which are commercially supplied mainly by the chemical deacylation of cephalosporin C (CPC), produced by the fungus *Acremonium chrysogenum* (Velasco et al., 2000; Oh et al., 2003). However, the chemical process requires several complicated steps using toxic compounds and produces a lot of chemical wastes. The innovation of an enzymatic process involving two enzymes has recently become the new deacylation process of CPC on an industrial scale. This enzymatic process involves no toxic raw materials, proceeds under mild reaction conditions, and reduces waste significantly. In this process, D-amino acid oxidase converts CPC to 7-(5-carboxy-5-oxopentanamido)-cephalosporanic acid, followed by auto-conversion to glutaryl-7-ACA (GL-7-ACA). Cephalosporin acylase then deacylates GL-7-ACA to 7-ACA (Kumar et al.,1993; Battistel et al.,1998; Li et al., 2001). The critical enzyme of this bioprocess is cephalosporin acylase, and extensive screening for this enzymatic activity is extremely important. However, this enzyme has been found only in a limited number of bacterial strains (Franzosi et al., 1995; Lee et al., 1998; Chen et al., 2001; Elander, 2003; Sonawane, 2006). The cephalosporin acylase can be found in several *Pseudomonas* species (such as *Pseudomonas putida*, *Pseudomonas cepacia*, *Pseudomonas nitroreducens*, *Pseudomonas syringae* and *Pseudomonas paucimobilis*) and other bacteria including *Bacillus cereus*, *Escherchia coli* and *Achromobacter xylosooxidans* (Shi et al., 1991; Saito et al., 1996; Linda et al., 2002; Zha and Yang, 2003; Tanomand et al., 2008, 2009; Ren, 2013; Ren et al., 2014). The discovery of such an industrially useful cephalosporin acylase is dependent on the availability of a sensitive assay which allows detection of even low acylase activity in crude biological samples. Different enzymatic methods for detecting microorganisms capable of producing cephalosporin C (CPC) acylase and/or 7-(4-carboxybutanamido) cephalosporanic acid (GL-7-ACA) acylase have been developed (Ishii et al., 1994; Velasco et al., 2000; Plhácková et al., 2003; Zhu et al., 2003). For example a method is based on the degradation of 2-nitro-5-(6-bromohexanoylamino) benzoic acid (NBHAB), a chromogenic substrate, into yellow 2-nitro-5-aminobenzoic acid by the action of the CPC acylase or the GL-7-ACA acylase (Akio and Keh-ichi, 1985; Aramori et al., 1991). This research was carried out to investigate the ability of soil fungi to produce endocellular and exocellular cephalosporin C acylase enzymes as well as the effect of different environmental and nutritional factors affecting the production of the enzyme by the most active fungal isolates.

MATERIALS AND METHODS

Screening of fungi for production of cephalosporin C acylases

Fifty fungal isolates representing thirty one (31) species and eight (8) genera were recovered from soil and screened for their abilities to produce intracellular and extracellular cephalosporin C acylases. These isolates belong to several genera including *Aspergillus* (10 species), *Emericella* (1), *Fusarium* (7), *Gibberella* (3), *Nectria* (1), *Paecilomyces* (1), *Penicillium* (7) and *Trichoderma* (1).

Production medium

Cephalosporin C acylase was investigated by using the following standard medium which contained the following constituents (g/L): Glucose, 20g; peptone, 5g; yeast extract, 5g; K_2HPO_4, 1g; $MgSO_4.7H_2O$, 0.2g and cephalosporin C 0.3 g as inducer. The pH was adjusted to 5.8.

Cultivation and culture conditions

The medium was distributed in Erlenmeyer flasks (250 ml), each flask contains 100 ml of the medium. The flasks were sterilized at 121°C for 20 minutes. Each flask 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 conditions at 30°C for seven (7) days. At the end of the incubation period, the content of each flask was filtrated.

Determination the activity of cephalosporin C acylase

Three grams of wet biomass was weighted and suspended in a 6 ml mixture of 0.1M phosphate buffer (pH=7), 20% sucrose and 1mM EDTA. 0.5g of glass beads were added and the microtubes were placed in a bead mill at 4°C for 25 minutes followed by centrifugation at 13000 rpm for 5 minutes in the case of interacellular enzyme but in case of extracellular enzyme the supernatant was used directly after filtration of sample. The colorimetric method for

determination of 7-aminocephalosporanic acid described by Balasingham et al. (1972) was employed. To 0.25 ml of 0.1M phosphate buffer (pH=7) containing 2.5 mg of cephalosporin C, 0.25 ml of cell suspension (intracellular enzyme) or supernatant (extracellular enzyme) was added. After incubation at 37°C for 30 minutes, 3 ml of solution made by mixing 2 ml of glacial acetic acid with 1 ml of 0.05M NaOH was added to the reaction mixture and centrifuged. 0.5 of 0.5% P-dimethylaminobenzaldehyed in methanol was added to the supernatant, and the absorbance was measured at 415 nm. One unit of the enzyme activity was defined as the quantity of enzyme which produced 1umol of 7-aminocephalosporanic acid per 1 minute. Standard curve of 7-aminocephalosporanic acid was shown in Figure 1.

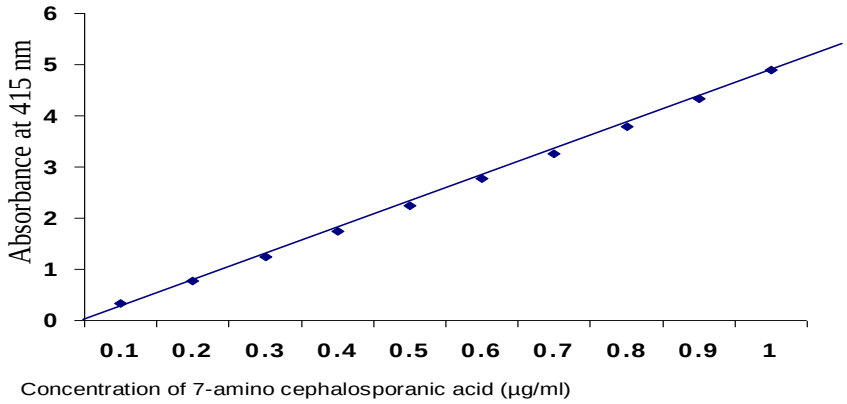

Figure 1. Standard curve of 7-amino cephalosporanic acid

Effect of incubation periods on cephalosporin C acylase production by *Gibberella intricans* and *Nectria haematococca*

The influence of different incubation periods (2, 4, 6, 7, 8, 10 and 12 days) on the production of cephalosporin C acylase and fungal dry weight were tested. The flasks were sterilized at 121°C for 20 minutes, inoculated with 10mm 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 at 30°C. After 48 hours intervals, fungal mycelium was separated from the growth medium by suction filtration through pre-weighed whatman #1 filter paper placed in buchner funnel. The filter paper with mycelium was dried in an oven at 85°C for 24 hours. The mycelial dry weight was recorded. The activity of cephalosporin C acylase was determined by using supernatant, according to method described by (Balasingham *et al.,* 1972).

Effect of temperatures on cephalosporin C acylase production by *Gibberella intricans* and *Nectria haematococca*

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

Effect of pH value on cephalosporin C acylase production by *Gibberella intricans* and *Nectria haematococca*

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

Effect of different carbon sources on cephalosporin C acylase production by *Gibberella intricans* and *Nectria haematococca*

The two fungal species were grown in Erlenmeyer flasks (250ml) containing 100 ml liquid medium. Nine carbon sources were added individually to the basal medium as follow (g/L): Glucose, 20 g; Fructose, 20 g; Maltose, 7.9 g; Lactose, 7.9 g; Sucrose, 8.4 g; Cellulose, 20g; Starch, 20g; Dextrin, 20g and Glycerol, 7.8g.. The flasks were sterilized at 121°C for 20 minutes, inoculated with 10mm disc cut out from five (5) days fungal colony grown on glucose-Czapek's agar

medium. Two replicates were used for each carbon source. Inoculated flasks were incubated under shaking condition at 30°C for seven (7) days. At the end of the incubation period, the mycelial dry weight was recorded. The activity of cephalosporin C acylase was determined by using supernatant, according to the method described by Balasingham et al. (1972).

Effect of different nitrogen sources on cephalosporin C acylase production by *Gibberella intricans* and *Nectria haematococca*

Gibberella intricans and *Nectria haematococca* were grown in Erlenmeyer flasks (250 ml) containing 100 ml liquid medium. Nine nitrogen sources were added individually to the basal medium as follow (g/L): Peptone, 5g; NH_4Cl, 5g; $(NH_4)_2SO_4$, 5g; NH_4NO_3, 5g; $(NH_4)_2HPO_4$, 5g; $NaNO_3$, 5g; KNO_3, 5g; $Ca(NO_3)_2.4H_2O$, 5g and CH_3COONH_4, 4g. Flasks were sterilized at 121°C for 20 minutes, 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 at 30°C for 7 days. At the end of the incubation period, the mycelial dry weight and the activity of cephalosporin C acylase were determined, according to the method described by Balasingham *et al.* (1972).

Effect of inoculum size on cephalosporin C acylase production by *Gibberella intricans* and *Nectria haematococca*

The influence of inoculum size on the production of cephalosporin C acylase was measured by adding 1, 2 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 inoculated flasks were incubated for 7 days at 30°C in liquid synthetic medium. After the incubation period, cephalosporin C acylase was measured, according to method described by Balasingham *et al.* (1972) in two replicates and fungal dry weight was determined.

Effect of shaker speed on cephalosporin C acylase production by *Gibberella intricans* and *Nectria haematococca*

The production of cephalosporin C 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 three (3) groups: The first group placed on rotary shaker at 160 rpm and 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).

Effect of medium quantity on cephalosporin C acylase production by *Gibberella intricans* and *Nectria haematococca*

This experiment was occurred by using two (2) volumes of liquid synthetic medium (50 and 100 ml) in 250 ml Erlenmeyer flasks. The flasks were inoculated by 10mm mycelial disc of tested fungi and incubated at 30°C for seven (7) days. After the incubation period, cephalosporin C 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 cephalosporin C acylase enzyme

Screening of fifty fungal isolates for production of endocellular cephalosporin C acylase showed that, sixteen isolates (represent 32% of total isolates) exhibited high enzyme production and 9 isolates (18%) had moderate ability. However, seventeen isolates (34%) were low producers and eight isolates (16%) had no ability to produce the enzyme. For exocellular enzyme, twenty seven isolates (represent 54% of total isolates) exhibited high enzyme production and six isolates (12%) were found to be moderate. However, nine isolates (18%) were low producers and eight isolates (16%) had no production (Table 1). For exocellular enzyme, twenty seven isolates (represent 54% of total isolates) exhibited high enzyme production and six isolates (12%) were found to be moderate. However, nine isolates (18%) were low producers and eight isolates (16%) had no production. The most active fungal isolates for exocellular enzyme were *Gibberella intricans* and *Nectria haematococca.* They produced 998 and 1000 units/ml, respectively (Table 1).

Table 1. Screening of fungi for their abilities to produce end- and exocellular cephalosporin C acylase

Fungi	Endocellular cephalosporin acylase			Exocellular cephalosporin acylase		
	UV-Absorbance at 415 nm	Enzyme concentration	Enzyme activity	UV-Absorbance at 415 nm	Enzyme concentration	Enzyme activity
Aspergillus flavus	0.31	88.00 L	3194.7	0.31	88 L	3194.7
	0.11	50.00 L	1815.2	-	-	-
A. fumigatus	0.02	9.09 L	330.0	0.55	155 M	5627.1
	-	-	-	0.35	91 L	3303.7
A. galaucus	0.21	70.00 L	2541.2	1.84	420 H	15247.7
A. niger	2.45	525.00 H	19059.6	1.36	315 H	11435.8
	0.18	55.00 L	1996.7	0.38	115 M	2087.5
A.ochraceus	-	-	-	1.39	317 H	11508.4
A.sydowii	0.02	9.09 L	330.0	1.31	300 H	1089.1
A.tamarii	0.76	200.00 M	7260.8	0.01	4.54 L	164.8
	1.2	280.00 H	10165.1	0.82	221 H	8023.2
A.terreus	4.55	925.00 H	33581.2	2.04	450 H	16336.8
	1.18	275.00 H	9983.6	1.50	350 H	12706.4
A.ustus	0.34	90.00 L	3267.4	2.89	620 H	22508.5
A.versicolor	1.03	250..00 H	9076.0	1.39	317 H	11508.4
	0.61	165.00 M	5990.2	0.31	88 L	3194.7
Emericella nidulans	0.40	100.00 M	3630.4	1.20	280 H	10165.1
	0.04	18.18 L	660.0	0.75	200 M	7260.8
	0.90	225.00 H	8168.4	1.77	390 H	14158.6
Fusarium chlamydosporum	0.03	13.63 L	494.8	-	-	-
F.compactum	0.86	215.00 H	7805.4	0.66	175 M	6353.2
F.heterosporum	0.11	50.00 L	1815.2	3.61	765 H	27772.5
F.mersmipoides	0.75	200.00 M	7260.8	3.28	700 H	25412.8
F.oxysporum	0.94	235.00 H	8531.4	-	-	-
	0.29	99.00 L	3594.1	0.43	140 M	5082.6
F.sambucinum	-	-	-	-	-	-
F.tricinuctum	0.02	9.09 L	330.0	0.74	200 M	7260.8
Gibberella avenacea	-	-	-	-	-	-
G. fujikuroi	-	-	-	-	-	-
	-	-	-	-	-	-
G. intricans	0.27	90.00 L	1225.3	4.92	998 H	36231.4
	0.44	135.00 M	4901.0	2.89	620 H	22508.5
	1.59	355.00 H	12887.9	1.80	400 H	14521.6
Nectria haematococca	0.11	50.00 L	1815.2	5.00	1000 H	36304.0
	-	-	-	0.19	53.93 L	1957.9
Paecilomyces lilacinus	2.11	450.00 H	16336.8	2.07	460 H	16699.8
Penicillum aurantiogriseum	1.64	365.00 H	13251.0	0.89	240 H	8713.0
P.brevicompactum	1.53	350.00 H	12706.4	1.81	400 H	14521.6
	1.98	440.00 H	15973.8	3.31	715 H	25957.4
P.chrysogenum	0.40	100.00 M	3630.4	3.15	655 H	23779.1
	0.85	212.50 H	7714.6	0.82	221 H	8023.2
	1.00	250.00 H	9076.0	0.31	88 L	3194.7
	0.38	115.00 M	4175.0	3.51	750 H	27228.0
	0.57	140.00 M	5082.6	1.59	365 H	13251.0
P.duclauxii	0.20	69.00 L	2505.0	0.01	4.54 L	165.0
P.funiculosum	0.50	150.00 M	5445.6	3.23	690 H	25049.8
	1.25	290.00 H	10528.2	0.02	9.09 L	330.0
P.purpurogenum	0.33	89.00 L	3231.0	0.07	31.81 L	1154.8
P.roqueforti	0.35	91.00 L	3303.7	0.86	230 H	8349.9
Trichoderma harzianum	-	-	-	-	-	-

High production (H) > 200, Moderate production (M) = 100-200, Low production (L) < 100, No production (-).

The cephalosporin acylase was found in several Pseudomonas spp. and other bacteria isolated from clinical and environmental specimens (Tanomand et al., 2008). Bashir et al. (2008) screened twelve (12) different strains of fungi for their abilities to produce penicillin G acylase under submerged conditions. Among fungi tested *Aspergillus niger* had the highest enzyme activity.

Effect of incubation periods on fungal growth and cephalosporin C acylase by *Gibberella intricans* and *Nectria haematococca*

Gibberella intricans and *Nectria haematococca* exhibited maximum cephalosporin C acylase production after seven (7) days of incubation. These fungi had enzyme activity 18846.13 and 34488.8 respectively. Incubation time of six (6) and eight (8) days produced relatively considerable amounts of cephalosporin C acylase. However decreasing of incubation period to four (4) days or increasing time to ten (10) and twelve (12) days decreased the production of the enzyme by the two tested fungi. No cephalosporin C acylase was recorded by *G. intricans* and *N. haematococca* after two (2) days of incubation. The results recorded for the effect of incubation time on the mycelial growth of *G. intricans* and *N. haematococca* was nearly similar to those reported for cephalosporin C acylase enzyme (Table 2). Maximum production of penicillin G acylase of *Aspergillus niger* was recorded between 20 to 24 hours of incubation under shaking conditions (Bashir *et al*., 2008).

Table 2. Effect of incubation periods on growth and cephalosporin C acylase production by *Gibberella intricans* and *Nectria haematococca*

Incubation periods (day)	*Nectria haematococca*		*Gibberella intricans*	
	Enzyme activity	Dry weight (mg/100 ml)	Enzyme activity	Dry weight (mg/100 ml)
2	-	-	-	-
4	14521.60	345.71	8653.78	345.17
6	32673.60	409.21	17115.52	409.87
7	34488.80	415.64	18846.13	451.24
8	30858.40	394.52	16153.82	429.09
10	27954.08	367.98	13846.34	390.92
12	12706.40	309.24	7307.63	308.71

Effect of temperatures on fungal growth and cephalosporin C acylase by *Gibberella intricans* and *Nectria haematococca*

Production of cephalosporin C acylase by *G. intricans* and *N. haematococca* was influenced by the incubation temperature. Maximum production of the enzyme was recorded at 30°C by the two tested fungi. The enzyme activity were 33874.53 and 32673.60 by G. *intricans* and *N. haematococca* respectively. Considerable enzyme activity was also recorded at incubation temperature of 25°C and 35°C by the two fungi tested. Incubation temperature below 25°C or above 35°C decreased the enzyme activity. Decreasing of the incubation temperature to 15°C prevent the secretion of the enzyme by the two fungi (Table 3). Abedi *et al*. (2004) showed that the optimum temperature for penicillin G acylase 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 *E. coli* penicillin acylase activity were 27.5°C and 31.5°C for free and immobilized enzyme respectively. However, the optimum temperature for maximal penicillin G acylase of *Aspergillus fumigatus* and *Mucor gryseoclanum* was at 40°C (Jose *et al.*, 2003).

Table 3. Effect of temperatures on growth and cephalosporin C acylase production by *Gibberella intricans* and *Nectria haematococca*

Temperature	*Nectria haematococca*		*Gibberella intricans*	
	Enzyme activity	Dry weight (mg/100 ml)	Enzyme activity	Dry weight (mg/100 ml)
15°C	-	-	-	-
20°C	23597.60	391.62	24669.65	354.43
25°C	27954.08	414.93	28719.73	365.68
30°C	32673.60	475.69	33874.53	411.85
35°C	32310.56	460.86	32033.56	384.25
40°C	25412.80	389.62	22092	342.89

Effect of pH values on fungal growth and cephalosporin C acylase by *Gibberella intricans* and *Nectria haematococca*

Activity of cephalosporin C acylase produced by *G. intricans* and *N. haematococca* was affected by the initial pH of the medium. Maximum activity of the enzyme was recorded at pH 7 by the two fungi. The enzyme activities were 29456.33 and 35577.92 respectively. Initial pH 6 and 8 exhibited good yield of the enzyme by the tested fungi. pH above 8 or below 6 exhibited an inhibitive effect on cephalosporin C acylase acidity by *G. intricans* and *N. haematococca*. No enzyme activity was recorded at pH 2 and 12 by the two fungal species. Data recorded for the effect of pH value on

mycelial growth of *G. intricans* and *N. haematococca* was nearly similar to those reported for cephalosporin C acylase enzyme (Table 4). Binder et al. (1994) reported that pH 8 was found to be optimum for the production of Glutaryl-7-aminocephalosporanic acid acylase of *Pseudomonas*. Javadpour *et al*. (2002) reported that the optimal pH of penicillin acylase produced by *Escherichia coli* was determined to be 8. The optimum pH rang for dialyzed preparations of penicillin G acylase produced by *Aspergillus fumigatus* and *Mucor gryseoclanum* was 7-8 and 7.5-8.5 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.

Table 4. Effect of pH values on growth and cephalosporin C acylase production by *Gibberella intricans* and *Nectria haematococca*

pH value	*Nectria haematococca*		*Gibberella intricans*	
	Enzyme activity	Dry weight (mg/100 ml)	Enzyme activity	Dry weight (mg/100 ml)
2	-	-	-	-
4	24686.72	399.45	17084.66	408.65
6	34851.84	380.22	28867.12	398.05
7	35577.92	373.37	29456.33	497.21
8	31947.52	373.37	25332.20	386.95
10	10891.20	370.98	9425.97	390.59
12	-	-	-	-

Effect of carbon source on fungal growth and cephalosporin C acylase by *Gibberella intricans* and *Nectria haematococca*

Carbon source is one of the most essential components in the microbial medium. The effect of different carbon sources on growth and cephalosporin C acylase acivity by *G. intricans* and *N. haematococca* was variable. Maltose and glucose were the best carbon sources for cephalosporin C acylase activity produced by *G. intricans* and *N. haematococca* respectively. Maximum activity of the enzyme was achieved by incorporation of maltose and glucose in the culture medium for enzyme production by *G. intricans* and *N. haematococca* respectively. The activity of cephalosporin C acylase were 22533.89 and 29043.20 in case of *G. intricans* and *N. haematococca* respectively. Addition of other carbon sources in the fungal cultures decreased the enzyme production by the two fungal species. Mycelial growth of *G. intricans* and *N. haematococca* did 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 *Aspergillus niger* (Bashir *et al.*, 2008). Other carbon sources such as glucose, sorbitol glycerol, sucrose, PAA and tryptone were increased the activity of Penicillin G acylase by *E. coli* and *B. megaterium* (Chandel *et al.* 2008).

Table 5. Effect of different carbon sources on growth and cephalosporin C acylase production by *Gibberella intricans* and *Nectria haematococca*

Carbon sources	*Nectria haematococca*		*Gibberella intricans*	
	Enzyme activity	Dry weight (mg/100 ml)	Enzyme activity	Dry weight (mg/100 ml)
Glucose	29043.20	368.35	20324.79	379.36
Fructose	26138.88	373.56	22313.16	382.41
Maltose	26190.74	373.00	22533.89	391.73
Dextrin	17531.13	379.51	19220.06	351.23
Lactose	19458.94	371.92	15243.68	379.33
Cellulose	17716.35	375.61	14286.35	132.97
Sucrose	20620.67	376.39	18630.84	398.02
Starch	23234.56	375.22	15022.59	401.56
Glycerol	23089.34	378.86	15493.94	362.10

Effect of nitrogen source on fungal growth and cephalosporin C acylase by *Gibberella intricans* and *Nectria haematococca*

Nitrogen source is one of the most essential components in the microbial medium because the nitrogen element participates in the formation of amino and nucleic acids and protein in microbial cells. Among nine nitrogen sources studied, peptone and ammonium sulphate were the best nitrogen sources for cephalosporin C acylase activity by *G. intricans* and *N. haematococca* respectively. Maximum activity of the enzyme was recorded by the incorporation of peptone or sodium sulphate in the culture medium for enzyme production. The enzyme activities were 32254.65 and 35214.88 by *G. intricans* and *N. haematococca* respectively. Considerable amounts of cephalosporin C acylase were also recorded

when potassium nitrate and ammonium chloride incorporated into the medium. However the other nitrogen sources decreased the enzyme activity with variable degrees. Mycelial growth of *G. intricans* and *N. haematococca* did 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.

Table 6. Effect of different nitrogen sources on growth and cephalosporin C acylase production *by Gibberella intricans* and *Nectria haematococca*

Nitrogen sources	*Nectria haematococca*		*Gibberella intricans*	
	Enzyme activity	Dry weight (mg/100 ml)	Enzyme activity	Dry weight (mg/100 ml)
$(NH_4)_2 SO_4$	35214.88	370.07	30929.19	350.44
$(NH_4)_2 HPO_4$	29769.28	386.05	19882.97	343.34
KNO_3	34488.80	366.31	27836.09	423.97
CH_3COONH_4	31947.52	380.71	24301.53	336.97
$Ca(NO_3)_2$	29043.20	390.52	17673.51	486.02
NH_4NO_3	33036.64	361.97	26510.63	408.48
$NaNO_3$	33762.72	368.29	27394.27	345.21
NH_4Cl	34851.84	370.06	28719.73	353.31
Peptone	32310.56	372.42	32254.65	450.94

Effect of inoculum size on fungal growth and cephalosporin C acylase by *Gibberella intricans* and *Nectria haematococca*

Maximum yield of cephalosporin C acylase was achieved by inoculating of liquid synthetic medium with 3 discs (10 mm diameter) of fungi. *G. intricans* cephalosporin C acylase activity was 18461.67 corresponding to 38482.24 for *N. haematococca*. Decreasing of the inoculum size to 2 or 1 disk decreased the production of cephalosporin C acylase enzyme by the two fungal species. Mycelial growth of *G. intricans* and *N. haematococca* did not greatly affected by inoculation size of fungi (Table 7). Spore concentration 1.5 x 10^7 spores/ml and germination during 24 and 72 hours showed maximum Penicillin G acylase activity by *Bacillus megaterium* (Chandel *et al.* 2008).

Table 7. Effect of inoculum size on growth and cephalosporin C acylase production by *Gibberella intricans* and *Nectria haematococca*

Inoculum size	*Nectria haematococca*		*Gibberella intricans*	
	Enzyme activity	Dry weight (mg/100 ml)	Enzyme activity	Dry weight (mg/100 ml)
1 disc	34851.84	368.65	15384.54	382.45
2 discs	37756.16	367.03	17307.93	389.55
3 discs	38482.24	378.25	18461.67	380.16

Effect of medium volume on fungal growth and cephalosporin C acylase by *Gibberella intricans* and *Nectria haematococca*

Cephalosporin C acylase activity was greatly influenced by the amount of cultivation medium. The enzyme activity increased with the increasing of medium volume by the two tested fungi. The enzyme activity was 16538.65 and 33399.68 for *G. intricans* and *N. haematococca* cultivated on 100 ml medium. Decreasing of the amount of medium to 50 ml decreased the enzyme activity by nearly half (7307.63 and 14521.60 for *G. intricans* and *N. haematococca*, respectively). Mycelial growth of *G. intricans* and *N. haematococca* did not greatly affect by the amount of medium (Table 8).

Table 8. Effect of medium quantity on growth and cephalosporin C acylase production by *Gibberella intricans* and *Nectria haematococca*

Medium quantity	*Nectria haematococca*		*Gibberella intricans*	
	Enzyme activity	Dry weight (mg/100 ml)	Enzyme activity	Dry weight (mg/100 ml)
100 ml	33399.68	382.90	16538.65	390.16
50 ml	14521.60	369.88	7307.63	386.67

Effect of shaker speed on fungal growth and cephalosporin C acylase by *Gibberella intricans* and *Nectria haematococca*

Speed of shaker had little effect on growth and Cephalosporin C acylase activity by *G. intricans* and *N. haematococca*. Maximum activity of Cephalosporin C acylase was recorded at 180 rpm (32767.99 and 36304.0 for *G. intricans* and *N. haematococca* respectively) (Table 9).

Table 9. Effect of shaker speed on growth and cephalosporin C acylase production by *Gibberella intricans* and *Nectria haematococca*

Shaker speed rpm	*Nectria haematococca*		*Gibberella intricans*	
	Enzyme activity	Dry weight (mg/100 ml)	Enzyme activity	Dry weight (mg/100 ml)
160	35287.48	369.15	29086.76	384.55
180	36304.00	370.41	32767.99	388.16
200	36158.78	372.00	29471.58	385.11

CONCLUSION

Cephalosporin acylase is the critical enzyme for production of cephalosporin antibiotics, and extensive screening for this enzymatic activity is extremely important. However, this enzyme has been found only in a limited number of bacterial strains. This research was carried out to investigate the ability of soil fungi to produce endocellular and exocellular cephalosporin C acylase enzymes as well as the effect of different environmental and nutritional factors affecting the production of the enzyme by the most active fungal isolates.

Discovery of new sources of the enzyme lead to improvement of antibiotic production and the development of antibiotic industries.

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