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EXTRACTION OF PENICILLIN G IN SOME ASPERGILLUSBY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Penicillin is one of the most important metabolite among these compounds.In this study, at first fungi was performed incubation in PDB media and then 7dayes of complete growth was extracted the antibiotic. After that the samples solve in in one milliliter acetonitrile and prepared the chromatogram of HPLC. After comparing the obtained chromatograms with its standard chromatogram found a completely match. Extracted penicillin content in*A. parasiticus,A. sclerotiorum*, was estimated 74.9 and 29.2 µg/ml, respectively. The results could be provide some information about absence or essence of major gens in some important species in industrial microbiology. Also molecular studies can be applied in manipulation of industrial fungi in order to improve their productions.

INTRODUCTION

Penicillin and cephalosporin are members of b-lactam antibiotics. As identified in some of filamentous fungi, they find in larger groups like microorganisms. Fungi synthesis both

penicillin and cephalosporin whereas bacterial synthesis larger group of cephalosporin and cephamycin but they not synthesis penicillin as final product(4). Nowadays, molecular genetic of antibiotic biosynthesis is one of challenging research field in bioactive microbial products. This subjects same in cephalosporin and penicillin. Now, chemical structure, function and biochemistry pathways of these important materials are well known. The genetic ability of bacillus, actinomycetes (especially streptomycete) and fungi is extraordinary because of production of antibiotic and other secondary metabolites(20,13). Secondary metabolites are synthesis by multi step pathways from precursors to special parts of these metabolites(3).

Antibiotic Specific enzymes or special metabolites have a key role in biosynthesis process. during the several years ago, many of genes involved in biosynthesis of antibiotic and other secondary metabolites were characterized(5,7,11,14,19). Understanding about expression of these genes which, of course, not essential for growth, is so important to their evolution than other operons of primary biosynthesis genes. In addition, knowledge about regulation mechanism of gene expression is necessary for surplus and industrial production of these metabolites. Some of special coding enzymes involved in antibiotic biosynthesis are located on clusters of bacterial or plasmid chromosome(6,8,9). In eukaryote such as plants and filamentous fungi, genes which are located on different chromosome may code the secondary metabolites(12). One of interest finding about this case is link between coding genes for resistance against genes involved in antibiotic biosynthesis.

Most of these elements which determine the resistance of antibiotic bind to related antibiotics genes. Also, Investigation of resistant genes in coding gene clusters in secondary metabolites without antibiotic activity (such as alkaloids, plant growth regulator and pigments) is remarkable. Link between antibiotic biosynthesis genes and resistant genes is a robust tool for cloning of antibiotic biosynthesis genes, because usually it can simplify the selection of transformants which carry the antibiotic biosynthesis determinant elements. Most of biosynthesis steps of penicillin, cephalosporin and cephamycin categorize in enzymeticlevels (2,15,1,16,18).

So far, many attempts performed to define and categorize of genetic systems which determine biosynthesis of penicillin and cephalosporin. This tendency is due to two reason including : 1- a comprehensive

knowledge of genetic basis of b-lactam products may be help for designing novel penicillin and cephalosporin and allow us to develop them. Secondary, it is possible to obtain a new insight in basic biological problems related with role and nature of these antibiotics. Undoubtedly, only fundamental reason of these progresses is create genetic - molecular robust tools to clone of antibiotic specific genes and also, genetic manipulate of organisms which produce antibiotics.

MATERIAL AND METHODS

Isolation of penicillin G

In this study, fungi prepared from fungi collection of mycology laboratory at Sari Agricultural Sciences and Natural Resources. Basic media for growth of Penicillium fungi was included: 21 g sucrose, 3 g extract yeast which solved in one liter deionized water. Fungi were inoculated under laminar flow. After that fungi media incubated at room temperature in a shaker with 120 rpm.

Antibiotic Extraction

In order to penicillin extraction from culture media of Aspergillus fungi, fungi cultured in liquid medium and produced antibiotic extracted by appropriate solvents from media. At first, culture medium was filtered to remove the hyphae, spoors and impurities from it. In order to better solving antibiotic with solvent, PH of media was brought to 2 by sulfuric acid at 0-4°C. Then added about 10 cc acetate ethyl to medium and after that by separating funnel and centrifuge, the solvent isolated from medium. Afterwards, phosphate buffer with 7.5 acidity added to separated solvent and then acidify again by phosphoric acid. Finally, phases for the last time separated from each other by centrifuge and acetate ethyl which was included penicillin remain in up of phases. Then, using of air flow, solvent evaporated and finally, extracted penicillin remain in vessel. In order to next evaluations sediment solved in one milliliter acetonitrile.

High-performance liquid chromatography

For detection of penicillin G used sodium acetate buffer 50 mM and acetonitrile. Also, in this study was used from C18 column, detector of UV 205 nm, flow rate 1.3 ml/min and 100 µl injection content that in each time 15 µl injected to column.

RESULTS AND DISCUSSION

In evaluation of chromatograms to determine Penicillin G content in obtain extract from samples, the desired peak was identified by comparing with retention time of penicillin G in control sample. Then for all of obtained peaks was calculated peaks area and height. **Figure 1** show chromatogram of control sample that penicillin G peak was shown by down arrow.

Chromatograms of Aspergillus strains

In this study were tested eight Aspergillus strains. According to obtained chromatograms can observed penicillin G content **(Figure 2, Figure 3)**.

Penicillin G content in*A. parasiticus,A. sclerotiorum*, was estimated 74.9 and 29.2 µg/ml, respectively. Antibacterial properties of fungi can be examined to antimicrobials, as well as secondary metabolites that have been known. Species are not able to control the growth of bacteria *P. viridiflava* and Gram-positive bacterium *R. iranicus* has been resistant to the fungus. Gram-positive bacteria are more sensitive to beta-lactam agents than Gram-negative bacteria and structural differences between their cell walls (e.g. the peptidoglycan-associated receptors and lipids, the nature of the relationship between the activities of the enzymes Autolytic) is concerned. Whenever bacteria is against antibiotics and antibiotics bind to PBPs, Autolytic wall enzymes are activated and this may lead to bacterial cell death. In Gram-positive bacteria, penicillin releases teichoic acid lipids are (which do not exist in gram-negative bacteria) and they enable autolytic process.

In another survey, the production of penicillin G (benzyl penicillin) was evaluated in fungal culture media that the results showed extraction and quality of antibiotic production increased by using ultrafiltration (21). The recent study in metabolome of *P. chrysogenum*indicated that using different inter cell concentrations of Amino acid precursors for penicillin production (AAA, Val and Cys) can evaluate the level of penicillin production. The results show that often the production speed is depended on several factors such as energy providing or the level of enzymes or transformers all of which are not well known. In previous studies, different media were used to penicillin production. In another study, other media which including yeast extract, sucrose on deionized water, and ethyl acetate were applied for antibiotic extraction (10).A study was conducted in 2009 by Philipe Petit and Esther Lucas where fungus Penicillium isolated from fifteen 200 soil samples were collected randomly which had been separated under cultivation (17). The antimicrobial activity of penicillin was observed. The fungi were cultured in medium containing 2 g glucose, 10 g peptone, 5 g of yeast extract per liter of distilled water. Antibiotics for separation of ethyl acetate and butanol were used.

Considering the mentioned chromatograms, many other metabolites except penicillin G were observed. Maybe these metabolites are

precursors of penicillin G such Penicillic acid, Penicilloic acid, Isopenicillic acid , Penicilloaldehyde, Ampicillin, Penicillin V, Oxacillin, Dicloxacillin and Cloxacillin which were identified by HPLC and providing their metabolite standards can be identified clearly.

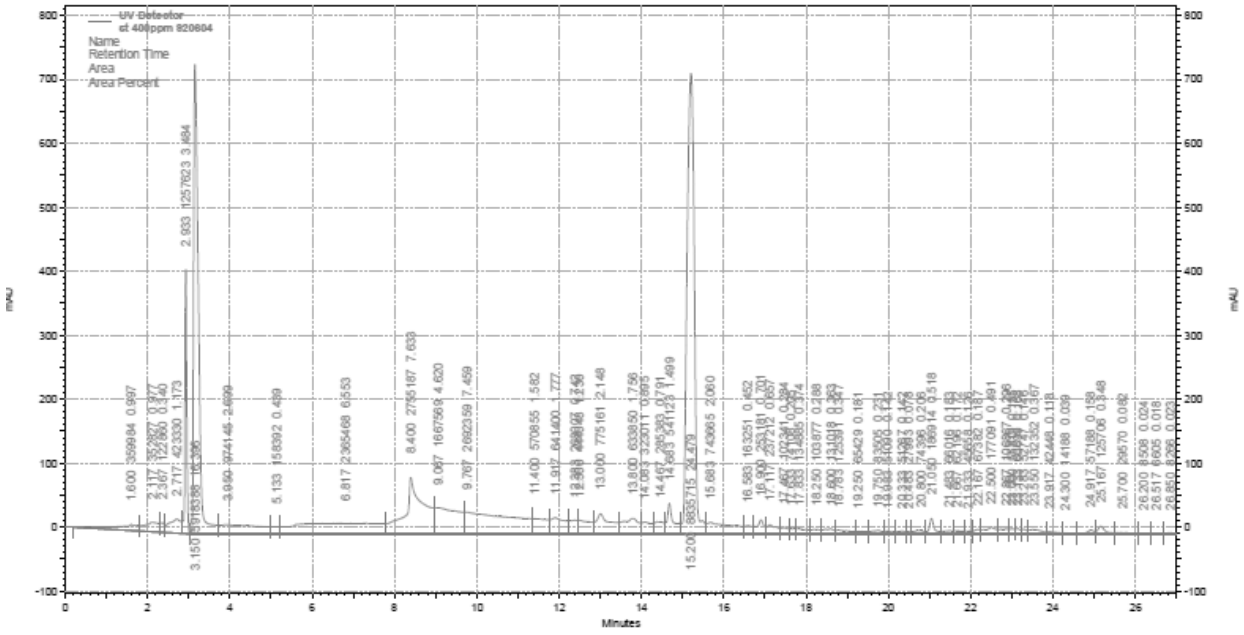

Figure 1: Standard chromatogram of penicillin G prepared from Sigma Company.

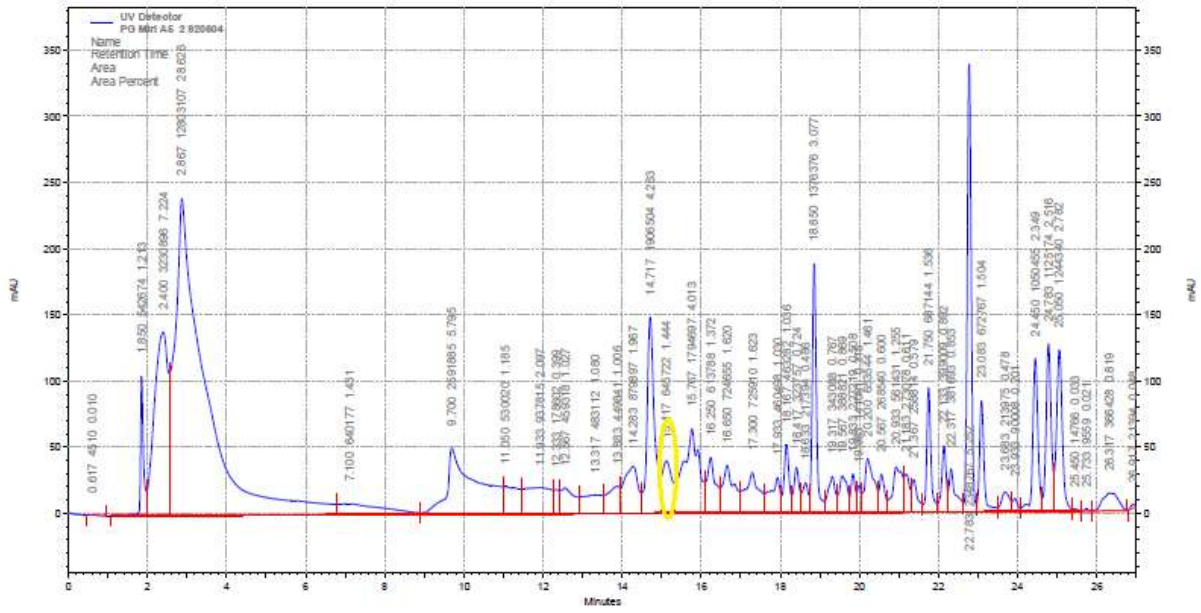

Figure 2: The obtained Chromatogram from extracted culture filtrates of *A.parasiticus.* The marked oval shows Pen G peak.

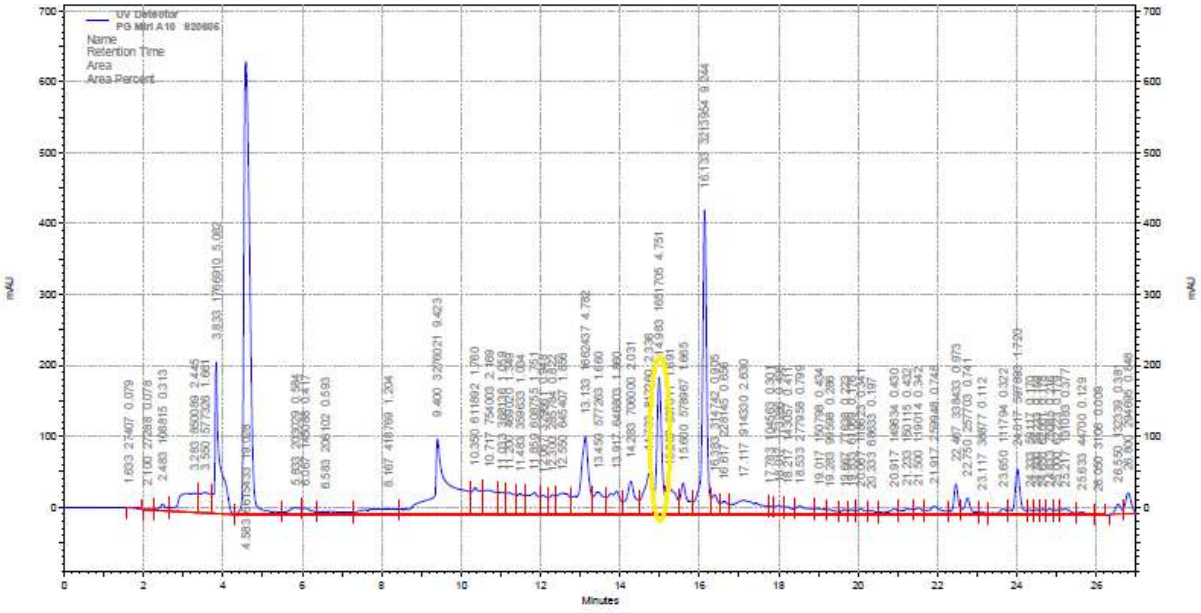

Figure 3: The obtained Chromatogram from extracted culture filtrates of *A.sclerotiorum.*

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