ISSN 0976 – 3333

Available Online at www.ijpba.info

International Journal of Pharmaceutical & Biological Archives 2011; 2(4):1190-1195

ORIGINAL RESEARCH ARTICLE

Effect of Minerals on Penicillin Production by *Penicillium chrysogenum* Strains Isolated from Coastal Marshes of Tamilnadu

A.Reena*[1], A. Panneerselvam[2], P.VinothKumar[1], OS.Aysha[1]

[1]*PG and Research Dept. of Microbiology Mohamed Sathak College of Arts and Science, Sholinganallur, Chennai-600119, TN.*

[2]*PG and Research Dept. of Botany &Microbiology, A.V.V.M. Sri Pushpam College,Poondi,Thanjavur Dist, TN.*

Received 22 May 2011; Revised 05 Aug 2011; Accepted 09 Aug 2011

ABSTRACT

Several studies revealed that mineral nutrients, including microelements, could stimulate the secondary metabolism in fungi. The importance of mineral elements in the production of penicillin was studied from three wild strains of *Penicillium chrysogenum* (MPPS1, MPPS2 and MPPS3), isolated from marsh soils of Muthupet coast, Tamilnadu. *P. chrysogenum* strains were cultured in PDA medium to evaluate their penicillin productivity levels after substituting different minerals ($Mgso_4$, $ZnSo_4$, KH_2Po_4). The results showed remarkable variations in the levels of penicillin productivity among the minerals and the strains. Among the three inorganic minerals used to test the optimum penicillin productivity by the three strains of *P. chrysogenum*, K_2HPO_4 showed higher productivity levels at 120h of fermentation period (*P. chrysogenum* MPPS1: 398.63 unit/ml; MPPS2: 442.60 unit/ml; MPPS3: 387.64 unit/ml). The penicillin production was comparatively low in the culture supernatants of $MgSo_4.7H_2O$ treated cultures. Optimum penicillin productivity was observed at 120h of fermentation period with all the three strains of P. *chrysogenum* (MPPS1: 392.24 unit/ml; MPPS2: 398.64 unit/ml; MPPS3: 380.22 unit/ml). The penicillin productivity data showed that zinc exhibited a moderate effect on penicillin production with all the three *P. chrysogenum* strains (MPPS1, MPPS2 and MPPS3). Penicillin production is increasing up to 394.16 unit/ml in the MPPS2 strain, when compared to MPPS1 (367.48 unit/ml) and MPPS3 (336.28 unit/ml) strains. The pH levels of the culture media treated with $MgSo_4.7H_2O$, K_2HPO_4 and $ZnSo_4$ are exhibiting similar trends in their increase towards alkalinity, during the course of fermentation period.

Key words: *Penicillium chrysogenum*, Penicillin, $Mgso_4$, $ZnSo_4$, KH_2Po_4.

INTRODUCTION

It is clear that the industrial microbiology field has utilized only a very minor portion of nature's microbial arsenal for the discovery of useful molecules. The reason is the inability of microbiologists to culture the vast majority of microbes in nature. It is estimated that only 1% of bacteria and 5% of fungi have been cultivated in the laboratory. This problem is being studied by a number of groups [1]. Penicillin biosynthesis is regulated by environmental factors such as carbon, nitrogen and phosphate content of the medium [2]. Fermentation development in the laboratory needs the determination of carbon, nitrogen, inorganic, and if necessary, complex nutrients supporting growth, and then modifications of the medium to support product biosynthesis. Several studies revealed that mineral nutrients, including microelements, could stimulate the secondary metabolism in fungi [3]. Mineral salts are usually used in enzymatic production [4,5,6], studied effect of media composition on the penicillin production, and reported that the penicillin activity was not affected, due to the addition of carbonate (0-1%). They also reported that the omission of cupric, magnesium, manganese, zinc sulphates and acetic acid did not affect the penicillin activity, while the omission of ammonium nitrate and potassium dihydrogen phosphate decreased the penicillin activity in the medium. The uptake of sulphate, the first step in the pathway, has been studied by using mycelium and isolated plasma membrane vesicles from *P. chrysogenum* [7]. These experiments indicate that sulphate is actively transported across the plasma membrane via a

***Corresponding Author:** Dr.A.Reena, **Email:** reena.denzil@yahoo.com, **Phone No:** +91-9176942150

sulphate / proton symport mechanism. It has been reported that inorganic phosphate in the culture medium controls the synthesis of a large number of secondary metabolites belonging to different biosynthetic groups such as, macrolides, tetracyclines, anthracyclines, polyether compounds, amino glycosides, and amino acid derived metabolites such as clavulani C acid and many other compounds [8]. The negative control exerted by inorganic phosphate on the biosynthesis of antibiotics and other secondary metabolites has been reported by many investigators [9]. Mineral salts (NaCl, KCl, $MgSO_4$, $Feso_4$, $MnCl_2$, $ZnSo_4$, $CaCl_2$ and $CuSo_4$) are usually used in enzyme production [4, 5] Effect of different salts on Xylanase production in solid-state fermentation of *Penicillium* sp. has been studied by [10]. It was assumed that potential isolates of *P. chrysogenum* may exist in the soil as natural source and penicillin G may be found higher in the quality and quantity than existing isolates of *P. chrysogenum.* The coastal soils of India [11], especially Tamilnadu [12], potentially have enormous biodiversity of *Penicillium* sp. However, they have not been extensively explored to identify the commercially viable species of *P. chrysogenum* strains and to evaluate their ability to form penicillin [13]. The successful development of strains for large-scale industrial production of heterologus proteins [14], and low value fuels, chemicals and materials [15], merit the composition of cultivation media in various steps of strain development. Therefore, there is need of a systematic screening program to isolate the potential antibiotic producing strains of *P. chrysogenum* from different soil samples and to determine the effect of different minerals through quantitative assays for its optimal productivity.

MATERIALS AND METHODS

Penicillium chrysogenum strains and Inoculums preparation

P. chrysogenum strains were isolated from soil in Mangrove marshes and agricultural lands of Muthupettai area. The cultures used throughout the experiment were maintained on a Potato Dextrose Agar (PDA) slant. Slants were inoculated, incubated at 30°C for 7 days and then stored at 4°C for inoculum preparation. The organism grown on the PDA medium in Petri plates were transferred to the seed culture medium by punching out 6mm of the agar plate culture with a sterilized cutter. The seed culture was grown in 250ml Erlenmeyer flask containing 50ml of corn steep broth: (Corn steep – 35 gm, Lactose – 35gm, Glucose 10gm, $Cacl_2$ – 5gm, KH_2Po_4 – 5gm in 1 litre of distilled water. pH is adjusted to 6.8 with NaoH (30% w/v) medium at room temperature on a rotary shaker (Lab Line Orbital Shaking Incubator) at 200 rpm for 48 h.

Effect of Mineral salts

Magnesium (Mg^{2+}) and Zinc ($Zn\ 2^{+}$) in the form of sulfate salts ($Mgso_4.7H_2O$, $ZnSo_4.7H_2O$) and potassium (K^{+}) in the form of mono and dipotassium hydrogen phosphate are used. Each metal ion at concentration of 5mg/l is added to Cornsteep medium containing 35 g/l Cornsteep and 35-g/l lactose to investigate the effect of these ions on penicillin yield. A control culture medium was prepared, without enhancing the amount of any one of the minerals, followed in the treated cultures.

Determination of Penicillin

Penicillin production in the broth extract was estimated by the volumetric analysis of Perret's iodometric method [16], depending upon the reduction of iodine by benzylpenicillin hydrolysis, since it is the sophisticated iodometric assay method, using spectrophotometric assay of β-lactamase activity.

Statistical analysis of data

Data was analysed by one way analysis of variance (ANOVA) followed by fischer's LSD post hoc test using spss 10.0 software (spss Inc,Chicago). The values are expressed as mean ± SEM for triplicates in each bacterial strain.

RESULTS AND DISCUSSION

In the present study, an attempt has been made to find out the optimum penicillin productivity of *P.chrysogenum* strains, by supplementing the culture medium with minerals viz., KH_2Po_4, $MgSo_47H_2O$ and $Znso_4$.

Control Culture

The result of the penicillin productivity is given in (**Table 1**). The highest penicillin productivity value of 361.64 unit/ml was obtained at 120 h from the culture for MPPS1 strain. This is higher than the peak values of MPPS2 (358.64 unit/ml) and MPPS3 (334.64 Unit/ml) strains. During the course of fermentation period the pH level of the culture media increased from 6.4 to 8.2, for all the three strains of *Penicillium chrysogenum.*

Three strains of *P. chrysogenum* (MPPS1, MPPS2 and MPPS3 were cultured in PDA medium to evaluate their penicillin productivity levels after substituting different minerals ($MgSo_4$, $ZnSo_4$, KH_2Po_4). The results showed remarkable variations in the levels of penicillin productivity among the minerals and the strains (**Fig.1, 2 & 3**).

Among the three inorganic minerals used to test the optimum penicillin productivity by the three strains of *P. chrysogenum*, K_2HPO_4 showed higher productivity levels at 120h of fermentation period (*P. chrysogenum* MPPS1: 398.63 unit/ml; MPPS2: 442.60 unit/ml; MPPS3: 387.64 unit/ml) (**Table 2**). The penicillin production of the *P. chrysogenum* strains was comparatively low in the culture supernatants of $MgSo_4.7H_2O$ treated cultures. Optimum penicillin productivity was observed at 120h of fermentation period with all the three strains of *P. chrysogenum* (MPPS1: 392.24 unit/ml; MPPS2: 398.64 unit/ml; MPPS3: 380.22 unit/ml) (**Table 3**). The penicillin productivity data showed that zinc exhibited a moderate effect on penicillin production with all the three *P. chrysogenum* strains (MPPS1, MPPS2 and MPPS3). Penicillin production is increasing up to 394.16 unit/ml in the MPPS2 strain, when compared to MPPS1 (367.48 unit/ml) and MPPS3 (336.28 unit/ml) strains (**Table 4**). The pH levels of the culture media treated with $MgSo_4.7H_2O$, K_2HPO_4 and $ZnSo_4$ are exhibiting similar trends in their increase towards alkalinity, during the course of fermentation period.

Among the minerals, KH_2Po_4 shows higher penicillin productivity (*P.chrysogenum* MPPS1: 435.48u/ml; MPPS2: 497.64u/ml; MPPS3: 419.81u/ml). Earlier, Foster [17], has studied the effect of phosphate concentration on penicillin formation and observed that phosphate gives maximum growth, and when much more phosphate is assimilated (0.03 percent), this leads to increased penicillin accumulation. Thus, the favorable effect of phosphorus on penicillin formation appears not only to synthesis of more cell material but also to effects on the metabolism of the mold. Very recently [10], have observed that phosphorous mono-pottassic induces a fall of secondary metabolite productivity in *Penicillium* sp., whereas sources of phosphorous di-pottassic enhance their productivity. According to [10], sources of phosphorous mono-pottassic cause a fall in the initial pH of the fermentation medium whereas sources of phosphorous di-pottassic increase the initial pH towards neutrality. In order to maintain the elemental balance of the growing mycelia and to provide optimal conditions for penicillin-G biosynthesis, a novel strategy of phosphorous feeding is reported by [18]. The required phosphorous to feed has been calculated by the mass flow distribution and the phosphorous composition of the growing mycelia. The optimal amount of supplied phosphorous has been experimentally determined for repeated fed-batch process. Initially, [18], have found that inorganic phosphorous source such as KH_2Po_4 is effective. But a combination of inorganic KH_2Po_4 with organic phosphorous, such as Cornsteep liquor, has showed the best results. Feeding KH_2Po_4 together with Cornsteep liquor after the first withdrawal has improved the penicillin titers by 16.1% on average. Their fermentation experiments and validation processes has showed a similarity with the results of the present study, which proves that feeding phosphorous in the cornsteep liquor culture medium is an efficient way to improve the production of penicillin-G. This phosphorous feeding strategy might also be useful to improve the fermentation processes of other industrial biological products [10, 18]. One of the transport processes, which play an important role in the biosynthesis of penicillins by filamentous fungi, is the uptake of sulfate. Current penicillin production processes yield final penicillin titers, which are more than 500 times higher than those, obtained with the parental *Penicillium chrysogenum* strain NRRL 1951 [19]. Sulfate is the primary sulfur source in industrial production of penicillin and is the precursor of cysteine that together with valine forms the backbone of the penicillin molecule [19]. Therefore, the strongly increased demand for cysteine during penicillin production requires an elevated rate of sulfate uptake from the environment. In the present investigation, *P.chrysogenum* strains cultured in magnesium sulphate ($MgSo_4.7H_2O$) supplemented medium produce (MPPS1: 392.24u/ml; MPPS2: 398.64u/ml; MPPS3: 380.22u/ml) optimum level of penicillin at 120h of incubation. Earlier works on the sulphur metabolism of moulds are limited and they mainly concerned with *Aspergillus niger*. The sulphate uptake system in *P. chrysogenum* has been characterized in detail either using mycelium [20],or using isolated plasma membranes [19]. In the current observation, optimum penicillin productivity has been achieved significantly from the *P.chrysogenum* strains in the medium with ZnSo4 (MPPS1: 367.48u/ml; MPPS2: 394.16u/ml; MPPS3: 336.28u/ml). There are similar earlier reports on the effect of zinc on penicillin production, which is increasing up to 1 to 3 mg ZnSO4-7H20/l in the medium, and falling off markedly at higher concentrations [17]. This concentration of zinc remains optimum, irrespective of the presence of different amounts of the other heavy metals in various combinations.

Growth is also markedly increases up to the 10 mg ZnSO4-7H20/l level. However, higher concentrations of zinc become toxic. Thus, in effect, zinc simply accelerates the formation of penicillin, probably because it accelerates the rise in pH through oxidation of gluconic acid by the mold. Our data bear out the idea that the role of zinc in penicillin production appears to be concerned with oxidative metabolism of the mold. Since the optimum pH range for penicillin production lies between 6 and 8, the effect of zinc in accelerating the rise in pH is critical.

Table.1. Penicillin production by *Penicillium chrysogenum* strains isolated from different soils in the control culture medium.

Time (Hours)	MPPS1		MPPS2		MPPS3	
	Unit/ml	pH	Unit/ml	pH	Unit/ml	pH
72	313.33 ± 5.77	6.5	306.66 ± 5.77	6.6	293.33 ± 5.77	6.4
96	336.28 ± 7.21	6.8	324.38 ± 6.22	7.0	308.26 ± 6.22	6.8
120	361.64 ± 8.24	7.1	358.64 ± 8.21	7.2	334.64 ± 8.24	7.2
144	322.82 ± 6.22	7.5	332.28 ± 7.24	7.6	289.62 ± 7.21	7.7
168	304.44 ± 10.55	8.0	306.58 ± 6.22	8.0	261.22 ± 6.24	8.2

Values are expressed as standard mean ± SEM

Table.2. Effect of dipotassium hydrogen phosphate (K_2HPO_4) on penicillin yield by different strains of *Penicillium chrysogenum*.

Time (Hours)	MPPS1		MPPS2		MPPS3	
	Unit/ml	pH	Unit/ml	pH	Unit/ml	pH
72	333.33 ± 5.77	6.4	346.66 ± 5.77	6.3	326.66 ± 5.77	6.3
96	367.46 ± 6.22	6.9	382.28 ± 8.24	6.8	362.61 ± 7.28	6.8
120	398.63 ± 5.77	7.3	442.60 ± 7.24	7.2	387.64 ± 8.22	7.3
144	362.18 ± 6.24	7.8	375.28 ± 6.22	7.6	357.04 ± 6.24	7.8
168	328.62 ± 7.28	8.3	338.48 ± 7.22	8.1	311.36 ± 7.24	8.2

Values are expressed as standard mean ± SEM

Table.3. Effect of magnesium sulphate ($MgSo_4.7H_2O$) on penicillin yield by different strains of *Penicillium chrysogenum*

Time (Hours)	MPPS1		MPPS2		MPPS3	
	Unit/ml	pH	Unit/ml	pH	Unit/ml	pH
72	323.33 ± 5.77	6.4	326.66 ± 5.77	6.5	303.33 ± 5.77	6.4
96	351.62 ± 6.22	6.9	342.72 ± 6.24	6.9	340.28 ± 6.21	6.8
120	392.24 ± 8.24	7.4	398.64 ± 7.21	7.3	380.22 ± 6.22	7.2
144	346.62 ± 7.21	7.8	338.28 ± 6.22	7.8	328.48 ± 7.21	7.8
168	306.48 ± 6.22	8.3	312.62 ± 7.21	8.3	288.36 ± 6.24	8.3

Values are expressed as standard mean ± SEM

Table.4. Effect of Zinc sulphate ($ZnSo_4$) on penicillin yield by different strains of *Penicillium chrysogenum*

Time (Hours)	MPPS1		MPPS2		MPPS3	
	Unit/ml	pH	Unit/ml	pH	Unit/ml	pH
72	286.66 ± 11.54	6.4	296.66 ± 5.77	6.5	273.33 ± 5.77	6.4
96	310.26 ± 6.24	6.9	338.08 ± 7.22	6.9	298.62 ± 6.24	6.9
120	367.48 ± 8.22	7.2	394.16 ± 8.44	7.3	336.28 ± 6.22	7.3
144	291 ± 7.44	7.6	302.64 ± 8.22	7.8	228.55 ± 7.21	7.8
168	252.12 ± 8.24	8.2	282.48 ± 6.24	8.3	262.24 ± 6.24	8.3

Values are expressed as standard mean ± SEM

Fig.1. Penicillin Production by *Penicillium chrysogenum* strains MPPS1 under the influence of different Minerals

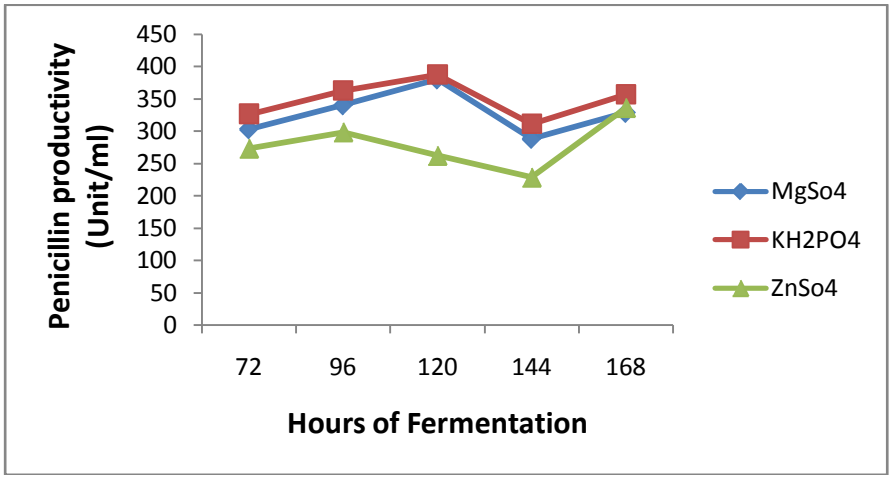


Fig.2. Penicillin Production by *Penicillium chrysogenum* strain MPPS2 under the influence of different Minerals

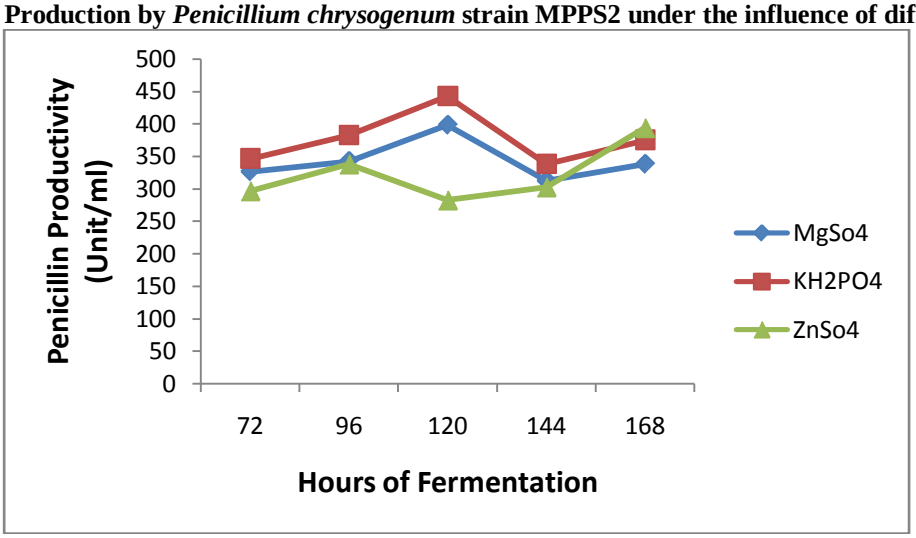
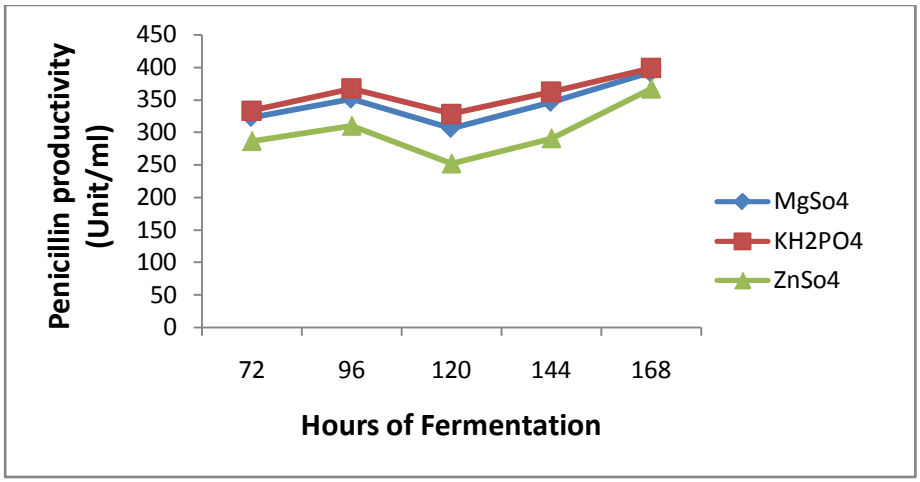


Fig.3. Penicillin Production by *Penicillium chrysogenum* strain MPPS3under the influence of different Minerals



IJPBA, July - Aug, 2011, Vol. 2, Issue, 4

ACKNOWLEDGEMENTS

The authors wish to thank PG and Research Dept. of Botany &Microbiology, A.V.V.M. Sri Pushpam College, Poondi, Thanjavur Dist, TN. For providing us the laboratory facility to carry out the part of work. We also grateful to the Mohamed sathak college of Arts and Science, Sholinganallur, Chennai. For giving us the support and constant encouragement for doing the research activity.

REFERENCE

1. Stevenson BS, Eichrost SA, Wertz JT, Schmidt TM, Breznak JA. New strategies for cultivation and detection of previously uncultured microbes. *Appl. Environ Microbiol*. 2004, 70: 4748-4755.
2. Feng B, Friedlin E, Marzluf GA. A reporter gene analysis of penicillin biosynthesis gene expression in *P. chrysogenum* and its regulation by nitrogen and glucose catabolite repression. *Applied and Environmental Microbiology*. 1994, 60(12), 4432-4439.
3. Lovkova MY, Buzuk GN, Ponomareva SM. Molecular-level regulation and the role of cobalt and zinc Ions in the metabolism of Isoquinolines, Indoles and Tropans. *Prikl. Biokhim. Microbiol*. 1995,31(1): 809-86.
4. Henning J, Astrid M, Kristain BRK, Lisbeth O. Production of cellulases and hemicellulases by three *Penicillium* species effect of substrate and evaluation of cellulose adsorption by capillary electrophoresis. *Enzyme Microb. Technol*. 2005, 36: 42-48.5.
5. Virupakshi S, Gireesh BK, Satish RG, Naik GR. Production of xylanolytic enzyme by a *thermoalkaliphilic Bacillus* sp. JB-99 in solid-state fermentation. *Process Biochem*. 2005, 40:431-435.

6. Marsafy E, Abdel-Akher M, El-Saied H. Effect of media composition on the penicillin production. *Zentralbl Bakteriol Parasiteen kd infections Kr Hyg*. 1977, 132(2): 117-22.
7. Hillenga DJ, Versantvoort HJM, Driessen AJM, Konings WN. Sulfate transport in *P. chrysogenum* plasma membranes. *J. Bacterial.* 1996, 178: 3953-3956.
8. Lounes A, Lebrihi A, Benslinane C, Lefebvre G, Germain P. Regulation of spiramycin synthesis in *Streptomyces ambofaciens*. Effect of glucose and inorganic phosphate. *Appl. Microbiol. Biotechnol*. 1996, 45: 204-211.
9. Liras P, Asturias JA, Martin JF. Phosphate control sequences involved in transcriptional regulation of antibiotic biosynthesis. *Trends. Biotechnol.* 1990, 8:184-189.
10. Assamoi AA, Delvigne F, Aldric JM, Destain J. et, Philippe Thonark, 2008. Improvement of xylanase production by penicillin camescens 10-10c in solid-state fermentation. *Biotechnol. Agron. Soc. Environ*. 12(2): 111-118.
11. Kumari GR, Mahrora S, Rao PS. Prevalence of Non-keratinophilic fungi in the soil. *Ind. J. Medi. Microbio*. 2005, 23: 144-145.
12. Kathiresan K, Manivannan S. α-amylase production by *Penicillium fellutanum* isolated from mangrove rhizosphere soil. *Afri. J. Biotech.* 2006, 5(10): 829-832.
13. Jayashree R, Sivagurunathan M, Sara Parvin Banu K, Ramasamy K. Value added products from penicillium Biomass. *Asian J. Microbial. Biotech & Env. Sc.* 1999, 1(1-2): 53-56.
14. Patrick MS, Fazenda ML, Mc Neil B, Harvey LM. Heterologus protein production using the *Pichia pastoris* expression system. *Yeast.* 2005, 22: 249-270.
15. Ostergaard S, Olsson L, Nielsen J. Metabolic engineering of *Saccharomyces cerevisiae. Microbiol Mol Biol Rev*. 2000, 64: 34-50.
16. Alicino JF. Iodometric assay of natural and synthetic penicillins, 6-aminopenicillanic acid and cephalosporin. *C. Anal. Chem*. 1961, 33:648-657.
17. Foster JW. Heavy metal nutrition of fungi. *Botan. Rev*. 1939, 5: 207-239.
18. Bing-li X, Rongzhaw G, Yuan YJ. A strategy of phosphorus feeding for repeated fed batch fermentation of penicillin G. *Biochemical engineering J*. 2005, 27(1):53-58.
19. Jorgensen H, Nielsen J, Villadsen J, Mollgaard H. Metabolic flux distributions in *P. chrysogenum* during fed-batch cultivations. *Biotechnol. Bioeng.* 1995,46: 117-131.
20. Bradfield G, Somerfield P, Meyn T, Holby M, Babcock D, Brdley D, Segel IH. Regulation of sulfate transport in filamentous fungi. *Plant Physiol*. 1970, 46: 720-727.