Comparative study on production of penicillin by *Penicillium notatum* and *Penicillium chrysogenum* & its antimicrobial property

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***Abstract*—** In the present study the antimicrobial activity of penicillin against the isolated bacteria from soil sample was studied. The bacterial species were isolated from the soil sample and identified by various biochemical tests. After that penicillium species were isolated from citrus fruits sample for isolation of penicillin. Finally the antibacterial activity was checked from the penicillin on the isolated bacterial species and was found that out of four bacterial species, penicillin showed remarkable effect on *Bacillus subtilis* but a small extend of inhibitory effect on *Bacillus cereus, Proteus vulgari and Neisseria flavescens.*

***Keywords*—** *Penicillium notatum, Penicillium chrysogenum, Bacillus subtilis, Bacillus cereus Proteus vulgari, Neisseria flavescens*, Penicillin and antimicrobial activity.

***Abbreviations*—** *PDA media: Potato Dextrose agar media, ZOI: Zone of inhibition, OD: optical density.*

I. INTRODUCTION

Fungi are an important component of the soil micro biota typically constituting more of the soil biomass than bacteria, depending on soil depth and nutrient condition [1]. Penicillium is a fungus, common in temperate and subtropical regions and can be found on salted food products but it is mostly found in indoor environments, especially in damp or water damaged buildings. It has rarely been reported as a cause of human disease. It is the source of several β-lactam antibiotics, most significantly penicillin. Other secondary metabolites of Penicillium include various penicillins, roquefortine C, meleagrin, chrysogine, xanthocillins, secalonic acids, sorrentanone, sorbicillin, and PR-toxin [2]. Penicillium leading to a large pharmaceutical industry of antibiotics [3]. It is estimated that there are 1.5 million fungal species on earth, of which only about 70 000 have been described till recently [4]. Penicillium has been used industrially to produce penicillin and xanthocillin X, to treat pulp mill waste, and to produce the enzymes polyamine oxidase, phospho-gluconate dehydrogenase, and glucose oxidase [5-6]. Penicillin falls under the beta-lactam group of antibiotics due its four-membered beta lactam ring structure. It inhibits the formation of peptidoglycan cross-links which weakens the bacterial cell wall. Therefore, bacteria will be vulnerable to cell lysis which is caused by osmotic pressure. The present study was aimed to explore the mycoflora diversity of antibiotics producing Penicillium species from citric source and to check their antimicrobial activity.

II. MATERIALS AND METHODS

Sample collection and Identification of Penicillium

The rotten citrus fruit sample i.e., rasbhari (*Physalis peruviana*) was collected for the isolation of *Penicillium spp.* in potato dextrose agar media (pH 7.2±0.2). The *Penicillium spp.* was identified from the colonies in petriplates by its blue green mold and white mycelium. Such colonies were visualized under binocular compound electronic microscope (Olympus) using lactophenol blue mounting (viz. slide plate technique) to confirm the mycelium and conodia of the fungi.

The bacterial species were also isolated from soil sample viz. serial dilution method and identification was done by gram's staining and biochemical test onto which the antimicrobial activity of isolated penicillin was assayed.

Production of Penicillin

Method of production of penicillin in the two different fermentation media was done viz. glucose (Glaxo laboratories (India) ltd.) and sucrose (RFCL limited, New Delhi) as substrate. The fermentation media was prepared and sterilized at 15psi, 121^{o}C for 15-20 minutes. The sterilized media was cooled to room temperature before inoculation. The flask with fermentation media were transferred to the clean air laminar flow chamber and 2ml of fungal culture was added to it and shaken well. After inoculation the flask with culture were kept in incubator for 7-14 days at 25-28^{o}C until harvest.

Sugar utilization analysis & Antibiotic assay

The sugar utilization analysis provided the data of how *Penicillium spp.* utilized the sugar content. DNS (3, 5-dinitrosalicylic acid) (BDH laboratory reagent) method is simple method for reducing sugar analysis by taking OD with PC based double beam

spectrophotometer2202 (Systronics) at 560nm. Antimicrobial activity of the crude penicillin extracts was determined by Agar well assay methods as previously described by Rojas et al. (2006).

II. Results and Discussion

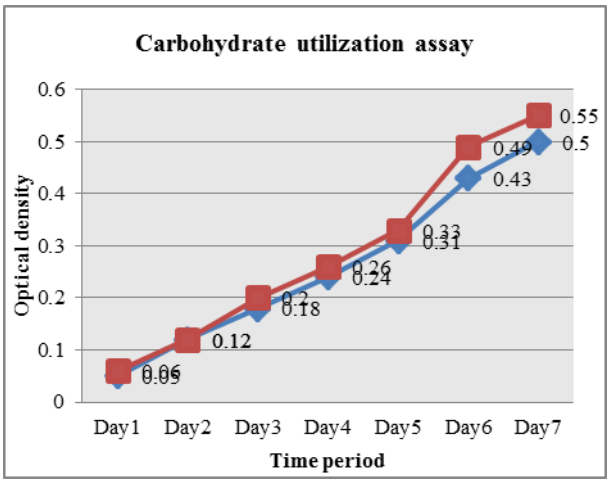
From the rotten citrus fruit sample i.e., rasbhari (*Physalis peruviana*) two types of *Penicillium species* were isolated viz.; *P. notatum and P.chyrosegnum* (Figure 1 a & b respectively). These strains of *Penicillium* were further processed for pure culture used in the production of penicillin.

(a)

(b)

Figure 1: (a) Isolated culture *of Penicillin notatum* (b) Isolated culture of *Penicillin chrysogenum* from *Physalis peruviana* on PDA (potato dextrose agar media) (pH7.2±0.2) media

The production of penicillin was done in two different fermentation media and the carbohydrate sugar source alone was changed. Both fermentation media consisted of 2gm/l brown sugar, 2gm/l casein, 0.68gm/l sodium hydrogen phosphate, 0.06gm/l ammonium chloride, 6gm/l ammonia and 2gm/l citric acid. The carbohydrate source for media 1 was 20gm/l glucose and media 2 was 20gm/l sucrose. The results of the penicillin production were estimated based on the routine carbohydrate utilization assay (Graph 1).



Graph 1: Carbohydrate utilization assay for penicillin production from Penicillium spp. viz. glucose and sucrose as substrate.

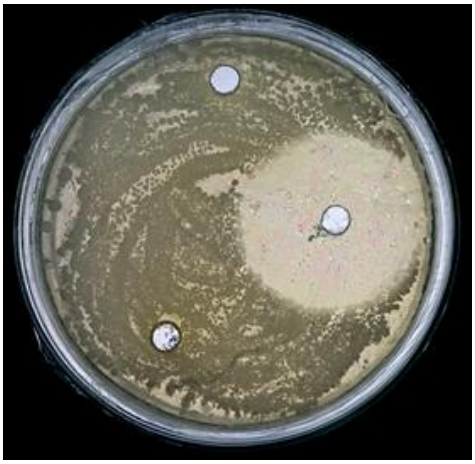
The antibacterial activity of isolated penicillin was checked on four bacterial species viz. Bacillus subtilis, Bacillus cereus, Proteus vulgaris and Neisseria flavescens by agar well diffusion method. The maximum inhibition of penicillin was showed on Bacillus subtilis while on other bacterial species (Bacillus cereus, Proteus vulgaris and Neisseria flavescens) no remarkable effect of inhibition was observed (Figure 2 (a), (b), (c) and (d)) (Graph 2 (a) & (b)).

TABLE 1

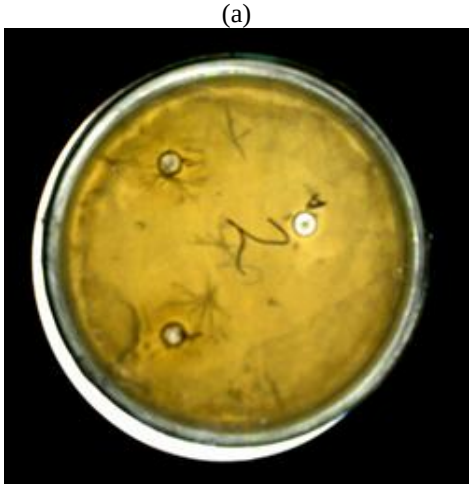
Antibacterial effect of Penicillin on Bacterial species

S.No.	Bacterial species	ZOI of penicillin
1.	*Bacillus subtilis*	****
2.	*Bacillus cereus*	**
3.	*Proteus vulgaris*	**
4.	*Neisseria flavescens*	**

, No remarkable effect of penicillin; *, Inhibitory effect of penicillin*

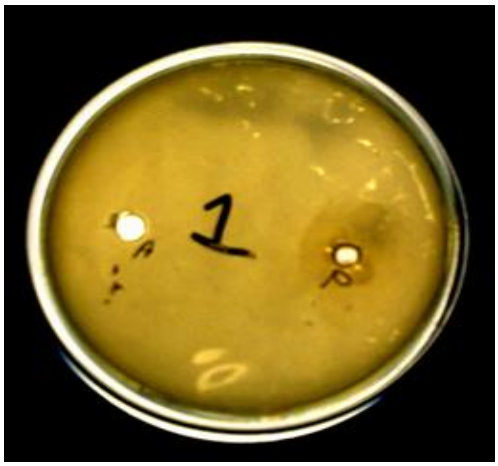


(a)



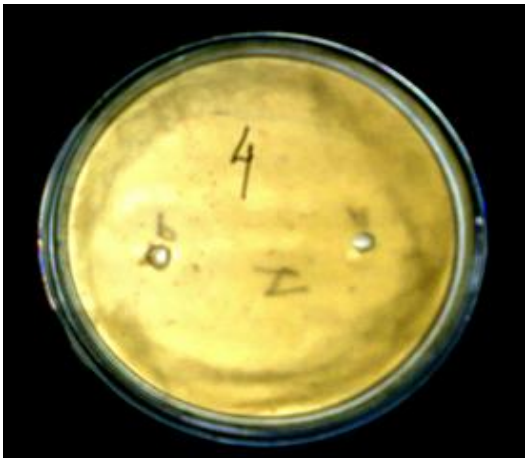
(b)

Figure 2: (a) Inhibitory effect of penicillin on *Bacillus subtilis*
(b) No Inhibitory effect of penicillin on *Proteus vulgaris*



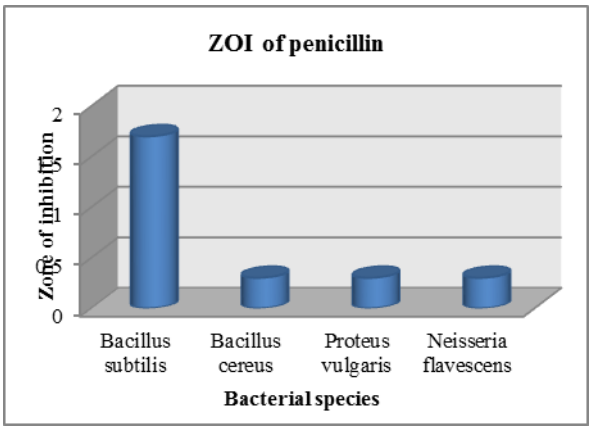
(c)

Figure 3: (c) No Inhibitory effect of penicillin on *Bacillus cereus*



(d)

Figure 3 (d): No Inhibitory effect of penicillin on *Neisseria flavesence*



Graph 2: (a) Zone of inhibition of penicillin on Bacterial species (*Bacillus subtilis, Bacillus cereus, Proteus vulgaris and Neisseria flavescens*);(b) Maximun remarkable effect of penicillin was observed in *Bacillus subtilis* while no remarkable effect was observed in *Bacillus cereus, Proteus vulgaris and Neisseria flavescens.*

Conclusions

From the present study it can be concluded that the antimicrobial activity of penicillin against the isolated bacteria have shown diverse effects. In this study first different bacterial species were isolated from the soil sample and identified by various biochemical tests. The bacterial species were isolated are named as follows- *Bacillus subtilis, Proteus vulgaris, Neisseria flavescens, Bacillus cereus.* After that *Penicillium species* were isolated from citrus fruits sample for isolation of penicillin. Finally the antibacterial activity was checked from the Penicillin on the isolated bacterial species and was found that out of four bacterial species, penicllin showed remarkable effect on Bacillus subtilis but a small extend of inhibitory effect on *Bacillus cereus, Proteus vulgari and Neisseria flavescens*. Results of this study suggest that citrus fruit sample seem to be good source of

Penicillium species and penicillin produced from them showed remarkable effect on microbes.

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REFERENCES

[1] Ainsworth, G.C. and Bisby, G.R. (1995): Dictionary of the fungi. 8th ed. Wallingford: CABI; p. 445.
[2] Diana, W.F. (1994). Soil biodiversity: its importance to ecosystem processes. Fort Collins: Colorado State University.
[3] Hawks worth, D.L. and Rossman, A.Y (1997): Where are all the undescribed fungi? Phytopathology 87: 888-891.
[4] Philippe, P., Esther, M. F. L. and Lucas, M. A. and Ludwig, H. P. (2009): Novel antimicrobial secondary metabolites from a Penicillium sp. isolated from Brazilian cerrado soil. 12.
[5] Rojas, J.J; Ochoa, V.J ; Ocampo, S.A. and Munoz, J.F. (2006). Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine: A possible alternative in the treatment of non-nosocomial infections. BMC Complement Altern Med. 6: 2.
[6] Yoon, C.S. and K.S. Bae (1995): Genetic Relationship among Penicillium Species by Characterizing RAPD Markers. J. Microbiol., 33: 171-177.