

Mutational effect of *Penicillium chrysogenum* on Antibiotic Production

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Abstract

-Lactams like penicillin and cephalosporin are among the oldest known antibiotics used against bacterial infections. Industrially, penicillin is produced by the filamentous fungus *Penicillium chrysogenum*. The most common method used to obtain high yielding mutants by strain improvement with classical mutagenesis is by treating a population with a mutagenic agent and plating out the colonies random selected. The present study deals with testing the survival ability of *Penicillium chrysogenum* spores against UV irradiation and antibiotic production. (Stauffer and Backus 1954) reported the high yielding *Penicillium chrysogenum* Q-176, obtained by mutagenesis with UV followed by resting of random survivors and of morphological (colour) mutants. The present work describes the different morphological mutants and its antibiotic production ability at different doses of UV. Selected mutant colonies were mass cultivated by rice flask preparation. The activity of spores from mutated colonies was studied by enhancing the spore growth in seed media for 44 hour and fermentation media for one sixty eight hours by shake flask method. By Penicillin activity was studied by HPLC. Mutant strains of *Penicillium chrysogenum* treated with both chemical mutagen EMS with high sporulation and morphology variation can able to produce more amount of antibiotic penicillin.

Keywords : Antibiotic, Penicillin, Strain improvement, Mutation, *Penicillium chrysogenum*

Introduction

-lactam antibiotics like penicillins and cephalosporins belong to one of the largest-selling classes of drugs worldwide with a production of 45,000 tons in the year 2000 [Bruggink and Roy 2001]. Penicillins and cephalosporins are produced by the filamentous fungi *Penicillium chrysogenum* and *Acremonium chrysogenum*, respectively, as well as some filamentous bacteria. These antibiotics possess as common structural motif the -lactam ring [Brakhage 1998]. There are many species of *Penicillium*, and a search was started to find other species that could be tested for penicillin production (Hosler and Johnson 1953). *Penicillium*

chrysogenum, produces approximately 200 times as much penicillin that *Penicillium notatum*. Scientist the began to increase the amount of penicillin produced by *Penicillium chrysogenum*, by irradiating it with X-Rays and UV rays in order to induce mutations of this species. This eventually lead to a mutant that produces 1000 times the amount of penicillin than Fleming's original culture. *Penicillium chrysogenum* isolated from fermented and cured meat products can grow well in submerged culture.

The colonies of *Penicillium Chrysogenum* are rapid growing flat, filamentous and velvety, wooly or cottony in texture. The colonies are initially white and become blue green, grey, green, Olive grey, yellow or pinkish in time.

The plate reverse is usually pale to Yellowish. Spores of *Penicillium chrysogenum* obtained from ATCC were suspended in sterile distilled water. Then the spore suspension were treated with UV, EMS, DMS and both UV and EMS at varying intervals and plated on LCS agar medium. Mutant strains of *Penicillium chrysogenum* treated with chemical mutagen EMS produces high amount of antibiotic penicillin.

Materials and Methods

The ampoule containing the spores of *Penicillium chrysogenum* obtained from ATCC was cut and opened at the neck aseptically. The contents were dissolved in 10 ml of sterile distilled water to make a spore suspension and stored at 4°C (The suspension can also be distributed in 2ml volumes in to small test tubes stoppered with cotton plugs and refrigerated). One tube culture was then removed and 0.5 ml of spore suspension was diluted upto 500 ml with sterile water. 10 ml of the suspension was transferred aseptically into a glass petridish and irradiated under the UV lamp with constant agitation for the various time intervals. At the end of each time intervals 1 ml of suspension was taken and serially diluted upto 10⁻⁵ dilution 100 µl of each dilutions were taken and plated in Lactose corn steep (LCS) agar medium. The plates were kept for incubation at 25°C for 12 days after which the selected colonies were transferred to rice flask.

Spore suspension was centrifuged at 3500 rpm for 10 min. The pellet were washed with sterile water and resuspended in 2ml of .05 M (PH-8) TM buffer. 0.1ml was spore

suspension was transferred separately to eppendroff containing EMS and DMS at 1mg per ml concentration and kept for 15 min. Then suspension was serially diluted to 10⁻⁵. From each dilution 100 µl was plated in LCS agar and incubated at 25°C for 12 days.

10 ml of the suspension was transferred aseptically into a glass petridish and irradiated under the UV lamp with constant agitation for the 15 minutes. At the end of the each time interval 1 ml of suspension was taken and centrifuged at 350 rpm for 10 minutes. The pellet were washed with sterile distilled water and resuspended in 2ml of .05 M (PH-8) TM buffer. 0.01ml of spore suspension was transferred in eppendroff containing EMS and DMS at 1mg per ml concentration separately and kept for 15 min and serially diluted to 10⁻⁵. From each dilution 100 µl was plated in LCS agar and incubated at 25°C for 12 days.

100g of polished Basmati rice was washed with 1lit of distilled water and excess water was then drained off air-drying the rice on a filter paper under room temperature for 15 minutes (the rice should not stick with one another). After partial drying the wet weight of the rice was measured. 25g of the soaked rice was then transferred to separate conical flasks and autoclaved at 121°C for 20 minutes, and weight of the individual flasks after sterilization was determined. The moisture content was adjusted by aseptically adding spore suspension and sterile water in the ratio (1:2) to the rice. After inoculation under aseptic conditions, the flasks were shaken thoroughly to disperse the spores

The conidiophores were recovered by shaking rice flask. Spore suspension was serially diluted and 0.1ml was plated onto the viability count medium (VCM) and Sabourand's Dextrose Agar (SDA), Nutrient Agar (NA) plates. The plates were incubated at 25°C for 5 days. After 5 days incubation, the colonies were counted and expressed as CFU into 10⁸ / ml-1, NA & SDA slants were incubated at 37°C and 25°C for 48 and 72 hours and the viability of the spores in the suspension were observed.

The culture was inoculated aseptically in 35 ml of seed medium in 500mL Erlenmeyer flask and incubated at 25°C in shaker at 220

rpm for 66 hours. After incubation, pH, PMV (Packed Mycelial volume) and microscopical observation were observed.

The Inoculum from seed medium was transferred aseptically to 35ml, of production medium in 500mL Erlenmeyer flask and incubated at 25°C in a shaker at 220 rpm for 164 hours. After incubation, pH, PMV and microscopically observations were noted. Then the broth was filtered through whatmann No.1 filter paper and the filtrate was assayed for productivity by HPLC analysis.

Results

In the present study the Spores of *Penicillium chrysogenum* were irradiated with physical mutagens like UV at 254 wave length, chemical mutagens like EMS and DMS. Physical and chemical Mutagens were enhancing the production of penicillin. Mutated Colonies were selected based on highest percentage of killing and production was analyzed by HPLC assay. Observation of rice flask shows that whitish green colored spores were observed on the third day of incubation, followed by green colored sporulation. Maximum sporulation were

obtained twelfth day of incubation with bluish green spores, which adhere on the rice grains. The survival percentage of the spores exposed to UV was found to be 30 at 15 min treatment and 5 at 60 min treatment. Here also, the lethality of the spores increases with increased exposure to UV at 254 nm. It is found to be highly sporocidal and powerful than exposure to UV at 254 nm, and killing rate of spores was found to be 45% at 15 min treatment and 90% at 60 min treatment. When spores of *Penicillium chrysogenum* were treated with EMS only seven colonies survive when compared to the control it is 64 and the percentage of killing is 93%. Similarly with DMS it is nine when compared to the control it is 65 CFU survive and the percentage of killing is 92%. Spores of *Penicillium chrysogenum* treated with UV and EMS only four colonies survive when compared to the control it is 66 and the percentage of killing is 94%. Similarly with UV and EMS it is nine when compared to the control it is 65 CFU survive and the percentage of killing is only 86%. Morphology mutated colonies were observed and isolated. Then they were analysed for penicillin assay by HPLC method.

S.No.	UV	CFU	%KILL
1	Control	55	
2	15 Min	30	45.45
3	30 Min	20	63.63
4	45 Min	15	72.72
5	60 Min	5	90.90

S.No.	UV	Activity IU/ml	pH	PMV
1	Control	36679	5.8	32%
2	C ₁	23389	6.1	28%
3	C ₂	24596	6.0	30%
4	C ₃	21461	6.0	29%

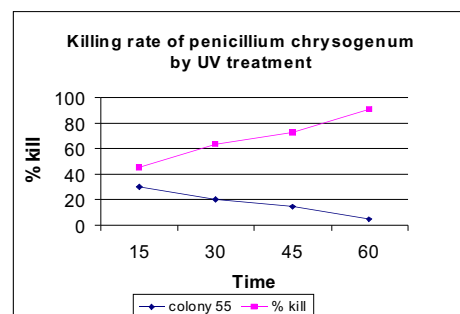


Figure-1 Effect of *Penicillium chrysogenum* by UV treatment

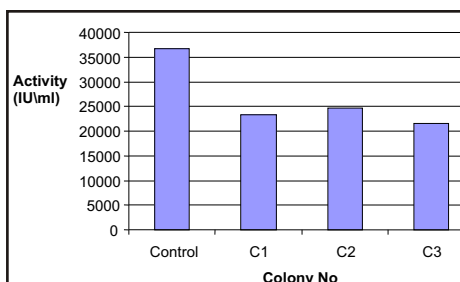


Figure -2 Production of penicillin by UV mutated *Penicillium chrysogenum*

Table 3 Production of penicillin by EMS mutated *Penicillium chrysogenum*

S.NO	EMS	Activity IU/ml	PH	PMV
5	Control	34533	5.9	35%
6	C ₄	29990	6.2	27%
7	C ₅	35056	5.7	36%
8	C ₆	36487	5.7	34%
9	C ₇	36809	5.8	33%
10	C ₈	31626	5.3	35%

Table 4 Production of penicillin by UV and EMS mutated *Penicillium chrysogenum*

S.NO	UV & EMS	Activity IU/ml	PH	PMV
11	Control	36679	5.7	31%
12	C ₉	34478	5.9	32%
13	C ₁₀	31221	5.8	35%
14	C ₁₁	32548	5.4	32%
15	C ₁₂	32537	5.7	33%

Discussion

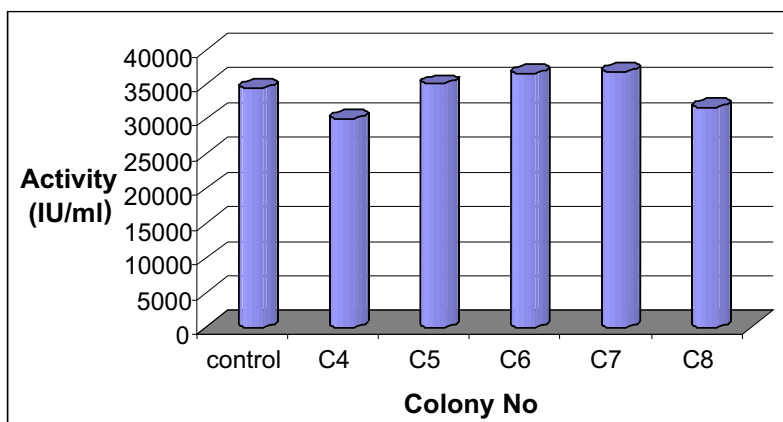
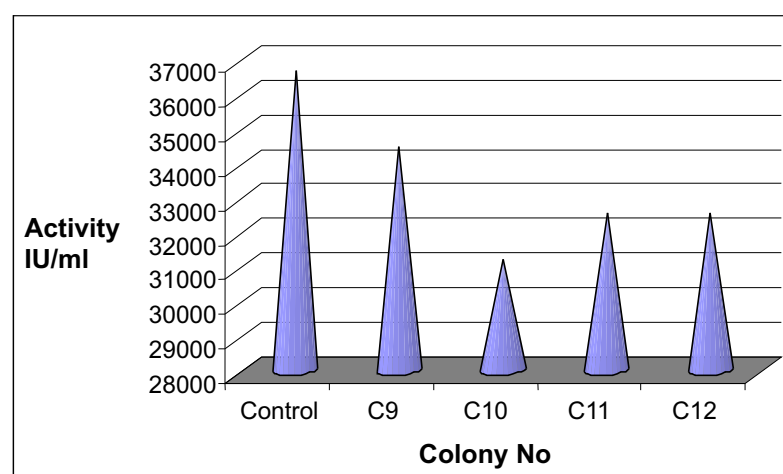
The most common method used to obtain high yielding mutants by strain improvement with classical mutagenesis is by treating a population with a mutagenic agent and plating out the colonies random selected. The present study deals with testing the survival ability of *Penicillium chrysogenum* spores against UV irradiation spores against UV irradiation selecting the mutants on the basis of random survivors by morphology and highest of antibiotic production (Stauffer and Backus 1954) reported the high yielding *Penicillium chrysogenum* Q-176, obtained by mutagenesis with UV followed by resting of random survivors and of morphological (colour) mutants. The penicillin biosynthetic pathways have the non-ribosomal peptide synthetase (L- -aminoadipyl)- L-cysteinyl-D-valine synthetase (ACVS) forms the tripeptide ACV. The formation of ACV acts as the committed step in both penicillin and cephalosporin biosynthesis. ACV is subsequently converted into isopenicillin N (IPN), which has the characteristic -lactam backbone, by the enzyme isopenicillin N synthase (IPNS). Both ACVS and IPNS have been shown to be located in the cytosol in *P. chrysogenum* (van der Lende *et al.*, 2002, Tobin *et al.*, 1995). Similarly on the other hand, epimerization of the α -amino

adipoyl moiety followed by ring expansion leads to cephalosporin biosynthesis in *A. chrysogenum* (Ullan *et al.*, 2007).

The present work describes the different morphological mutants and its antibiotic production ability at different doses of UV (Yerokkhina 1961) correlate the morphology variation and variation in antibiotic production in streptomycin producing strain LS-1 increasing UV exposure time of spores of *Penicillium chrysogenum* and also treatment of spores markedly increase the mortality of spores. Mortality of spores to physical chemical, (UV + EMS) and (UV + DMS) combined treatment.

Effect of combined mutation (physical + chemical) on the mutant C₉ in formed to be the second highest antibiotic producer (3669 & IU/ml-1) from selected colorizes alinkhanian *et al.*, 1961. Analyzer the effect of two

mutagens (physical + chemical) in three variant of streptomycin. Production strain with the survival rate of 93% respectively. Varant (1860) of *Actinomyces streptomycin* showed the greatest number of plus variants with minimal UV doses where as the variant (C₂) showed positive effect with higher dosage. The mutant C₇ has been produce highest amount of antibiotic (36809 IU/ml -1) with EMS treatment. The mutagenic effect of chemicals like EMS showed its greatest superiority of antibiotic production. (Alikhanian and Zhadanova 1960). Genetic engineering were employed to improve the efficiency by the genes coding for enzymes involved in biosynthesis of penicillin into yeast successfully (Gidijala *et al.*, 2007, Lutz *et al.*, 2005). Overproduction of a single protein, Pc-Pex11p, results in 2-fold enhanced penicillin production by *Penicillium chrysogenum*. (Kiel *et al.*, 2005)

Figure -3 Production of penicillin by EMS mutated *Penicillium chrysogenum*Figure -4 Production of penicillin by UV and EMS mutated *Penicillium chrysogenum*

Thus from this work it can be inferred that the selection of mutant strains of *Penicillium chrysogenum* with high sporulation and morphology variation can able to produce more amount of antibiotic penicillin which can be used for industrial production.

References

- Alikhanian, S.I., and Zhdanova, N.I. 1960. Antibiotic production with effective mutagens. *Doklady Akad Nauk S.S, S.R*, **133**; 454-456.
- Brakhage AA: Molecular regulation of beta-lactam biosynthesis in filamentous fungi. *Microbiol Mol Biol Rev* 1998, **62**(3): 547-585.
- Bruggink.A, Roy PD: Synthesis of B-lactam Antibiotics, Chemistry, Biocatalysis and Process Integration. In Industrial synthesis of semisynthetic antibiotics Edited by: Bruggink A. Dordrecht, Kluwer, The Netherlands; 2001:12-55.
- Gidijala L, van der Klei IJ, Veenhuis M, Kiel JA: Reprogramming *Hansenula polymorpha* for penicillin production: expression of the *Penicillium chrysogenum* *pcb* gene. *FEMS Yeast Res* 2007, **7**(7): 1160-1167.
- Hosler, P., and Johnson, M.J., 1953. Penicillin from chemically defined media. *Ind Eng. Chem.*, **45**:871.
- Kiel JA, van der Klei IJ, van den Berg MA, Bovenberg RA, Veenhuis M: Overproduction of a single protein, Pc-Pex11p, results in 2-fold enhanced penicillin production by *Penicillium chrysogenum*. *Fungal Genet Biol* 2005, **42**(2): 154 -164.
- Lutz MV, Bovenberg RA, van der Klei IJ, Veenhuis M: Synthesis of *Penicillium chrysogenum* acetyl-CoA:isopenicillin N acyltransferase in *Hansenula polymorpha*: first step towards the introduction of a new metabolic pathway. *FEMS Yeast Res* 2005, **5**(11):1063-1067.
- Stauffer, J.F., and Backus, M.P. 1954. Production of family Strains in *Penicillium chrysogenum* and their antibiotic effect. *Ann. N.Y.Acad.Sci.*, **60**:35.
- Tobin MB, Cole SC, Miller JR, Baldwin JE, Sutherland JD: Amino-acid substitutions in the cleavage site of acyl-coenzyme A:isopenicillin N acyltransferase from *Penicillium chrysogenum*: effect on proenzyme cleavage and activity. *Gene* 1995, **162**(1): 29-35.
- Ullan RV, Campoy S, Casqueiro J, Fernandez FJ, Martin JF: Deacetylcephalosporin C production in *Penicillium chrysogenum* by expression of the isopenicillin N epimerization, ring expansion, and acetylation genes. *Chem Biol* 2007, **14**(3):329-339.
- van der Lende TR, van de Kamp M, Berg M, Sjollem K, Bovenberg RA, Veenhuis M, Konings WN, Driessen AJ: delta-(L-alpha-Aminoadipyl)-L-cysteiny-D-valine synthetase, that mediates the first committed step in penicillin biosynthesis, is a cytosolic enzyme. *Fungal Genet Biol* 2002, **37**(1): 49-55.
- Yeroknina, L.I., 1961. Variation in antibiotic production. *Trudy inst. Mikrobiol. Akad. Nauk S.S.S.R.*, **10**: 169-173.

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