SHORT COMMUNICATION

Intergeneric Cosynthesis of Penicillin by Strains of *Penicillium chrysogenum*, *P. chrysogenum/notatum* and *Aspergillus nidulans*

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A number of mutants impaired in penicillin production have previously been isolated from *Penicillium chrysogenum* and *Aspergillus nidulans*. During cofermentation of osmotically fragile mycelia derived from these strains, in the presence of inhibitors of cell wall regeneration, intergeneric cosynthesis has been demonstrated between mutants which are probably impaired in different parts of the penicillin biosynthetic pathway.

INTRODUCTION

There are several ways in which mutants impaired in antibiotic production can be used to obtain information on the production process. Genetic techniques can be used to identify the number of loci affecting biosynthesis, while biochemical investigations of the accumulation or utilization of postulated intermediates can define the steps involved in the pathway. A combination of both approaches can be used to demonstrate control processes associated with penicillin biosynthesis. In this way information has been gained about the production of penicillin by *Aspergillus nidulans* and *Penicillium chrysogenum*. The generally higher penicillin titre of *P. chrysogenum* makes it the preferred organism for biochemical investigations, while the extensive knowledge of the genetics of *A. nidulans* and the fact that it possesses a sexual cycle makes it more suitable for genetic studies (Macdonald & Holt, 1976). Cosynthesis between two different non-producer mutants could occur when the mutants are cofermented under suitable conditions such that an intermediate produced by one mutant is used as a penicillin precursor by the other mutant. While earlier attempts to achieve cosynthesis of penicillin have failed (Bonner, 1947; Normansell *et al.*, 1979), we have recently reported a procedure using osmotically fragile mycelia to demonstrate cosynthesis by certain paired combinations of mutants derived from *A. nidulans* (Makins *et al.*, 1980). This technique depends for its success on the treatment of mycelia with cell wall lytic enzymes and subsequent culture in osmotically buffered media containing inhibitors of cell wall regeneration. The present study shows that the same procedure, with some modifications, allows the cosynthesis of penicillin by some pairs of mutants derived from *P. chrysogenum*, *A. nidulans* and *P. chrysogenum/notatum*.

Mutants of *A. nidulans* and *P. chrysogenum* altered in penicillin production have been isolated and assigned to complementation groups and the mutations have been genetically mapped (Normansell *et al.*, 1979; C. Blake, unpublished; Holt *et al.*, 1976; Ditchburn *et al.*, 1976; J. F. Makins, unpublished). Investigations of non-producer mutants have revealed the existence of four complementation groups in *A. nidulans* (A, B, C and D) and five in *P. chrysogenum* (V, W, X, Y and Z) (Edwards *et al.*, 1974; Normansell *et al.*, 1979). Most (9/15) of the non-producer (Npe) mutants of *P. chrysogenum* isolated were members of

Fig. 1. The biosynthesis of penicillin and stages in the pathway at which some mutations impairing production are thought to act. [*Results from Normansell *et al.* (1979). †Unpublished results of J. F. Makins.]

complementation group Y while 20/28 mutants of *A. nidulans* belonged to the group A. Certain wild isolates of *A. nidulans* with negligible penicillin titres are also apparently impaired in production at the *npeA* locus (Cole *et al.*, 1976). More recently, biochemical investigations have shown that mutants of *P. chrysogenum* bearing *npeY* are unable to produce the tripeptide α-aminoadipoylcysteinylvaline (ACV) (Normansell *et al.*, 1979), and that strains carrying the *npeA* mutation are able to produce penicillin when supplied with ACV (J. F. Makins, unpublished). Hence, the suggestion has been made that *npeA* and *npeY* mutations representing the complementation groups most frequently encountered in both organisms affect the same step in penicillin production, namely, the formation of ACV tripeptide. Figure 1 shows the generally accepted biosynthetic pathway for penicillin and the various steps in this pathway which are affected by *npe* mutations.

In addition to the above mutants the present study also included a penicillin non-producing mutant (P56) derived from a commercial, high titre strain of *P. chrysogenum*, as well as two non-producing *P. chrysogenum/notatum* isolates (DF3 and DF7).

METHODS

Strains. Details of the origin and full genotypes of the strains used in these experiments are given in the references: *A. nidulans* strains designated GH are described in Edwards *et al.* (1974), *P. chrysogenum* strains designated HP in Normansell *et al.* (1979) and *A. nidulans* strain M5 in Cole *et al.* (1976) (where it is designated 118/5). The *A. nidulans* strain S1 is a riboflavin-requiring derivative of a natural isolate (no. 49 of the Birmingham collection; Grindle, 1963) impaired in penicillin production at the *npeA* locus (Cole *et al.*, 1976). The *P. chrysogenum* strain P56 is a penicillin non-producing mutant of a high titre industrial strain (the latter kindly provided by Dr Lein, Panlabs). The collection of strains from which the non-producing *P. chrysogenum/notatum* isolates DF3 and DF7 (kindly provided by the late Dr D. Fennel) were taken is described in Raper *et al.* (1944). It has been suggested that these *P. chrysogenum/notatum* isolates and *P. chrysogenum* are members of the same species (A. Onions, personal communication).

Cosynthesis tests. The procedure was essentially that described previously (Makins *et al.*, 1980). Mycelium of each strain was harvested after 3 d growth in penicillin production medium (PPM) and treated with a mixture of cell wall lytic enzymes to render it osmotically fragile. Mixtures of mycelia representing all possible paired combinations of strains were grown in osmotically buffered (1 M-KCl) PPM containing polyoxins (2.5 g l⁻¹) and 2-deoxyglucose (0.2 g l⁻¹). After 5 d shaken culture at 25 °C the filtrates were assayed for penicillin content by bioassay (Brownlee *et al.*, 1949).

RESULTS AND DISCUSSION

The nature of the impairments in a selection of strains was investigated by cosynthesis experiments. The average penicillin titres of each strain after growth as osmotically fragile mycelium in osmotically buffered PPM containing polyoxins and 2-deoxyglucose are listed in

Table 1. *Cosynthesis of penicillin by pairs of mutant strains*

The lower left section shows the penicillin titres in benzylpenicillin equivalent units ml^{-1} , and the upper right section shows (as + or -) whether or not the value is evidence of statistically significant production. The italicized numbers on the diagonal are the titre of each strain grown alone under the same conditions. All values shown are the average for at least three flasks.

<i>A. nidulans</i>							<i>P. chrysogenum</i>					<i>P. chrysogenum/notatum</i>		
S1	M5	GH79	GH49	GH50	GH117		HP83	HP144	HP154	HP96	HP78	P56	DF3	DF7
<i>npeA</i>	<i>npeA</i>	<i>npeA</i>	<i>npeB</i>	<i>npeC</i>	<i>npeD</i>		<i>npeV</i>	<i>npeW</i>	<i>npeX</i>	<i>npeY</i>	<i>npeZ</i>	<i>npe002</i>	<i>npe003</i>	<i>npe007</i>
<i>A. nidulans</i>	<i>npeA</i>	0.1	—	+	+	+	+	+	+	—	+	—	—	—
	<i>npeA</i>	0.0	—	+	+	+	+	+	+	—	+	—	—	—
	<i>npeA</i>	0.0	0.2	+	+	+	+	+	+	—	+	—	—	—
	<i>npeB</i>	1.8	2.0	1.0	0.0	—	—	+	—	—	—	+	+	+
	<i>npeC</i>	2.4	2.8	2.4	0.4	0.3	—	+	—	+	—	+	+	+
<i>P. chrysogenum</i>	<i>npeD</i>	2.8	2.8	3.5	0.4	0.0	0.2	—	+	+	—	+	+	+
	<i>npeV</i>	4.4	8.1	7.1	0.1	0.0	5.1	0.2	—	+	+	+	+	+
	<i>npeW</i>	6.8	4.1	9.5	4.1	4.2	0.8	1.5	1.1	+	—	+	+	+
	<i>npeX</i>	5.7	4.1	4.1	0.0	0.1	0.4	0.2	7.8	0.1	+	+	+	+
	<i>npeY</i>	0.0	0.0	0.1	0.8	6.0	5.6	3.8	8.8	4.6	0.0	—	—	—
<i>P. chrysogenum/notatum</i>	<i>npeZ</i>	5.1	5.1	4.1	0.0	0.2	0.1	6.0	0.0	4.6	0.0	0.0	+	+
	<i>npe002</i>	0.0	0.1	0.0	9.0	8.5	9.5	4.2	8.2	3.4	0.0	9.0	0.0	—
	<i>npe003</i>	0.0	0.0	0.0	3.4	2.4	4.1	4.2	5.1	5.2	0.0	3.4	0.0	—
	<i>npe007</i>	0.0	0.0	0.0	2.9	2.8	4.0	4.2	6.0	6.0	0.0	3.8	0.0	0.0

Table 1 (diagonal, italicized figures). The characteristic penicillin titres of the untreated parents of the strains are: for *A. nidulans* strains designated GH, 5 units ml⁻¹, or designated M, 12 units ml⁻¹; for strains of *P. chrysogenum* designated HP, 50 units ml⁻¹, or designated P, 5000 units ml⁻¹ (1 unit = 0.6 µg benzylpenicillin). After mixed culture of all possible paired combinations of the Npe strains as osmotically fragile mycelia, the filtrates were examined for penicillin content by bioassay (Table 1).

Some combinations of strains of the same species that complement in heterozygous diploids do not exhibit cosynthesis in mixed culture. This may be due to interference by regulatory mechanisms or insufficient quantities of a particular intermediate produced. These results suggest that the failure of cosynthesis alone is not sufficient evidence to classify two mutations as non-complementary. Conversely, however, where cosynthesis does occur it is clear that the individual *npe* mutations involved must affect different steps in the biosynthetic pathway. The lack of cosynthesis by any combination of strains bearing *npeA*, *npeY*, *npe002*, *npe003* or *npe007* together with their patterns of production in combination with strains bearing other *npe* mutations, suggests that these genes affect the same step in penicillin biosynthesis. As both *npeA* and *npeY* have been implicated in the formation of ACV tripeptide, this could indicate that the mutants P56 (*npe002*), DF3 (*npe003*) and DF7 (*npe007*) are similarly impaired in this reaction.

Biochemical studies have not so far characterized the nature of the impairments to penicillin synthesis resulting from the mutations *npeB* or *npeD* in *A. nidulans*. The patterns of complementation shown by both diploid analysis and cofermentation suggest that strains bearing *npeB* are competent in the cyclization of ACV tripeptide into isopenicillin N, whereas *npeD* mutants may be impaired in this function.

No cosynthesis was observed between strains bearing *npeC*, *npeV* and *npeX*. All of these mutations have been associated with altered acyl exchange activities (Normansell *et al.*, 1979; J. F. Makins, unpublished).

We assume that where cosynthesis occurs it results from the diffusion of intermediates from one strain to another. We have investigated other possibilities such as the formation of heterokaryons or the reversion of *npe* mutations and have not found any evidence to support these alternative explanations. It is possible that the suggested diffusion occurs in this system, but not in normal mixed cultures, because the enzymic treatment produces areas of discontinuity of cell wall structure which in the presence of polyoxin and 2-deoxyglucose are maintained. It is suggested that these areas are permeable to the intermediates of penicillin biosynthesis.

The cosynthesis of penicillin found in these experiments suggests that many of the steps of penicillin production are similar if not the same in the two genera studied. Differences in regulatory mechanisms may, however, occur between organisms.

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