Pakistan J. Sci. Ind. Res., Vol. 29, No. 1. February 1986

STUDIES ON THE ISOLATION AND SCREENING OF INDIGENOUS PENICILLIN PRODUCING STRAINS: PART I

Najma Murtaza, Shaheen A. Husain, and Izhar H. Qureshi*

PCSIR Laboratories, Karachi-39

(Received February 27, 1985)

Out of twenty locally isolated strains of the genus Penicillium, only four strains were shown to produce penicillin. Comparative study of penicillin production by these strains under different sets of cultural conditions is also described.

INTRODUCTION

The discovery of penicillin [1] by Sir Alexender Fleming in 1929 and later the demonstration of its clinical application [2] in 1941, marked the begining of a new era in medical therapeutics. By 1942, penicillin became available in sufficient quantity to researchers for detailed chemical investigations. As a result of these studies, it was possible to elucidate the structure ofpenicillin [3] and also to accomplish its synthesis [4].

Following the successful application of penicillin as a powerful therapeutic agent, massive research efforts were directed towards isolating new antibiotics. Today many hundreds of penicillins have been discovered and more than 20 penicillins are commercially available. All penicillins contain a common bicyclic nucleus, consisting, of fused β -lactam and thiazolidine rings with different acylamino side chains at C-6 position. (Table 1). Penicillins have a characteristic gram positive antibacterial activity. Recently several penicillins have been found to show both gram negative and gram positive activity.

Literature survey showed that penicillin is produced only by Penicillium and Aspergillus species, the most important ones being *Penicillium notatum* and *Penicillium chrysogenum.* The latter species is used in the manufacture of penicillin.

For the economic production of microbial derived compounds, it is necessary to use a special strain capable of producing the desired compound in good yield with minimum number of side products. However, commercial strains are a closely guarded secret and it is necessary to adopt indigenous laboratory strains for commercial production either through continuous growth in a given environment or by mutation caused by chemical or physical treatment.

The present studies deals with the isolation and screening of the indigenous strains of the genus Penicillium. Comparative studies of the production of penicillin by selected strains under different sets of cultural conditions are also described.

MATERIAL AND METHODS

Twenty strains (P_1 to P_{20}) of the genus Penicillium were isolated from local soil and tested for antimicrobial activity following the method of Florey *et al* [5] and for the production of penicillin by the method described by Betina [6]. The result recorded in Table 2 indicate that seven strains show appreciable antimicrobial activity. Out of these 4 strain were also found capable of producing penicillin in modest yield. These penicillin producing strains (P_3, P_6, P_{13}, and P_{18}) all belonging to *Penicillium chrysogenum* were maintained on Czapeck Dox-A slants for further studies.

In order to obtain favourable cultural conditions for the production of penicillin, different sporulation, seed and fermentation media were tried and the media giving best results were selected for further studies. The composition of the media are described below.

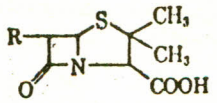
Sporulation media

Four sporulating media having the following composition were tried:

1. *Medium A.* Sucrose, 20 g; $NaNO_3$, 6 g; KH_2PO_4, 1.5 g; $MgSO_4$, $7H_2O$, 0.5 g; $CaCl_2$, 2.5 g; agar, 20 g; distilled water to make up to 1 litre.

* To whom all correspondence may be addressed to:

Table 1. List of some commercially available penicillins



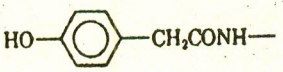
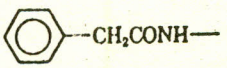
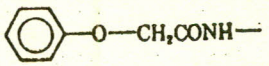
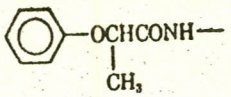
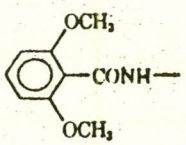
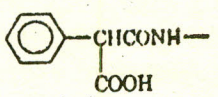
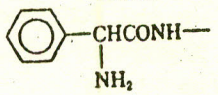
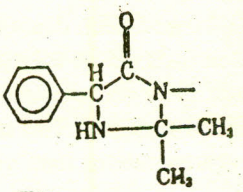
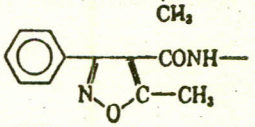
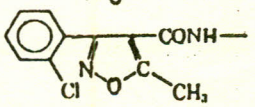
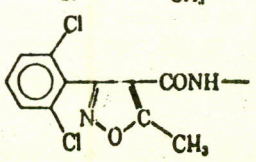
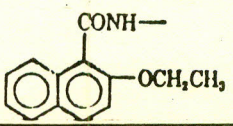
S.No.	Common or generic name	Side chain group, R
1.	Penicillin F	$CH_3CH_2CH{=}CHCH_2CONH-$
2.	Penicillin K	$CH_3(CH_2)_6CONH-$
3.	Penicillin X	
4.	Penicillin G	
5.	Penicillin O	$CH_2{=}CHCH_2SCH_2CONH-$
6.	Penicillin V	
7.	Phenethicillin	
8.	Methicillin	
9.	Carbenicillin	
10.	Ampicillin	
11.	Hetacillin	
12.	Oxacillin	
13.	Cloxacillin	
14.	Dicloxacillin	
15.	Nafcillin	

Table 2. Screening of locally isolated strains of the genus penicillium

S.No.	Organisms	Antimicrobial activity	Penicillin produce
1.	P – 1	+	–
2.	P – 2	+	–
3.	P – 3	+++	+
4.	P – 4	–	–
5.	P – 5	+++	–
6.	P – 6	+++	+
7.	P – 7	++	–
8.	P – 8	+	–
9.	P – 9	–	–
10.	P – 10	++	–
11.	P – 11	–	–
12.	P – 12	+	–
13.	P – 13	+++	+
14.	P – 14	++	–
15.	P – 15	+++	–
16.	P – 16	+	–
17.	P – 17	++	–
18.	P – 18	+++	+
19.	P – 19	+++	–
20.	P – 20	–	–

2. *Medium B.* Peptone, 6 g; glycerine, 15 g; glucose 15 g; KCl, 5 g; NaCl, 5 g; $FeSO_4.7H_2O$, 0.05 g; $CuSO_4.5H_2O$, 0.02 g; KH_2PO_4, 0.3 g; $MgSO_4.7H_2O$, 0.05 g; $MnSO_4$, 0.0002 g; agar, 30 g; distilled water to make upto 1 litre.

3. *Medium C.* Glycerol, 9.2 g; sucrose, 1 g; NaCl, 4 g; yeast extract, 50 g; phenylacetic acid, 20 g; agar, 20 g; distilled water to make upto 1 litre.

4. *Medium D.* 20 g of clean rice were taken in a 250 ml conical flask and autoclaved. A solution containing honey (15%) and peptone (2%) was prepared and distributed in test tubes (15 ml) and sterilized. This sterilized solution was poured into the rice-containing flask at the time of inoculation.

Seed media

Four seed media having composition detailed below were tried.

1. *Medium A.* Glucose, 50 g; peptone, 5 g; NaCl, 4 g;; KH_2PO_4, 0.1 g; $MgSO_4.7H_2O$ 0.05 g; distilled water to make upto 1 litre. Distributed 60 ml in each 500 ml. conical flask.

2. *Medium B.* Sucrose, 20 g; $NaNO_3$, 6.0 g; KH_2PO_4, 1.5 g; $MgSO_4.7H_2O$, 0.5 g; $CaCl_2$, 2.5 g; distilled water to make up to 1 litre. Distributed 60 ml in each 500 ml conical flask.

3. *Medium C.* Granulated sugar, 18 g; corn steep liquor, 35 g; $CaCO_3$, 5 g; distilled water to make upto 1 litre. Distributed 60 ml in each 500 ml conical flask.

4. *Medium D.* Lactose, 2.8 g; corn steep liquor, 45 g; maize gur, 6.3 g; KH_2PO_4, 0.3 g; $(NH_4)_2SO_4$, 0.3 g; $MgSO_4.7H_2O$, 0.07 g; NH_4NO_3, 0.6 g; phenylacetic acid, 2 g; distilled water to make upto 1 litre. Distributed 60 ml in each 500 ml conical flask. Subsquently $CaCO_3$, 0.15 g was added in each flask.

Fermentation media

Four fermentation mdia of the following composition were tried.

1. Medium A. Glucose, 40 g; $(NH_4)_2SO_4$, 0.4 g; KH_2PO_4, 7.5 g; Na_2SO_4, 1 g; $MgSO_4.7H_2O$, 0.7 g; $FeSO_4.7H_2O$, 0.18 g; $MnSO_4.4H_2O$, 0.05 g; $ZnSO_4.7H_2O$, 0.05 g; $CuSO_4.5H_2O$, 0.008 g; $CaCl_2$ g; Distilled water to make upto 1 litre. Distributed 60 ml in each 500 ml conical flask.

2. *Medium B.* Lactose, 35 g; corn steep liquor, 20 g; $(NH_4)_2SO_4$, 6 g; distilled water to make up to 1 litre. Distributed 60 ml in each 500 ml conical flask.

3. *Medium C.* Lactose, 110 g; corn steep liquor, 25 g; $(NH_4)_2SO_4$, 5 g; diammonium phosphate, 4 g; urea, 1 g; phenylacetic acid (1.2% solution), 4 ml/litre; distilled water to make upto 1 litre. Distributed 60 ml in 500 ml conical flasks. Then $CaCO_3$, 0.36 g and cottonseed cake, 0.6 g were added to each flask.

4. *Medium D.* Lactose, 85 g; corn steep liquor, 32.5 g; maize gur, 5 g; NH_4NO_3, 1.5 g; $(NH_4)_2SO_4$, 2 g; diammonium phosphate, 2 g; $MgSO_4.7H_2O$, 0.07 g; phenylacetic acid (1.2% solution), 2 ml/litre; distilled water to make upto 1 litre. Distributed 60 ml in 500 ml conical flasks. Then $CaCO_3$ (0.36 g) and cottonseed cake (0.6 g) were added to each flask.

General procedure

Selected strains of *Penicillium chrysogenum* species were inoculated on sporulating medium and incubated at 24-25° for 7 days. The growth was observed visually and the spores produced were used in the preparation of seed culture.

1. *Preparation of seed culture.* In a typical batch, fifteen 500 ml conical flasks containing 60 ml of seed medium were inoculated with the spores and incubated at 24-25° for 48 hr. on an orbital shaker. The growth of seed culture was observed visually.

2. *Fermentation:* A set of five 500 ml conical flasks containing the fermentation medium was inoculated with

Table 3. Effect of different media on sporulation

S.No.	Medium	Strain No. P-3	Strain No. P-6	Strain No. P-13	Strain No. P-18
1.	A	–	–	–	–
2.	B	+++	+++	+++	+++
3.	C	+	+	+	+
4.	D	++	+++	++	+++

Table 4. Effect of different media on the growth of seed culture

Medium	Strain No. P-3	Strain No. P-6	Strain No. P-13	Strain No. P-18
A.	–	–	–	–
B.	+	+	+	+
C.	+++	+++	+++	+++
D.	++	++	++	++

Table 5. Production of penicillin in different fermentation media

Medium used	Yield of penicillin (iu/ml)			
	Strain P-3	Strain P-6	Strain P-13	Strain P-18
A.	995	657	876	1210
B.	292	146	–	1241
C.	2044	2126	–	2701
D.	2177	2236	511	2847

the seed culture and incubated at 24-25° for 162 hr. on an orbital shaker. The quantiative estimation of penicillin produced was made by iodometric titration [7].

RESULTS AND DISUSSIONS

The selected strains (P_3, P_6, P_{13} and P_{18}) of *penicillium chrysogenum* species were first grown on four different sporulation media. The results are shown in Table 3. It was concluded that whereas strain P_3 and P_{13} gave best results in medicum B only, the strains P_6 and P_{18} showed maximum sporulation in medium B as well as in medium D.

The spores of the selected strains were grown on four different seed media. Te results recorded in Table 4 indicate that spores of P_3, P_6, P_{13}, and P_{18}, grow best in seed medium C.

The seed culture of the selected strains were then grown on four different fermentation media. The results are shown in Table 5. It was concluded that P_{18} was the most promising strain giving the best yield of penicillin in medium D.

From the above data, it is concluded that sporulation medium B, seed culture medium C and fermentation medium D constitute the most favourable cultural conditions for the production of penicillin from locally isolated strains of *Penicillium chrysogenum*. Amongst the four strain studied, P_{18} is the most promising producing penicillin in an yield of 2847 i.u./ml. Development of this strain into commercially viable strain through mutation is under investigation.

Acknowledgement. The authors are grateful to M/s. Glaxo Laboratories Pakistan Limited for the generous gift of dextrose monohydrate. Their thanks are also due to Dr. Tahira B. Sarfaraz for critically evaluating the manuscript. Technical assistance rendered by Mr. Omer Farooq and Mr. Sher Nauroze Khan is also acknowledged with thanks.

REFERENCES

1. Sir Alexander Fleming, Brit. J. Exptl. Pathol., **10**, 226 (1929).
2. E.P. Abraham, E. Chain, C.M. Fletcher, A.D. Gardner, N.G. Healtley, M.A. Jennings, and H.W. Florey, Lancet, **2**, 177 (1941).
3. H.T. Clarke, J.R. Johnson, and R. Robinsons, (eds), "*The Chemistry of Penicillin*" (Princetone Univ. Press, Princeton, New Jersey, 1949) E. Chain, Ann. Rev. Biochem, **17**, 657 (1948), Endeavour, 7, 152 (1948). A. H. Cook, *Quart. Rev.*, (London), **2**, 203 (1948).
4. Vincent du Vigneaud, Frederick H. Carpenter. Robert W. Holley, Arthur H. Livermore, and Jullian R. Rachele, Science, **104**, 431 (1946).
5. S.C. Prescott, and C.G. Dunn, "Industrial Microbiology", McGraw-Hill Boc k Company, Inc. New York, Toronto, London (1959), p. 766.
6. Vladimir Betina, 'Naturwissenschaften', **44**, 378 (1957). (1957).
7. M.M. Bethel, and C.R. Bond, Analyst, **86**, 448-457 (1961).