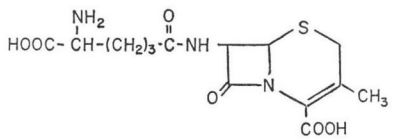
Communications to the editor

THE OCCURRENCE OF DEACETOXYCEPHALOSPORIN C IN FUNGI AND STREPTOMYCETES

Sir:

The production of β-lactam antibiotics with a cephalosporin nucleus has been associated with either the cephalosporin C-producing genus, *Cephalosporium*[1] along with its sexual stage *Emericellopsis*[2], or the actinomycete genus, *Streptomyces*, which produces the 7-methoxycephalosporins[3,4]. Penicillin N, having the penicillin nucleus, is produced also by *Cephalosporium*[5] and by *Streptomyces*[3,6]. Another cephalosporin, identified as deacetylcephalosporin C possessing a D-α-aminoadipoyl side chain at C-7, was recently reported from a *Cephalosporium* species[7]. Other penicillins which commonly are synthesized by *Penicillium* have not been reported from the above two genera.

Fig. 1. Structure of deacetoxycephalosporin C



The possibility that penicillin N is associated with the production of cephalosporin compounds prompted a study in our laboratories to screen for new cephalosporins from penicillin N-producing cultures in our collection. As a result of this program, deacetoxycephalosporin C (Fig. 1) was obtained from several strains of fungi and two species of *Streptomyces*. The majority of these organisms also produce deacetylcephalosporin C, cephalosporin C and occasionally the lactone of deacetylcephalosporin C. Deacetoxycephalosporin C may be used in the preparation of other cephalosporin antibiotics. Chemical deacylation removes the aminoadipoyl side chain to yield 7-aminodeacetoxycephalosporanic acid (7-ADCA). The 7-ADCA can be re-acylated with various agents, for example phenylglycyl derivatives, to produce new antibiotics such as cephalexin. Deacetoxycephalosporin C was produced by 22 strains of fungi and 2 streptomycetes from our collection. These cultures represent the genera *Cephalosporium*, *Emericellopsis*, *Scopulariopsis*, *Paecilomyces*, *Diheterospora* and *Streptomyces*.

Cultures were grown on a variety of shake-flask media. The formulation of the vegetative medium and one of the most productive

Table 1. Vegetative and fermentation media for production of deacetoxycephalosporin C by fungi

	Component	g/liter
Vegetative medium pH 6.5	Peanut meal	20.0
	Malt extract	20.0
	Corn steep liquor	5.0
	$MgSO_4 \cdot 7H_2O$	0.25
	KH_2PO_4	1.0
	K_2HPO_4	0.5
	$CaCl_2 \cdot 2H_2O$	0.1
Fermentation medium pH 6.5	Sucrose	40.0
	Glycerol	10.0
	Na-Glutamate	5.0
	Peanut meal	20.0
	$(NH_4)_2(SO_4)_2 \cdot 6H_2O$	3.0
	KNO_3	20.0
	$CaCO_3$	3.0

Table 2. Sporulation, vegetative, and fermentation media for production of deacetoxycephalosporin C by *Streptomyces lipmanii*

	Component	g/liter
Sporulation medium pH 6.5	Tomato paste	20
	Baby oatmeal	20
	Washed agar	25
Vegetative medium pH 6.7	Glucose	5
	Dextrin 700	10
	Tryptone	5
	Yeast extract	5
	$MgSO_4 \cdot 7H_2O$	2
Fermentation medium pH 6.0	Dextrin 700	55
	Soybean grits	40
	Molasses	20
	NaH_2PO_4	1.3
	KCl	1

screening media for fungi are presented in Table 1. Fungal cultures were maintained on a vegetable juice-agar medium. Sporulation and vegetative media as well as a typical fermentation medium, for *Streptomyces lipmanii* are shown in Table 2. The media and growth conditions for *Streptomyces clavuligerus* have been published previously[8]. All cultures were grown at 25°C except *S. clavuligerus* which was grown at 30°C.

Antibiotic activity in fermentation broths was detected by a disc-plate agar diffusion technique. Strains of either *Bacillus subtilis*, *Salmonella gallinarum* or *Pseudomonas solanacearum* were used as assay organisms.

Initial detection and identification of deacetoxycephalosporin C was performed with paper chromatography. The principal method employed Whatman #1 paper in a descending system in which the solvent front is run off the paper. The developing solvent was acetonitrile - water (4 : 1). A bioautograph was obtained with *Pseudomonas solanacearum* as the test organism. An Rf of 1.0 was arbitrarily assigned to cephalosporin C. In this system the Rf value for deacetoxycephalosporin C is 0.72, while for deacetylcephalosporin C it is 0.55. Penicillinase was incorporated into the agar to inactivate penicillin N.

Deacetoxycephalosporin C was isolated from the fermentation broth by a series of chromatographic procedures. The broth was adjusted to pH 2.0 and allowed to stand for one hour at room temperature to inactivate acid-labile antibiotics. After readjustment of the pH to 6.0, the broth was filtered and the filtrate was extracted with *n*-butanol to remove impurities. The aqueous phase was concentrated *in vacuo* to remove the butanol and the resulting concentrate was applied to an activated carbon column. After a water wash, the activity was eluted with 50 % acetone. The elution was monitored with thin-layer and paper chromatography. The active fractions were combined and concentrated *in vacuo*. The concentrate was chromatographed on a column of anion-exchange resin (Amberlite IRA 68-Acetate cycle), and the column was developed with 0.15 N aqueous sodium acetate. Active fractions were combined and applied to an activated carbon column. After the column was washed with water, the activity was eluted with 50 % aqueous acetone. The active fractions were dried, yielding crude deacetoxycephalosporin C, sodium salt. This material was further purified by chromatography on a cellulose column developed with acetonitrile - water (4 : 1). Active fractions were pooled, dried, dissolved in a minimum volume of isopropanol, and poured into 20 × volumes of ether. The sodium salt of deacetoxycephalosporin C formed as a precipitate. Excellent agreement of physical-chemical properties was obtained between this material and an authentic sample prepared from cephalosporin C by hydrogenolysis[9].

Table 3. Deacetoxycephalosporin C-producing fungi and actinomycetes

Organism	Culture number
Cephalosporium chrysogenum	ATCC 14615
Cephalosporium sp.	NRRL 5445
Cephalosporium sp.	NRRL 5712
Cephalosporium sp.	NRRL 5716
Cephalosporium sp.	NRRL 5718
Cephalosporium sp.	NRRL 5719
Cephalosporium sp.	NRRL 5720
Cephalosporium sp.	NRRL 5721
Cephalosporium sp.	NRRL 5722
Cephalosporium sp.	NRRL 5723
Cephalosporium sp.	NRRL 5724
Cephalosporium sp.	NRRL 5725
Emericellopsis sp.	NRRL 5446
Emericellopsis sp.	NRRL 5447
Emericellopsis sp.	NRRL 5713
Emericellopsis sp.	NRRL 5714
Emericellopsis sp.	NRRL 5717
Paecilomyces carneus	ATCC 16329
Paecilomyces carneus	NRRL 2622
Paecilomyces carneus	NRRL 5711
Diheterospora chlamydosporia	NRRL 5728
Scopulariopsis sp.	NRRL 5715
Streptomyces lipmanii	NRRL 3584
Streptomyces clavuligerus	NRRL 3585

The cultures producing deacetoxycephalosporin C are listed in Table 3. More than 50 % of our penicillin N-producing microorganisms also produced deacetoxycephalosporin C. Remaining strains, which did not appear to produce deacetoxycephalosporin C under the conditions described, may synthesize

the antibiotic under more appropriate conditions, or its presence might be detected under conditions described if a more sensitive assay was used.

The microorganisms listed in Table 3 other than *Streptomyces* and *Emericellopsis*, the perfect stage of some *Cephalosporium* strains, are genera in the Moniliaceae of the Fungi Imperfecti.

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