Mutants of *Aspergillus nidulans* Impaired in Penicillin Biosynthesis

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(Received 20 June 1974)

INTRODUCTION

Mutants blocked in the production of a metabolite can provide information on intermediate products in the biosynthetic pathway of that metabolite and the number of genetic loci involved. Lemke & Nash (1972) have isolated mutant strains of *Cephalosporium acremonium* unable to yield β -lactam antibiotics and have reported on their ability to synthesize peptides implicated as intermediates in the elaboration of penicillin N and cephalosporin C. Bonner (1947) isolated 55 mutants of *Penicillium notatum* which produced less than 10 % of the normal amount of penicillin. Mixed cultures of all possible pairings of these mutants did not show normal penicillin production. Caglioti & Sermonti (1956) and Sermonti (1956) examined nine mutants of *Penicillium chrysogenum* with much reduced or no detectable penicillin activity. Heterozygous diploid strains were synthesized between different mutants and in only one case did complementation occur. These results indicated the existence of two loci affecting penicillin production.

The work of Holt & Macdonald (1968*a, b*) and Macdonald, Holt & Ditchburn (1972) on *Aspergillus nidulans* established a useful system for the genetic study of penicillin production. In this mould, although penicillin yields are low (Holt & Macdonald, 1968*a*) the interpretation of crosses is easier than in *P. chrysogenum*. Work was therefore undertaken to produce mutants of *A. nidulans* impaired in penicillin biosynthesis for their use in complementation analyses. This communication reports the isolation of such mutants using a range of mutagens to avoid possible locus specificity.

METHODS

Organisms. Mutants, derived from *Aspergillus nidulans*, strain NRRL 194 (Pontecorvo, Roper, Hemmons, Macdonald & Bufton, 1953) were kindly supplied by Dr A. J. Clutterbuck of the University of Glasgow. The domestic codes and phenotypes of the strains used are: G5 (*biA1*), G26 (*biA1*; *w-010*), G30 (*suAadE20 yA2 adE20; acrA1; galA1; pyroA4; facA303; sB3; nicB8; riboB2*), G31 (α ; *galA1; pyroA4; facA303; sB3; nicB8; riboB2*), GH8 (*biA1; fwA1*), GH11 (*yA2; pyroA4; cnxA5*), GH13 (*biA1; niaD17*), and GH26 (*biA1; methG1; w-005*).

Genetic symbols. For genes determining: inability to utilize a specific sugar as sole carbon source – *galA1* = galactose; nutritional requirements – *adE20* = adenine, *biA1* = biotin, *methG1* = methionine, *nicB8* = nicotinic acid, *pyroA4* = pyridoxine, *riboB2* = riboflavin; ability to suppress nutritional requirement – *suAadE20* = suppressor of *adE20*; inability to utilize specific nitrogen compounds as sole nitrogen source – *cnxA5* = nitrate and hypoxanthine, *niaD17* = nitrate; resistance – *acrA1* = acriflavine, *facA303* = fluoroacetate;

Table 1. Frequency of penicillinless mutants induced by different mutagens

Strain	Mutagen	No. of isolates screened	No. of isolates selected	Frequency of penicillinless mutants (%)
G5	FUV	500	1	} 0.19
G30	FUV	1000	2	
G31	FUV	1200	2	
G26	NTG	4100	3	0.07
GH8	EMS	4900	5	0.10
GH11	GC	1600	10	0.63
GH13	8MOP	5200	4	0.08
GH26	NA	1400	1	0.07
Total		19900	Total 28	Mean 0.14

mutant spore colour – *fwA1* = fawn, *w-oro* and *w-oo5* = white, *yA2* = yellow. The symbol α indicates that the strain may also carry *suAadE20*.

Media. Minimal medium (MM) and complete medium (CM) were based on those described by Pontecorvo *et al.* (1953) The fermentation medium (FM) for penicillin production was that used by Holt & Macdonald (1968*a*).

Production of mutants. The mutagens employed and references to the details of the methods used were as follows: far-u.v. light, FUV (Macdonald, Hutchinson & Gillett, 1963); *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, NTG (Martinelli & Clutterbuck, 1971); ethyl methane sulphonate, EMS (Yost & Chaleff, 1967); gamma rays from a ^{60}Co source, GC (Alderson & Scott, 1971; the irradiation unit described by Burlin & Chan, 1971, was used); near-u.v. light in the presence of 8-methoxypsoralen, 8MOP (Alderson & Scott, 1970); nitrous acid, NA (Siddiqui, 1962).

A rapid screening method for the isolation of mutants impaired in penicillin synthesis. After mutagenic treatment conidia were plated on CM agar and those surviving gave rise to individual colonies. One loopful of conidia from each colony was inoculated into 2 cm³ of FM contained in a screw-cap Universal bottle of 28.4 cm³ (1 oz) capacity. After static incubation for 5 days at 25 °C a single drop of the culture medium was spotted on the surface of a test agar seeded with spores of *Bacillus subtilis*. Following overnight incubation of the assay plate at 37 °C, single colony isolates which gave no inhibition zones were selected as putative mutants unable to synthesize penicillin. All were subsequently grown in submerged shaken cultures and their penicillin yields assayed (Holt & Macdonald, 1968*a*).

RESULTS AND DISCUSSION

All of the parent strains used in this investigation yielded 2 to 3 µg penicillin/cm³. After growth in submerged culture, mutant isolates were classified as 'penicillinless' when they gave titres of no more than 10% that of the strains from which they were derived. The frequency of mutants selected after different mutagenic treatments is shown in Table 1. Although the mutations were induced in different strains and hence in different genetic backgrounds, it can be seen that ^{60}Co γ -irradiation gave the highest frequency of penicillinless mutants. Das & Nandi (1972) compared a number of mutagens including FUV and GC. for their efficiency in producing mutants of *Aspergillus niger* with altered citric acid production. The most effective mutagen was GC. Gamma irradiation induces a high frequency of chromosomal aberrations in *A. nidulans* (Kafer & Chen, 1964). It is of interest that five of

the ten mutants induced by GC treatment in this experiment were morphologically different from the parent strain, GH11, when grown on surface culture.

Using standard genetic methods (Pontecorvo *et al.* 1953), diploid complementation tests were made between selected pairs of mutants, establishing that 20 of the 28 penicillinless mutants selected belonged to a single complementation group and that the remaining eight were representatives of at least four other complementation groups. All penicillinless mutants retaining normal morphology after treatment with the mutagens employed were members of one complementation group, designated *npeA*. Haploidization analysis (McCully & Forbes, 1965) enabled the locus *npeA* to be assigned to linkage group VI in *A. nidulans*.

These results show that the situation in *A. nidulans* with respect to mutations leading to impaired penicillin biosynthesis is similar to that found by Sermoniti (1956) in *Penicillium chrysogenum* in that most of these are within a single cistron. However, in *A. nidulans* at least four other loci affecting penicillin production in some way have now been identified.

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