

**Screening and Identification of Secondary
Metabolites Produced by Stress-tolerant
Penicillium Strains under Extreme
Environmental Conditions**

**SUMMARY
of
THESIS**

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SUMMARY

Production of secondary metabolites by microorganisms in extreme conditions is well documented. Fungi are well-known for their ability to synthesize wide variety of small bioactive molecules called as secondary metabolites; detrimental (toxins) and beneficial (pharmaceuticals) effects on human welfare (Keller, *et al.* 2005; Gawas-Sakhalkar, *et al.* 2012). Due to these bioactive properties, many fungal secondary metabolites have been adopted by humans for their use as Pharmaceuticals such as antibiotics, cholesterol-lowering agents, tumor inhibitors and immune-suppressants for transplant operations (Keller, *et al.* 2005). The production of secondary metabolites by fungi may depend upon the nutrient availability, physical and environmental conditions. However, the production of secondary metabolites in extreme environmental conditions could be a new approach and may be an area of interest as the production of bioactive molecules may get modified and enhanced.

The present study was an attempt to explore the potential aptitude of two *Penicillium* strains isolated from two different climatic zones, for the production of secondary metabolites under harsh environmental conditions such as, temperature, pH, salinity, heavy metal and nutrient stress. The identification of secondary metabolites produced by *Penicillium* strains were also studied. It is postulated that alterations in microbial membrane (protein, nucleic acids, lipid content) and production of secondary metabolites may occur under different environmental stress conditions, which has been analyzed in the present study by using FTIR, LC-MS, GC-MS, UV-Vis absorption and

HPLC-MS techniques. However, several studies have documented about the production of different secondary metabolites under extreme environmental conditions, but their potential applications in biomedical field is a topic of our interest.

The results are summarized as under:

- The two fungal strains were isolated from two different climatic zones of India, one from agricultural soil of dry temperate region of Leh, Ladakh (J & K), India and another from garden soil of tropical region of Lucknow (U.P.), India.
- The microscopic morphological characters were used for the identification of fungal strains and it was concluded that both fungal isolates belong to *Penicillium* genera.
- Based on sequencing of the ITS regions and comparison with NCBI GenBank database, strains were identified as psychrophilic *P. oxalicum* (**accession no. KR150256**) and mesophilic *P. citrinum* (**accession no. KR150257**).
- The present work revealed that the psychrophilic *P. oxalicum* has potential to grow at extremely low temperature (4°C) with growth optima at 15°C and acidic pH 4.0. The mesophilic *P. citrinum* showed zero tolerance to low temperature and showed optimum growth between 30°C- 35°C and acidic pH 5.0.
- Both *Penicillium* strains preferred 2% (w/v) NaCl for their optimum growth. Further, it was observed that psychrophilic *P. oxalicum* was able to tolerate NaCl concentrations (upto 15%, w/v) than mesophilic *P. citrinum* (10 %, w/v NaCl). Hence, psychrophilic *P. oxalicum* was found to be halotolerant in nature.
- The preferred source of carbon for *P. oxalicum* was glucose, followed by sucrose. For *P. citrinum*, sucrose was the best carbon source. Among the nitrogen sources,

glycine was found to be relatively better nitrogen source than Glycine and Sodium Nitrate in case of both the *Penicillium* strains.

- Biochemical characteristics of *Penicillium* strains revealed that psychrophilic *P. oxalicum* could efficiently produce extracellular *amylase* and *cellulase* enzymes, when compared with the counterpart mesophilic *P. citrinum*.
- Lipid Profiling revealed abundance of mono fatty glycerols and di-fatty acid derivative of glycerolipids in both *Penicillium* strains grown at 4°C. They were scarcely available when the *Penicillium* were grown at 35°C.
- Among the fatty acid, long chain (C₁₆-C₂₂) saturated fatty acids were abundantly present in both the *Penicillium* strains grown under two extreme temperature conditions. However, concentration of Palmitic acid was increased in *P. citrinum* due to rise in growth temperature (35°C).
- Among the unsaturated fatty acids, growth of both the *Penicillium* strains at low temperature (4°C) preferentially synthesized Linolenic acid, Linoleic, oleic acid and Erucic acid. High temperature (35°C) grown *P. citrinum* strain continued synthesis of most of the unsaturated fatty acid such as Docosahexanoic acid (omega-3) and Erucic acid (omega-9) while *P. oxalicum* showed reduced percentage of unsaturated fatty acid. Hence it is proved that the types of lipids in individual fungus may vary from organism to organism as they are known to change with the age, stages of development, nutrition, and environmental conditions.
- The high production of secondary metabolites in both *Penicillium* strains, were at higher growth temperature (35°C), when compared with that obtained in low

temperature grown cells. The temperature dependent increase in the quantity of secondary metabolites of psychrophilic *P. oxalicum* with rising temperature, might be a survival strategy of this fungus under stress conditions.

- The production of secondary metabolite were reached to its optimum level in *P. oxalicum* at 2% w/v NaCl concentration. Whereas, production of secondary metabolite in *P. citrinum* continued upto 5% (w/v) salt concentrations.
- Results of production of secondary metabolite by *P. oxalicum* and *P. citrinum*, showed that glucose and sucrose were relatively better carbon sources, for respective strain. Among organic nitrogen sources, L-glycine was better nitrogen source for production of secondary metabolite in both the *Penicillium* strains.
- The psychrophilic *P. oxalicum* was more tolerant to high concentration of Arsenic and Chromium than the mesophilic *P. citrinum*. Psychrophilic *P. oxalicum* was able to tolerate arsenic upto 1000 mg L⁻¹ and grew well at 100 mg L⁻¹ medium supplemented with arsenic. Whereas, mesophilic *P. citrinum* strain was able to tolerate arsenic concentration upto 50 mg L⁻¹.
- Psychrophilic *P. oxalicum* exhibited optimum growth at 01 mg L⁻¹ chromium concentration and showed chromium tolerance upto 10 mg L⁻¹ concentration. Whereas mesophilic *P. citrinum* was able to grow upto 01 mg L⁻¹ Chromium concentration, 2.5 mg L⁻¹ or higher concentration of Cr (VI) eliminated the growth.
- The production of secondary metabolites in the presence of heavy metal (As III and Cr VI) showed that higher conc. of As (III) 500 mg L⁻¹ and 1.0 mg L⁻¹Cr (VI)

promoted the production of secondary metabolites in both *Penicillium* strains when compared with the control (without metals), irrespective of growth.

- FTIR spectra of biomass of both *Penicillium* strains showed species specific as well as stress dependent compositional alterations in functional groups present in fungal membrane or cell such as lipids (especially in saturated fatty acids) and deformation in proteins, perhaps it was a part of their intracellular defense strategy.
- TLC analysis of EtOAc crude extract of secondary metabolites of *Penicillium* strains suggested that secondary metabolites of *P. oxalicum* include some unique metabolites not present in *P. citrinum*, whereas extracts of *P. citrinum* exhibited presence of alkaloids based on their absorbance and color of the chromatogram on TLC plates.
- The results of identification of temperature dependent production of volatile compounds of secondary metabolites using GC-MS/MS, revealed the presence of 53 major peaks by both *Penicillium* strains. The compounds were identified as derivatives of quinoline, quinolinone, azulene, and azetidinedione class of compounds with widely known applications in biomedical and therapeutical fields such as antiprotozoal activity, anti-inflammatory, antimicrobial, cytotoxic and anticancer properties.
- As per the results of HPLC fingerprinting of metabolites, 17 and 15 peaks were detected in case of both psychrophilic *P. oxalicum* and mesophilic *P. citrinum*, respectively. Among these peaks, 06 major peaks were identified with their characteristic UV-Vis absorption spectra in case of psychrophilic *P. oxalicum*, but

none of them were found to be known compounds as per the literature reported. On the other hand, metabolites produced by mesophilic *P. citrinum* were identified as Quinolactacin C, Abscisic acid, Anofinic acid, Conocenol B, and Aurofusarin with several extensive applications.

- The ^1H NMR study also confirmed about temperature dependent changes in the composition of secondary metabolites produced by psychrophilic *P. oxalicum*, while composition of secondary metabolite produced by *P. citrinum* remained the same except quantitative changes.
- These results of Cell viability and cell toxicity assay suggested that the secondary metabolite of *P. oxalicum* was more cytotoxic to A549 cancer cell lines than the metabolites of *P. citrinum*.
- The results on antibiotic susceptibility test showed about 8 fold reduction in the MIC of tetracycline that revealed the secondary metabolites of *Penicillium* strains have equivalent drug resistance reversal potential against bacterial strain tetracycline tolerant *P. aeruginosa*.
- Hence, foregoing results revealed that the unique characteristics of the psychrophilic *P. oxalicum* and its temperature stress dependent production of number of new secondary metabolites with potential industrial applications, particularly in pharmaceutical and therapeutical fields.