

# Investigation of penicillin G content in some *Penicillium* species

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**ABSTRACT:** Fungi are among the main biological producers in today's modern world. There are several cases of luxuries in universal trade with fungal origin. Antibiotics which produce more than 60 years, are among most valuable fungal productions which have lots of health consumption for humans. Based on current statistics, in our country approximately 700 tone antibiotics are consuming which 100% of them are imported from abroad. The origin source of these antibiotics is microorganisms like fungi and bacteria which could be taken into grant for antibiotic industry. In present study existence of genes involved in Penicillin biosynthesis were investigated in two species of *Penicillium*. In this study first *P. moldavicum*, *P. chrysogenum* fungi were cultured in PDB media and 8 days after their full growth, antibiotic extraction was conducted. After drying, the samples were solved in 1 milliliter of acetonitrile and were analyzed by HPLC. Chromatogram gained from injecting both two types was fully compatible with chromatogram of the standard sample. Level of penicillin G extracted in species *P. moldavicum* is 172.05 microgram in milliliter and in species *P. chrysogenum* is 75.04 microgram in milliliter. According to key role of penicillin in industry, these studies need to more research to optimization of Penicillin G biosynthesis.

**Keywords:** Antibiotics, Penicillin G Production, HPLC.

## INTRODUCTION

Penicillin identified by Feleming in 1928. Industry production of penicillin was improve after the Second World War, and until now was counted one of Important antibiotics in beta lactam group. These antibiotics were used to fight with human and animal diseases and they have a key role in therapeutic conditions (22). Due to being resistant of bacteria, the identification of new species is necessary. Penicillin biosynthesis is a good model for studying of secondary metabolites production in filamentous fungi. The biosynthesis of beta lactam antibiotics by microorganisms has an important role in molecular genetics and biochemistry studies. One of great aspects of filamentous fungi is production of secondary metabolites such as antibiotics, anti-viroid components (23). Sequencing of Fungi genomes had shown that numerous genes involved in Polyketide, non-ribosomal peptides and different enzymes involved in secondary metabolites biosynthesis (24). Our study showed the penicillin G content in *Penicillium* species.

In some plant-pathogenic fungi, secondary metabolites are extracted from plant materials. The plant replies to fungal infection by producing antifungal plant metabolites defenses against some fungal attack (2,12). Although, Auto-inducers are well known as regulators of secondary metabolism and differentiation factors in bacteria (12,13), they are largely unknown in fungi (14,15).

Nowadays, the HPLC coupled with a variety of detectors apply to most of the mentioned methods, The chromatographic efficiency of the reversed phase separations is superior, and the penicillin degradation is minimized by more moderate pH values (6).The absorbance spectrophotometry in the ultraviolet (UV) region is the most common detection method (1,5,7). The highest absorbance is in excitation wavelengths in the range of 20 & 230 NM. The detection limit has been measured as low as 0.1- 0.5 pg/ml (1,8) to determine directly. Due to the large number

of compounds display absorbance at these wavelengths, peak overlap can be complicated in complex biological samples.

## MATERIALS AND METHODS

### Culture conditions

The isolates were isolated from agriculture soils and their antibacterial properties were checked before. Then, the fungus isolate was inoculated on PDA (Potato Dextrose Agar) medium, (including: potato, dextrose and agar on 1 L deionized water). The agar plates were incubated at 25°C for 7 days. Then, spore suspensions were prepared and cultured on a liquid specific medium (yeast extract, sucrose on 1 L deionized water) to induce antibiotic production.

### Antibiotics Extraction

Chloroform and butyl acetate were used to separate antibiotics from culture media. After the filtration of impurities and mycelia and spores from the medium, temperature of the medium was reduced to 0 - 4°C with pH about 2. So, this condition, prepares better pen G transfer to ethyl acetate (17,18). At this step, solvent and the medium are in different phases and should be separated (19). Using a separating funnel, the ethyl acetate was included with penicillin extracted from the medium. to complete separation of the phases a centrifuge step at 5000 rpm (11).

### HPLC analysis

Pen G (sodium salt) was proceed from Sigma Chemical Co. (St. Louis, MO, USA). Formic acid of p.a. Grade, Acetonitrile of HPLC grade from Merck (Darmstadt, Germany) and ammonium acetate of HPLC grade were prepared from Fluka (Steinheim, Germany), and Purified water (18.2 MOcm) was from Aria Shimi (Tehran, Iran). The fungi were fermented in broth specific media of penicillin synthesis (3 gr yeast extract and 21 gr sucrose in one liter of pure distilled water). Samples from the supernatant were taken after 7 days incubation at room temperature on a rotary shaker, with 120 rpm. 50 µl of both the unprocessed supernatant and an ethyl acetate-extracted fraction were analyzed with a high performance liquid chromatography (HPLC) waters with a C18 column at 25°C, and the flow rate was 0.2 ml/min. Penicillin G at a concentration of 0.1 mg/ml was used as the control sample. The mobile phase consisted of 0.1% formic acid in water with 1 mM ammonium acetate (solvent A, pH 2.7) and acetonitrile (solvent B).

## RESULTS AND DISCUSSION

The chromatograms of extracted samples had complete coincidence with standard in Pen G related peaks (Figure 1). This result confirmed the presence of Penicillin G in obtaining extractions.

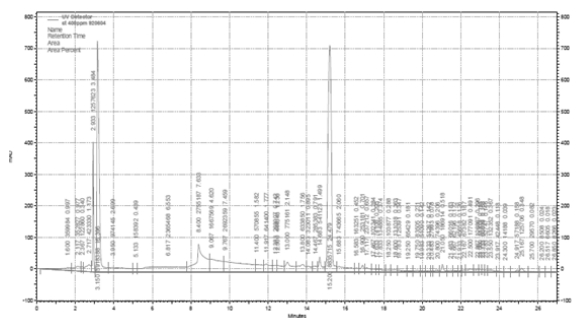
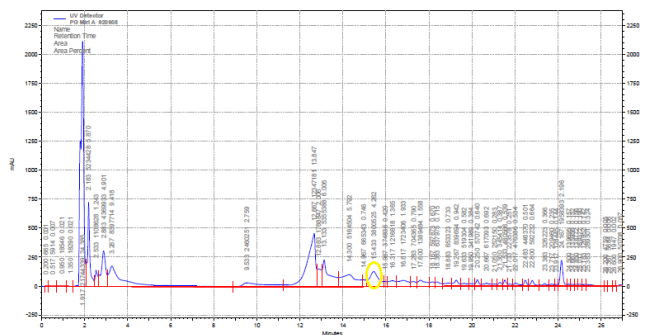


Figure 1. Chromatogram resulting from injection of penicillin G standard to HPLC system

In this study, two *Penicillium* spp were investigated. For all tested species, the extraction process was performed and their HPLC chromatograms were obtained. After comparing chromatograms with standard sample, the retention time of standard was similar with extracting samples from fungal media. Accordingly, chromatograms, the presence of penicillin G was confirmed in all surveyed isolates (Figure 2).



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