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Comparative study of wild and UV irradiated strain of *Penicillium chrysogenum* for penicillin production

Biological Science

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ABSTRACT

Penicillin is a well-known antibiotic that is produced by fungi belong to species *Penicillium*. Due to the immense applicability of penicillin antibiotic in the field of medicine, it has gained a very vital position in the industrial sector. In view of the importance of penicillin in the field of industry and medicine, the current works aim to compare and standardize the effect of penicillin antibiotic production by *Penicillium chrysogenum*. The mutant strain was prepared by exposing wild strain to Ultraviolet (UV) light exposure. The antibiotic produced was estimated using titrimetric protocol and the comparative study was done on the productivity of wild strain and mutant strain of *Penicillium chrysogenum*. In mutant strain, a productivity of penicillin at pH 5.5 and 2 ml of inoculum size is higher as compare to wild strain in the same condition.

1. Introduction:

The framework of penicillin began in the 1940s, and an era of antibiotic has been recognized as one of the greatest advances in therapeutic medicine (1). Penicillin is very effective for killing bacteria in the body. Antibiotic Penicillin is well known for treatment of pneumonia, syphilis, gonorrhea etc. Another thing penicillin can do is to protect your body from infections. This is very important in case you get a cut with deadly bacteria in it (2). Penicillin belongs to the beta - lactam family of antibiotics, the members of which use a similar mechanism of action to inhibit bacterial cell growth that eventually kills the bacteria. (3) Bacterial cells are surrounded by a protective envelope called cell wall. One of the primary components of the bacterial cell wall is peptidoglycan, a structural macromolecule with a net like composition that provides rigidity and support to the outer cell wall (4). In order to from the cell wall, a single peptidoglycan chain is cross-linked to other peptidoglycan chains through the action of the enzyme DD-trans peptidase (also called a penicillin binding protein-PBP) (5). Throughout a bacterial life cycle, the cell wall (and thus the peptidoglycan can cross-links) is continuously remolded in order to accommodate for repeated cycles of cell growth and replication. Penicillin and other antibiotics in the beta-lactam family contain a characteristic four-membered beta-lactam ring (6). Penicillin kills bacteria through binding of beta-lactam ring to DD-trans peptidase, inhibiting its cross-linking activity and preventing new cell wall formation. Without a cell wall, a bacterial cell is vulnerable to outside water and molecular pressures and quickly dies. Since human cells do not contain a cell wall, penicillin treatment results in bacterial cell death, without affecting human cells (10). A great deal of work has been directed towards increasing the yield of penicillin in fermentation with *Penicillium chrysogenum*. The spectacular increase in yield which has been achieved has resulted in the main, from two approaches. In the first approach, mutant strains of *Penicillium chrysogenum* produced by spontaneous variation or by the action of a mutagenic agent such as nitrogen mustard or ultraviolet light were selected for enhanced penicillin production. Further enhancement of the yield of penicillin has resulted from a complete definition of the environmental conditions (pH, temperature, constituents of the medium) necessary for the excretion of penicillin by the mold. However, changes in the sulfur metabolism which accompany an increase in penicillin synthesis brought about either by mutation of the mold or by changes in conditions of the fermentation, have not been investigated (11). The basic biochemical reaction behind penicillin production is as follows (12), here beta-lactam indicates the actual functional ring or core of the penicillin structure. The basic method of penicillin production involves following steps:

α-Ketoglutarate + AcCoA → Homocitrate → L-α-Aminoadipic acid → L-Lysine + β-Lactam

The present study deals with the mutation in *Penicillium chrysogenum* by UV irradiation and comparing its antibiotic production capacity with wild- type *Penicillium chrysogenum*.

2. Materials and method:

Microbial Culture

*Penicillium chrysogenum*fungi was kindly provided by bioprocess laboratory of Shree Ramkrishna Institute of Computer Education and Applied Sciences. The culture was confirmed to be *P. chrysogenum*based on the colony morphology as well as the microscopic observation.

Preparation of mutant strain using U.V irradiation

The plate containing the pure culture of *P. chrysogenum* were further subjected to U.V irradiation and one plate was preserved as a wild strain. The U.V mutations were induced by exposing the plate to the U.V light available in the UV chamber. The plates containing the pure culture of *P. chrysogenum* exposed to UV irradiation for 5 minutes and one plate preserved as wild type.

Preparation of production media and inoculation

To obtain penicillin antibiotic, fungi *P. Chrysogenum* were inoculated in production media with nutrient. Prepare various flask with the same composition of media. Fermentation medium was prepared

with different pH (3.5, 5.5, and 7.5). Inoculate different amount (1 ml, 2 ml, 3 ml) inoculum in mutant labeled flask as well as the wild labeled flask. Incubate flask at 120 rpm and 28° for 5-6 days. Fermentation media contained Yeast extract: 2.0 gms, Lactose: 2.5 gms, Glucose: 0.5 gms, Salt solution: 10.0 mL {Composition of Salt solution KH_2PO_4: 30.00 gms, $MgSO_4.7H_2O$: 2.50 gms, $Fe(NH_4)SO4.6H_2O$: 1.0 gms, $CuSO_4$: 0.05 gms, Na_2SO4: 5.00 gms, $MnSO_4$: 0.20 gms, $CaCl_2$: 0.10 gms, Distilled water: 1000 mL} $CaCO_3$: 0.40 gms, Na_2CO_3: 0.10 gms, for 100 ml of production media (13).

Quantitative estimation of penicillin by Iodometry method:

Iodometric titration of beta-lactam antibiotics is not based on oxidation of iodine directly but on oxidation of the products of the hydrolysis of the beta-lactam structure. The two products of hydrolysis – penaldic acid and penicillamine interact with the iodine molecule, applied as a titrant to form Schiff base and 2-amino-3-sulpho-3-methyl-butanoic acid, respectively (7). In brief, Starch added to the solution reacts with free iodine forming a blue colored complex. Different aliquot of penicillin solution was taken in flasks and made final volume up to 4ml with phosphate buffer. 1 ml of 1N NaOH was added in sample flasks of each aliquots and 1 ml of 1.1N HCL was added in blank flasks of each aliquot. Flasks were incubated in dark for 30 min. 1 ml of 1.1N HCL was added in each sample flask and 1 ml of 1N NaOH was added to blank flasks. 10ml of 0.1N I_2 solution was added in each flask. 1-2 drop of starch was added as an indicator. These solutions were titrated against $Na_2S_2O_3$ and found out the concentration of penicillin (9).

Quantitative estimation of penicillin activity by agar diffusion method:

Agar diffusion method was employed to estimate the antibiotic and antimicrobial activity of penicillin produced by mutant and wild *P. crysogenum*. The activity of the extract of antibiotic penicillin estimated against *Bacillus subtilis*. The pen assay seed agar with a spore suspension of 0.2 ml *Bacillus subtilis* is spread on to pre - solidified pen assay base agar and make well using cup borer. The crude extract of penicillin produced by mutant and wild is added in well of unknown high (U_H) and unknown low (U_L, sample 1:3 diluted with phosphate buffer) and standard penicillin which was collected commercially and was added in to well of U_H and U_L(1:3 dilute) then kept plate at 4°C for 15 min for distribution of penicillin in agar. Then incubate the plate at 37°C for 24 hours. After that measure the zone of inhibition and calculate the antibiotic activity by using standard formula (8).

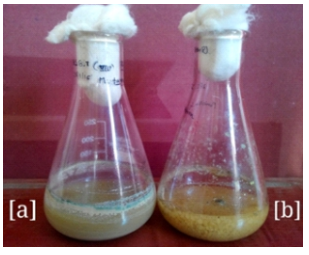

Figure 1: Production of Penicillin antibiotic by [a] Mutant strain *P. chrysogenum* [b] Normal strain *P. chrysogenum*.

3. Result and discussion:

After incubation period filter the media and collect filtrate then centrifuge and take supernatant. An iodometry method was used for the estimation of penicillin of different flask with different pH and different inoculum size.

The Quantitative and Qualitative tests have been performed to determine the yield and efficacy of the penicillin produced from wild and mutant strain using different pH and inoculums size of the *Penicillium chrysogenu*m culture. The quantitative yield was analyzed using iodometry and Qualitative method determine by well diffusion method and result are shown as follows:

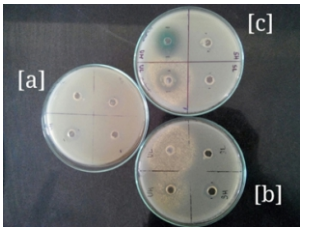

Figure 2: Qualitative Estimation by well diffusion showing the antibiotic efficiency of wild as well as mutant strains of penicillin. [a] Normal (S_H, S_L, U_H, U_L) [b] Wild type (S_H, S_L, U_H, U_L) [c] Mutant type (S_H, S_L, U_H, U_L).

The above figure shows the variation in the zone of inhibition in the zone of inhibition as obtained from a wild and mutant strain of pH 5.5 and inoculums size 2 ml.when 500µl of the crude extract was used for the test. The mutant strain produced a very high zone of inhibition which is exposed to UV light for 5 minutes compare to wild strain. zone of inhibition of wild type show higher in pH 5.5 and inoculum size 2 ml compare to pH 3.5 and 7.5 and inoculum size 1 ml and 3 ml but smaller than the mutant strain a pH 5.5 and inoculums size 2 ml.zone of inhibition was 15 mm (for unknown high) and 5 mm (for unknown low) in wild strain and 28 mm (for unknown high) and 20 mm (for unknown low) in the mutant strain.

Quantitative estimation of penicillin yield by iodometry:

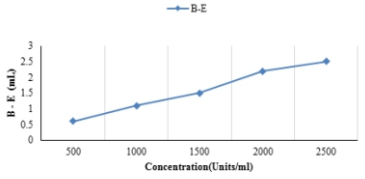

Figure 3: standard graph of penicillin estimation by iodometric method.

Titrimetric method was employed for analyzing the variation in the amount of penicillin produced by wild and mutant strains.

Table 1: The below table was shows that, in pH 5.5 and inoculum size 2 ml with expose to UV light for 5 min produced high amount of penicillin as compare to wild strain.

Sample	*Dilution*	*Aliquotes (mL)*	*Phosphate buffer (mL)*	*1 N NaOH (mL)*	*1.1 N HCl (mL)*	*INCUBATE At*	*1.1 N HCl (mL)*	*1 N NaOH (mL)*	*0.01 N I2 (mL)*		*0.01 N Na2SO3 (mL)*	*B – S (mL)*	*Conc. from graph (Units/ml)*	*Mean conc. (Units/ml)*
Wild type	Undiluted	B - 1	3.0	-	1.0	*ROOM*	-	1.0	10	*1 to2*	13.0	1.6	1475	1008.33
		S - 1	3.0	1.0	-		1.0	-	10		11.4			
	Dilution (1:5)	B - 1	3.0	-	1.0	*TEMP*	-	1.0	10	*DROP*	14.5	1.5	1375	
		S - 1	3.0	1.0	-		1.0	-	10		13.0			
	Dilution (1:10)	B - 1	3.0	-	1.0	*In*	-	1.0	10	*Of*	10.7	0.2	175	
		S - 1	3.0	1.0	-	*DARK*	1.0	-	10		10.5			
Mutant type	Undiluted	B - 1	3.0	-	1.0		-	1.0	10	*STARCH*	3.6	1.5	1500	1875
		S - 1	3.0	1.0	-	*for*	1.0	-	10		2.1			
	Dilution (1:20)	B - 1	3.0	-	1.0		-	1.0	10		3.8	2.4	2250	
		S - 1	3.0	1.0	-	*30 min*	1.0	-	10		1.4			

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4. 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 radiation is that the rate of Production of penicillin increases in mutant type *P. chrysogenum compare to wild type.* 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 the high yield from the stock cultures.One of the major efforts to modify microorganisms of biotechnological interest has been provided by the antibiotics industry. *P. chrysogenum* has been extensively mutated during the last decades to increase the penicillin titers, but despite the importance of this microorganism, the biochemical bases that have been modified during the strain improvement process remain poorly understood.

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