



Comparative estimation of Penicillin production by wild and UV irradiated mutant strains of *Penicillium chrysogenum*

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Abstract:

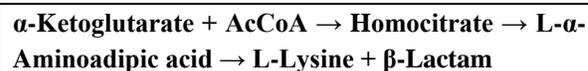
Penicillin is one of the well known antibiotics that are produced by *Penicillium* species of Fungi. Due to the immense applicability of penicillin antibiotic in the field of medicine it has gained a very vital position in the industrial sector. It is used to treat several bacterial infections due to its innate antibacterial activity. In view of the importance of penicillin in the field of industry and medicine the current work aims to standardize the effects of mutations on the *Penicillium* productivity by *P.chrysogenum*. The strain *chrysogenum* was selected as it is the well known organism commercially used for Penicillin production. The work involves the isolation of *P.chrysogenum* culture from the contaminated papaya followed by its pure culture preparation. The mutant strains were prepared by subjecting the wild strains to UV irradiation for various time durations. All the strains were inoculated into the Production medium respectively. The antibiotic produced was estimated using titrimetric protocol and the comparative study was done on the productivity of all the treated cultures. The work concludes that the effect of U.V mutations on the productivity of the Penicillin by *P.chrysogenum* depends upon the exposure time. The productivity increases regularly for a certain period of exposure time after which it starts decreasing. This can be a very useful method for the industrialist to analyze and standardize the specific mutational conditions which can produce high yield of the product with minimum source.

Introduction:

The “Wonder Drug” Penicillin is an antibiotic produced from *Penicillium chrysogenum* ^[1]. Antibiotics are created as part of the life process of one organism, which can kill or stop the growth of a disease-causing microbe ^[2]. Alexander Fleming was the first person to propose the concept of Antibiotics. Penicillin is the antibiotic produced by several species of *Penicillium* which has the capability to inhibit the growth of bacteria around its colony ^[3].

Keywords: Wild, Mutant, UV irradiation, *Penicillium chrysogenum*, Penicillin

Penicillin is also used in treating diseases such as pneumonia and blood poisoning ^[4] and in the treatment of several bacterial infections. Due to its immense medical importance Penicillin production at the industrial scale had gained importance. There are several variants of Penicillin that have been produced synthetically or have been modified from the natural penicillin available. They belong to the beta lactum group of antibiotics ^[5] as they contain an active lactum ring in their chemical structure which is attached to several different R groups that differ according to the type of penicillin like Penicillin G, Penicillin V, and Bezathine Penicillin etc ^[6]. All the variant types can be synthesized artificially by altering the side chain of the beta lactum ring. *P.chrysogenum* is the well known species of *Penicillium* which has maximum antibiotic producing ability when compared to the other species. Research shows that the mutant strains of *Penicillium.chrysogenum* treated with chemical mutagen EMS Produces high amount of penicillin when compared to the wild strain ^[7] This is the species upon which extensive research is in progress to develop the strain so as to increase the product yield. The basic biochemical reaction behind penicillin production is as follows ^[8], here beta lactum indicates the actual functional ring or core of the penicillin structure.



Considering these aspects the current work focuses on the effect of UV induced mutations on the penicillin productivity.

Materials and Methods:

Isolation of *Penicillium chrysogenum* from spoiled fruit:

Since *P.chrysogenum* is common fungi found in the spoiled food and moist soil, the stock culture was isolated from the spoiled papaya. The general plating

method was employed to isolate the organism onto the plates of Asthana hawker's media which is one of the recommended media for fungal growth. The culture was confirmed to be *P.chrysogenum* based on the colony morphology as well as the microscopic observation. Several replicas of the pure cultures were made in order to subject them to different durations of exposure to U.V rays.

Fig1: Colony Morphology of *P.chrysogenum*



Fig1: The figure shows the colony morphology of pure culture of *P.chrysogenum* on Asthanahawker's plate.

Preparation of Mutant strains using U.V Irradiation:

The plates containing the pure culture of *P.chrysogenum* were further subjected to U.V irradiation for various intervals of time and were labeled respectively. One of the plates was preserved as a wild strain. The U.V mutations were induced by exposing the plates for various durations to the U.V light available in the Laminar Flow.

Preparation of Production Media and Inoculation:

To obtain the penicillin the stocks were inoculated in production media with same composition in all the flasks. All the organisms which were subjected to pre-treatment were inoculated into the respective production media labeled according to the duration of exposure to U.V rays. Since Penicillin is the secondary metabolite of the organism the minimum incubation time required for product yield was 15days. The production media contained Glucose: 5gms, Potassium Nitrate: 3.5gms, Potassium dihydrogen phosphate: 1.75gms, Magnesium sulphate: 15gms for 1litre of the Production media.

Estimation of Penicillin yield from the broth:

After the incubation period the media was filtered and the mat was separated and the dry mat weight was calculated. A titrimetric method was employed to estimate the yield of Penicillin in the broth after the prescribed period of incubation. Since the inoculums in the different fermentation media were subjected to U.V irradiation for different intervals of time the product yield is also expected to differ among the cultures.

Quantitative estimation of Penicillin yield by Titrimetry:

Chemicals Required:

Hypo, Standard Penicillin solution, Phosphate buffer, Sodium acetate buffer, Starch solution, Iodine indicator.

Protocol:

To 1ml of broth in the clean conical flask 5ml of Phosphate buffer and 0.1N NaOH should be added. The flask is then incubated in dark for 5minutes. To this 5ml of Iodine solution should be added. Then 5ml of Acetate buffer and 2ml of 0.2N HCl are added and mixed. The above solution is then titrated against the Hypo solution from the burette till the end point is indicated by the change in the color from Blue to Colorless.

Principle:

Penicillin in the broth reacts with alkali to form Penicilloic acid, which forms a complex with iodine. Starch when added to the solution reacts with free iodine forming a blue colored complex. This is titrated against Hypo to get colorless end point. The hypo consumed is directly proportional to the free iodine and inversely proportional to the Penicillin produced.

Qualitative estimation of Penicillin activity by Well diffusion method:

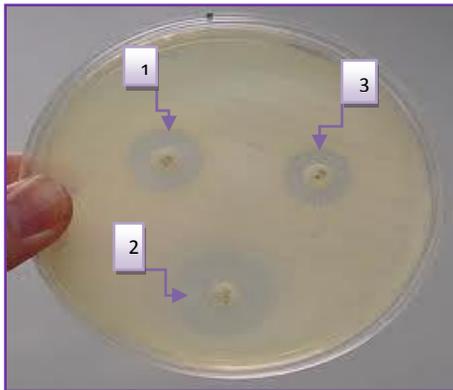
To estimate and compare the antibiotic activity of Penicillin produced by various mutants Well diffusion method was employed. The activity of the crude antibiotic was estimated against the *Bacillus* species isolated from soil. Onto the different labelled Nutrient agar plates 0.1ml of the *Bacillus* cultures were inoculated by spread plate method. Wells were made in the same plate and the crude antibiotic was

added to the wells. After incubation the inhibition zones were measured.

Results and Discussions:

The Quantitative and Qualitative tests have been employed to determine the yield and efficacy of the Penicillin produced from wild and mutant strains of the stock culture. The Quantitative yield was analyzed using Titrimetry and Qualitative by Well diffusion methods and the results are shown as follows.

Fig 2: Qualitative Estimation by Well diffusion Showing the antibiotic efficacy of mutant strains



- 1: 2min UV exposure,
- 2: 2.30min UV exposed and
- 3: 1min UV exposed

The above Figure shows the variations in the Zone of inhibition as obtained from wild and mutant strains. When 0.5ml of the crude extract was used for the test, Wild strain produced very little zone of inhibition. Highest zone was observed for the strain exposed for 1min to U.V. The strain that was exposed for 2min showed bigger zone when compared to wild strain and smaller when compared to the 1min mutant. The zones of inhibition were 0.2, 3.2, and 4.0, 0.08 cm respectively for wild, 1min, 2min and 2.30min exposed strains respectively. The zone of inhibition for wild strain was measured separately.

Quantitative Estimation of Penicillin Yield by Titrimetry

Titrimetric method was employed for analyzing the variations in the amount of penicillin produced by wild and mutant strains. The results are depicted in the table below.

Table 1: Shows the Results of estimation of Penicillin by Titrimetry

Sample	Standard Penicillin	Broth (ml)	Phosphate buffer (ml)	NaOH (ml)	I N C U B A T I O N	HCL (ml)	Iodine (ml)	Acetate buffer (ml)	Hypo Consumed (ml)
BLANK	1.0	0.00	5	2		2	5	5	1.0
WILD	0.00	1	5	2		2	5	5	1.1
UV exposed for 1min	0.00	1	5	2		2	5	5	0.8
UV exposed for 2min	0.00	1	5	2		2	5	5	1.0
U.V exposed for 2.30 min	0.00	1	5	2		2	5	5	1.2

Table1: The above table above shows that, with the increasing duration of UV exposure the penicillin productivity increased to a certain limit after which it started decreasing. From the above table it can be inferred that wild strain produced less amount of penicillin when compared to the Mutant strain that were exposed for 1 min and 2min but higher than the mutant strain that was exposed for 2.30min.

Conclusion:

From the results of quantitative estimation and qualitative efficacy study it can be concluded that the treatment with UV irradiation has a direct influence on the penicillin producing ability of the fungi. The effect of this radiation is dependant upon the exposure time in a variable manner. Initially the rate of Production of penicillin increases with the duration of exposure directly up to a certain limit. Later suddenly the rate of penicillin production decreases with the increased exposure time. From the above work it can be stated that the industrialist should initially analyze the optimum exposure time for the maximum penicillin production to obtain high yield from the stock cultures.

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References:

- 1) Web page of "Penicillium chrysogenum"
http://bioweb.uwlax.edu/bio203/s2008/kitzmann_step/
- 2) Fun facts about fungi
<http://herbarium.usu.edu/fungi/funfacts/penicillin.htm>
- 3) "Speciation despite globally overlapping distributions in *Penicillium chrysogenum*: the population genetics of Alexander Fleming's lucky fungus" PMID : 21951491
- 4) http://www.odec.ca/projects/2004/dieh4v0/public_html/project_info.htm
- 5) Wolters kluwer:
<http://www.uptodate.com/index>
- 6) Transtutors.com
<http://www.transtutors.com/chemistry-homework-help/chemistry-in-action/types-of-penicillin.aspx>
- 7) <http://www.advancedbiotech.in/36%20Mutational.pdf>
- 8) <http://www.news-medical.net/health/Penicillin-Production.aspx>.
