SHORT COMMUNICATION

Relationship of the Genetic Determination of Impaired Penicillin Production in Naturally Occurring Strains to that in Induced Mutants of *Aspergillus nidulans*

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INTRODUCTION

Variation in penicillin production among wild isolates of *Aspergillus nidulans* was first investigated by Holt & Macdonald (1968*a, b*) and subsequently this character was shown to vary continuously under genetic control (Merrick & Caten, 1975). In both studies, however, it was apparent that a class of isolates existed which showed no detectable penicillin production. Eighteen heterokaryon-compatibility (h-c) groups have been recognized in *A. nidulans* (Grindle, 1963; Jinks *et al.*, 1966) and the isolates that did not produce penicillin were mainly representatives of two of these, h-c groups F and G (Holt & Macdonald, 1968*a*).

Derivatives of the Glasgow strain of *Aspergillus nidulans*, NRRL194, have been used in work on the genetics of the biosynthesis and overproduction of penicillin (reviewed by Macdonald & Holt, 1976). Edwards, Holt & Macdonald (1974) isolated 28 mutants impaired in penicillin biosynthesis and used diploid complementation tests to determine whether the mutations were in the same or different genes. Twenty of the mutants belonged to one complementation group, designated *npeA*, and among the remaining eight there were representatives of at least three other complementation groups, *npeB*, *npeC* and *npeD*. These four loci are unlinked (Holt, Edwards & Macdonald, 1976, and unpublished data).

The aim of the present work was to examine the relationship of the genetic determination of impaired penicillin production in natural isolates of *A. nidulans* to the *npe* mutations induced in the Glasgow strain. Three independent natural non-producers were examined: a representative of h-c group F (strain 49), a representative of h-c group G (strain 143) and strain 113 which does not belong to any recognized h-c group. In addition an induced non-producing mutant (*npe-04*) of a high yielding natural isolate (strain 118) was examined (Holt & Macdonald, unpublished data). To overcome the heterokaryon incompatibility between derivatives of strain NRRL194 and other natural isolates, tests for allelism of the blocks in penicillin biosynthesis were made by sexual crosses.

METHODS

Strains. Details of the strains used are given in Table 1. Prototrophic strains 49, 113, 118 and 143 were isolated from various localities in the U.K. and were kindly provided by Professor J. L. Jinks of the University of Birmingham. Certain derivatives of strain NRRL194 were obtained through the courtesy of Dr A. J. Clutterbuck of the University of Glasgow.

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The gene symbols used are those of Clutterbuck (1974). Mutants were given the symbol *npe* when their penicillin yield was 10 % or less than 10 % of that of their parents. Derivatives of strain NRRL194 are prefixed G or GH, derivatives of the Birmingham strains are designated by the strain number followed by the mutant isolation number, e.g. 49/14 is the 14th mutant to be derived from Birmingham strain 49.

Media. Minimal medium (MM) and complete medium (CM) were based on those of Pontecorvo *et al.* (1953). The fermentation medium (FM) for penicillin production was that used by Holt & Macdonald (1968*a*). Sexual crosses were made by mixed conidial platings on malt extract agar (Oxoid).

Sexual crosses. Progeny from crosses were isolated by three methods (Pontecorvo *et al.*, 1953): A, by selecting green progeny on CM after random ascospore analysis of a cross between yellow- and white-sporing parents; B, by selecting recombinants on MM after random ascospore analysis of a cross between strains having different auxotrophic requirements; C, by analysis of individual hybrid cleistothecia. Method C was used where possible but hybrid cleistothecia were rare in certain crosses between derivatives of the Birmingham strains and multiply-marked derivatives of strain NRRL194.

Penicillin yield testing. Progeny were tested after growth in static liquid culture using the rapid screening method of Edwards *et al.* (1974), except progeny from crosses 49/14 × G24, 49/14 × G38, 49/14 × G39 and 49/14 × 118/6 which were grown in shake flasks of FM prior to assay (Holt & Macdonald, 1968*a*). Biological assays were as described by Holt & Macdonald (1968*a*). As a check that growth inhibition was due to penicillin, assays were performed in duplicate and, in one, penicillinase (Neutrapen; Riker Laboratories, Loughborough, Leicestershire) was added to the test agar at a concentration of 80 units ml⁻¹.

RESULTS AND DISCUSSION

In a cross between two independently produced *npe* mutants differing from their parents by single mutations, one-quarter of the progeny should produce wild-type levels of penicillin if the *npe* loci are unlinked. Where they are linked, however, this proportion will be reduced and if the mutations are allelic it will be zero in a small progeny sample. Intra-genic recombination is possible and rare recombinants would be expected if larger samples were taken.

The results of sexual outcrosses involving derivatives of various natural isolates of *A. nidulans* impaired in penicillin production and strains carrying *npeA*, *B*, *C* or *D* mutations are shown in Table 2. Strains from h-c groups F and G, strain 113/4 of unknown heterokaryon compatibility and derivatives of strain 118 carrying the *npe-04* mutation (118/5 and 118/6), all had mutations at the *npeA* locus. From crosses involving any of these strains and a strain possessing an *npeA* mutation, none of the progeny synthesized much more penicillin than 1 µg ml⁻¹. The absence of Npe⁺ strains demonstrated that the natural isolates were all defective at the *npeA* locus but did not show that this was the only defect in these strains. However, in outcrosses between these strains and strains carrying *npeB*, *C* or *D* mutations one-quarter of the progeny produced about 4 µg ml⁻¹. Thus the mutation at *npeA* was the sole cause of impaired penicillin synthesis in all the natural non-producers tested.

From the cross 49/15 (h-cF) × 143 (h-cG), about half of 300 progeny tested by the rapid screening method produced very low levels of penicillin whereas the remainder had no detectable antibiotic activity. Eighty-five of the progeny were assayed after growth in shake flasks: 50 produced no detectable penicillin, and the other 35 were uniform and all produced a trace of penicillin ($\chi^2_{0.1}$ for deviation from 1:1 = 2.28, $P > 0.1$). The mean titre of the 35 penicillin-producing segregants (0.69 ± 0.22 µg ml⁻¹) was not significantly different from that

Table 1. *Strains of Aspergillus nidulans used*

Domestic code	Known genotype	h-c group	Penicillin titre ($\mu\text{g ml}^{-1}$)	Origin
49/14	<i>meth-08; w-012</i>	F	0	Natural isolates or marked derivatives of natural isolates
49/15	<i>y-08; an-02</i>	F	0	
113/4	<i>an-001</i>	Unknown	0	
118/5	<i>ribo-02; npe-04</i>	A	0	
118/6	<i>ribo-02; npe-04; y-010</i>	A	0	
143	wild type	G	< 1*	
143/6	<i>w-044; nic-06</i>	G	< 1*	Derivatives of strain NRRL194 (penicillin titre 3 $\mu\text{g ml}^{-1}$)
G24	<i>biA1; npeA01; y-06</i>	Glasgow†	0	
G38	<i>suAadE20 yA2 adE20; acrA1; galA1; pyroA4; facA303; sB3 npeA02; nicB8; riboB2</i>	Glasgow†	0	
G39	<i>suAadE20 yA2 adE20; acrA1; galA1; pyroA4; facA303; sB3 npeA03; nicB8; riboB2</i>	Glasgow†	0	
GH80	<i>yA2; pyroA4; cnxA5; npeA0023</i>	Glasgow†	0	
GH117	<i>biA1; methG1; w-005; npeDoo45</i>	Glasgow†	0	
GH119	<i>biA1; wA3; npeCoo7</i>	Glasgow†	0	
GH124	<i>biA1; wA3; cnxA5; npeBoo6</i>	Glasgow†	< 1	

* In earlier experiments these strains showed no penicillin production (Holt & Macdonald, 1968a). Minor improvements in the fermentation medium probably account for the low level seen here.

† Incompatible with all known h-c groups.

Table 2. *Details of sexual crosses and segregation of impaired penicillin production*

Cross	Method of recombinant selection*	No. of progeny tested	Npe:Npe ⁺	$\chi^2_{1d.f.}$	Deviation from 3:1†
h-cF (49/14) $\times$ <i>npeA01</i> (G24)	A	150	150:0	—	S
h-cF (49/14) $\times$ <i>npeA02</i> (G38)	A	150	150:0	—	S
h-cF (49/14) $\times$ <i>npeA03</i> (G39)	A	150	150:0	—	S
h-cF (49/14) $\times$ <i>npe-04</i> (118/6)	A	200	200:0	—	S
h-cF (49/15) $\times$ <i>npeDoo45</i> (GH117)	C	194	139:55	1.16	NS ($P > 0.1$)
h-cF (49/15) $\times$ h-cG (143)	C	300	300:0	—	S
h-cG (143) $\times$ <i>npeA03</i> (G39)	C	200	200:0	—	S
h-cG (143) $\times$ <i>npeBoo6</i> (GH124)	C	193	144:49	0.01	NS ($P > 0.1$)
h-cG (143) $\times$ <i>npeCoo7</i> (GH119)	C	190	134:56	2.02	NS ($P > 0.1$)
h-cG (143) $\times$ <i>npeDoo45</i> (GH117)	C	189	129:60	4.58	NS ($P 0.05-0.02$)
h-cG (143/6) $\times$ <i>npe-04</i> (118/5)	C	189	189:0	—	S
113/4 $\times$ <i>npeA0023</i> (GH80)	B	192	192:0	—	S

* See Methods for details.

† S, Significant ($P < 0.001$); NS, not significant.

of strain 143 ($0.71 \pm 0.12 \mu\text{g ml}^{-1}$). It was concluded that the *npe* mutations in both parents were allelic but that the one carried by strain 143 was leaky. Simpson, Croft & Caten (personal communication) crossed a different member of h-c group F (strain 136), which had a negligible penicillin titre, with strain 109 (h-c group H), which had a penicillin titre of $8.6 \mu\text{g ml}^{-1}$. Of 50 progeny, 19 had negligible titres and the others had titres similar to that of strain 109. This bimodal distribution indicated that the primary difference between the strains was at a single locus, in agreement with the present finding.

The tests of allelism described here between natural isolates and laboratory mutants impaired in penicillin yield suggest that the failure of certain wild-type strains to produce the antibiotic was due to their carrying a spontaneous mutation at the *npeA* locus. Rather

than being randomly distributed among h-c groups, all but one of the impaired natural isolates were members of only two h-c groups, in which all of the strains examined produced little or no penicillin (Holt & Macdonald, 1968*a*). One explanation of this is a single gene with pleiotropic effects. A mutation at one of the loci determining heterokaryon compatibility (Caten, Butcher & Croft, 1972) may have impaired penicillin synthesis, or mutation at the *npeA* locus may have altered heterokaryon compatibility. The frequent occurrence of mutations at the *npeA* locus among wild-type and mutagen-treated strains, and therefore the likelihood that this gene plays an important role in penicillin biosynthesis in *A. nidulans*, is being investigated further.

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