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Mutagenesis and Enrichment of Auxotrophs in *Penicillium chrysogenum*[1]

PRAKASH S. MASUREKAR, MARC P. KAHGAN, AND ARNOLD L. DEMAIN

Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

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An efficient method for isolation of auxotrophs of *Penicillium chyrysogenum* involving mutagenesis with ethyl methanesulfonate followed by enrichment with sodium pentachlorophenate was developed. The auxotroph frequencies obtained were 30 to 40%.

During the course of our studies on the regulation of penicillin biosynthesis (3), we became interested in isolating lysine auxotrophs of *Penicillium chrysogenum* which were blocked early in the lysine biosynthetic pathway. For this purpose, it was desirable to use a method which could yield a high frequency of auxotrophs. We tested *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) at neutral *p*H but were unable to obtain general auxotroph frequencies above 1%. Therefore, the alkaline NTG method (1) was tried.

Conidia of *P. chrysogenum* Wis. 54-1255 (10^6/ml) in 0.1 M tris(hydroxymethyl)aminomethane (Tris)-maleate buffer (*p*H 9.0) were treated with 0.3% NTG for various lengths of time. It was found that a 15-minute treatment time produced 99.7% kill and an auxotroph frequency of 13%. Although this relatively high auxotroph frequency was obtained, many of the auxotrophs had either multiple requirements or their growth was stunted.

Ethyl methanesulfonate (EMS) was also tested. *P. chrysogenum* conidia (10^6/ml) in physiological saline were shaken (250 rev/min) with 0.8% EMS at 25 C. Auxotroph frequencies as high as 3% were obtained by the mutagenic treatment which lasted as long as 12 hr. Although these auxotroph frequencies are lower than those obtained with alkaline NTG, the fraction of multiple and stunted auxotrophs was very small.

We next attempted to apply an auxotroph enrichment technique to raise the frequency above 3%. Sodium pentachlorophenate (PCP) had been predicted by Ganju and Iyengar (2) to be a useful enrichment agent from the results of their model experiments using authentic auxotrophs and prototrophs. Their method was tested as follows. The conidia treated with EMS for 12 hr were allowed to germinate on Czapek agar (Difco) plates for 22 hr. The germinated and ungerminated conidia were recovered from the plates by repeated flushing with a total of 10 ml of sterile distilled water, accompanied by stroking of the agar surface with a glass rod. The recovered conidial population was shaken with 25 μg of PCP/ml (pentachlorophenol neutralized with NaOH) for 7.5 min. This exposure time was found to be optimal in our preliminary studies. PCP was removed by rapidly filtering the suspension through 0.22-μm membrane filters (Millipore Corp.) and washing five times with sterile distilled water. These conidia were then tested for auxotrophy by plating on complex and minimal media. Auxotroph frequencies up to 39% were obtained which represented an en-

TABLE 1. *Sodium pentachlorophenate enrichment[a] of mutagenized Penicillium chrysogenum conidia*

Expt no.	Survival[a]	No. of colonies tested	No. of auxotrophs	Auxotroph frequency[b] (%)	Enrichment factor
1	0.06	50	14	28	7.4
2	0.03	25	9	36	9.5
3	0.05	70	27	39	10.2
4	0.03	27	10	37	9.7

[a] EMS mutagenesis for 12 hr; 7.5-min PCP enrichment.

[b] EMS mutagenesis for 12 hr alone gave an auxotroph frequency of 3.8%.

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TABLE 2. *Classification of auxotrophs of Penicillium chrysogenum*

Treatment	Total no.	Auxotrophs				
		Amino acid no.	Vitamin no.	Purine or pyrimidine or both no.	Multiple no.[a]	Unclassified no.[b]
Alkaline NTG	26	9	1	2	10	4
EMS	18	11	0	2	2	3
EMS and PCP	89	54	8	8	15	4

[a] Multiple auxotrophs grow on a chemically defined medium containing amino acids, vitamins, purines, and pyrimidines, but not on the medium supplemented with amino acids alone, vitamins alone, or purines plus pyrimidines alone.

[b] Unclassified auxotrophs grow on complex medium, but not on a chemically defined medium containing amino acids, vitamins, purines, and pyrimidines.

richment of up to 10-fold (Table 1). The enrichment procedures did not markedly alter the broad distribution of auxotroph types (Table 2). By this method, we have been able to isolate the type of lysine auxotroph required to proceed with our studies on penicillin biosynthesis (P. S. Masurekar and A. L. Demain, Abstr. Annu. Meet. Amer. Soc. Microbiol., Philadelphia, p. 12, 1972).

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