NEW ANTHRACYCLINE GLYCOSIDES FROM *MICROMONOSPORA*

I. DESCRIPTION OF THE PRODUCING STRAIN

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During the course of successive mutagenic treatments of *Streptomyces peucetius* var. *caesius*, producer of anthracyclines, a novel mutant was isolated from a plate of the surviving population. This mutant showed remarkable morphological, cultural and biochemical differences when compared to the original strain. The new culture produced four new glycosides of a novel class within the group of the daunorubicin related anthracyclines, two of which were also present in the original strain.

To our surprise the taxonomical study carried out on this mutant allowed its assignment to the genus *Micromonospora* ØRSKOV (1923). The possibilities of whether this new strain has originated from a contamination or from a mutation are discussed. The type strain is *Micromonospora* sp. strain B 211 F.I. (=ATCC 31366; DSM 1190; FRI 4363).

Streptomyces peucetius, the daunorubicin producing microorganism[1,2], is characterized by a considerable morphological and cultural variability, as well as by a remarkable biosynthetic versatility. These correlated properties have been exploited through induced mutagenesis in order to obtain mutants capable of producing new antitumor anthracyclines. Examples along this line of investigation are the isolation of doxorubicin from *S. peucetius* var. *caesius*[3], of 13-dihydrodaunorubicin together with daunosaminyldaunorubicin from *S. peucetius* var. *carneus*[4] and of 13-dihydrocarminomycin I, from a mutant of *S. peucetius* labelled B 441 F.I.[5].

Following further this line of investigation, a complex of new anthracyclines has been recently isolated from cultural broths of a new strain labelled B 211 F.I.[6]. The taxonomical examination carried out on this mutant showed that it belongs to the genus *Micromonospora*[7]. In the present paper the results of the taxonomical study of this microorganism are reported.

Materials and Methods

Microorganism: *Streptomyces peucetius* var. *caesius* 106 F.I. (IMRU 3920; IMI 131502).

Mutagenic treatments: N-Methyl-N′-nitro-N-nitrosoguanidine (NTG) was employed according to DELIC *et al.* 1970[8]. Combined treatments using NTG and heat were also applied. In this case the treatment of the spore suspension with NTG at a concentration of 500 mcg/ml was made at 50°C for 45 minutes.

Macroscopic observations were made on cultures grown for several weeks at 27°C on the media listed in Table 1 and reported by WAKSMAN (1961)[9] unless otherwise specified.

Morphological studies were performed on liquid shaken cultures grown on any one of the media listed in Table 1 as they provided usually a better material for examination of hyphae and spores than the same cultures grown on the corresponding solid media.

The carbohydrate utilization properties were studied using the medium reported by PRIDHAM and

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GOTTLIEB[10], while the physiological properties were studied on the media reported by WAKSMAN[9].

The biochemical characteristics of the cell wall were determined at the Institut für Mikrobiologie, Technische Hochschule, Darmstadt (West Germany) according to the methods described by M. P. LECHEVALIER and H. LECHEVALIER[11] and by KROPPENSTEDT and KUTZNER[12].

Results and Discussion

Strain 106 F.I. of *Streptomyces peucetius* was subjected to a mutagenic treatment with NTG. Among the survivors a biochemical mutant was isolated and labelled 416 F.I. The main features of this strain were a yellow color of the soluble pigment and the production of new anthracycline glycosides. Recently a report has been made on mutants of the known anthracycline producing microorganism *Streptomyces coeruleorubidus* forming a yellow coloured pigment and from the fermentation broths of which yellow anthracyclinones have been isolated[13].

Strain 416 F.I. in turn, was subjected to a mutagenic treatment with NTG and heat and, this time, a strain with different morphological characteristics was isolated. This strain was designated B 211 F.I. and examined for its anthracycline production and its taxonomic characterization.

Description of Strain B 211 F.I.

Antibiotic production:

Strain B 211 F.I. was grown in shaken flasks in a medium containing (g/liter): glucose, 60; brewer's dry yeast, 30; NaCl, 2; KH_2PO_4, 1; $CaCO_3$, 2; $MgSO_4 \cdot 7H_2O$, 0.1; $FeSO_4 \cdot 7H_2O$, 0.001; $ZnSO_4 \cdot 7H_2O$, 0.001; $CuSO_4 \cdot 5H_2O$, 0.001; tap water, up to 1,000 ml. Under these conditions, the new strain produced four anthracycline glycosides designated A, B, C and D. Two of these glycosides, namely A and C along with small amounts of daunorubicin and doxorubicin, were also present in cultures of the parent strain 416 F.I. The isolation procedure and the physicochemical and biological properties of these four anthracyclines are reported in the accompanying paper[6].

S. peucetius produces, besides the anthracyclines, antifungal polyene antibiotics of the tetraene and pentaene type.[1,2] A chemical and biological investigation carried out on the culture broth of the strain B 211 F.I., demonstrated that this strain also possessed the ability to produce these same compounds.

Morphological properties:

Spores are of the following size: $0.9 \sim 1.1 \times 1.1 \sim 1.6\ \mu$. They are formed singly or very frequently in pairs (Fig. 1), rarely as clusters, terminally on short sporophores, arising mono-

Fig. 1. Photomicrograph of sporulated mycelium of strain B 211 F.I. grown for 10 days in liquid shaken glucose-yeast extract medium at 27°C. (×1,000)

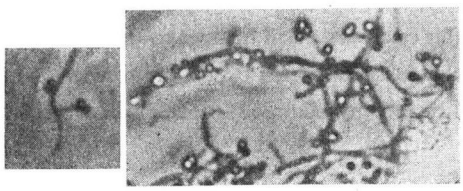

Fig. 2. Scanning electronmicrograph of spores of strain B 211 F.I. grown for 10 days in liquid shaken glucose-yeast extract medium at 27°C. (×14,200)

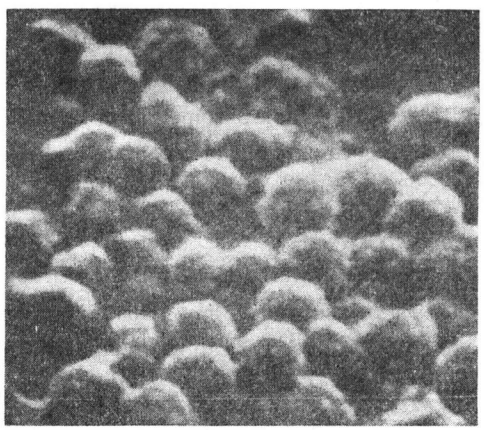

Table 1. Cultural characteristics of strain B 211 F. I. on various media.[9]

Medium	Response
BENNETT's agar	Growth good, raised and ridged, colour orange to terra-cotta or coral red, turning to black on aging.
EMERSON's agar	Growth fair, raised and ridged. Colourless to light terra-cotta throughout turning brown on aging.
Glucose-asparagine agar	Growth good, flat, colour light terra-cotta throughout.
1 % NZ-amine type A, 1 % glucose+agar[16]	Growth good, raised and ridged. Colour from orange terra-cotta to bright red, turning brown to black on aging.
Glucose-yeast extract agar	Growth poor, raised and ridged. Colour light terra-cotta throughout.
Starch-casein agar	Growth good, flat. Colour terra-cotta throughout.
Gelatin agar	Growth poor, raised and ridged. Colour terra-cotta to bright red.
Tyrosine agar[10]	Growth good, raised and ridged. Bright terra-cotta colour at first, deep reddish turning copper brown to almost black, on aging. A deep brown soluble pigment is produced.
Yeast extract-L-tyrosine agar	Growth fair, flat. Colour brown; a deep reddish to coffee brown soluble pigment is produced.
Peptone-iron agar[18]	Growth good, raised and ridged. Colour light terra-cotta throughout.
Potato-glucose agar	Growth moderate, raised and ridged. Colour from terra-cotta to brown turning almost black on aging.
CZAPEK's agar	Growth poor, flat. Colour light terra-cotta turning black on aging.
Glycerol-glycine agar	Growth moderate, flat. At first colourless, turning olive green to deep green on aging.
Inorganic-salts-starch agar[10]	Growth fair, flat. Colour from rose to terra-cotta turning orange brown on aging.
Glycerol-asparagine agar	Growth poor, flat. Colour light terra-cotta turning almost black on aging.

podially as branches on long, randomly produced hyphae 0.5~0.9 μ in thickness. Sessile spores are rarely observed. The spores are nearly spherical (Fig. 2). No polymorphic forms are seen. Aerial mycelium is absent.

Cultural properties:

The cultural characteristics of strain B 211 F.I. are given in Table 1. Growth is generally good on organic media, less on synthetic ones. On the former it is usually raised and ridged, rather moist but not slimy in appearance. On aging and sporulation the orange-terra-cotta coloured substrate mycelium turns brown to almost black. Generally no soluble pigment is produced. No growth is observed at a temperature above 40°C.

Physiological and biochemical properties:

The carbon utilization pattern as well as the physiological properties of strain B 211 F.I. are shown in Table 2. In Table 3 comparative data of the biochemical properties of strain B 211 F.I. and of *S. peucetius* var. *caesius* are reported and concern the type of diaminopimelic acid (DAP), the sugar composition and the fatty acid spectrum of the cell wall.

Identification of Strain B 211 F.I.

The main features of strain B 211 F.I. suggest that this microorganism belongs to the genus *Micromonospora* ØRSKOV (1923)[14]. This conclusion is unambiguously supported by, (1) the absence of aerial mycelium, (2) the fructification structures borne on the substrate mycelium, (3) the cell wall composition pattern.

A careful comparison of the characters reported by WAKSMAN[9,15], LUEDEMANN and BRODSKY[16]

and LUEDEMANN[17] for the known species belonging to this genus with those shown by strain B 211 F.I., did not allow us to identify our strain with any of the known species of this genus. Further studies on this line are in progress.

Table 2. Physiological properties of strain B 211 F. I.

Utilization of:	glucose	+
〃	sucrose	+
〃	D-xylose	+
〃	mannitol	+
〃	inositol	+
〃	L-arabinose	+
〃	D-fructose	+
〃	adonitol	+
〃	lactose	+
〃	*d*(+)mannose	+
〃	maltose	+
〃	raffinose	+
〃	L-rhamnose	+
〃	alpha-alpha-trehalose	+
〃	esculin	+
〃	glycerol	+
〃	Na-citrate	+
〃	NH_4-succinate	+
〃	Na-acetate	+
〃	NH_4-tartrate	+
〃	glycogen	+
〃	paraffin	−
Negative control		−
Liquefaction of gelatin		+
Tyrosine decomposition		+
Melanin formation		+
Hydrolysis of starch		+
H_2S formation		−
Nitrate reduction		+
Milk (peptonization and coagulation)		+
Antibiotics produced:	new anthracyclines of the daunorubicin group and antifungal antibiotics of the polyene type.	

+: positive reaction, −: negative reaction.

Discussion

During the course of a mutagenic program carried on *S. peucetius* var. *caesius*, producer of anthracyclines and polyene antibiotics, a microorganism was isolated which produced, along with the polyene antibiotics, four hitherto undescribed anthracyclines two of which are also produced by strain 416 F.I. This microorganism was found to belong to the genus *Micromonospora* and labelled strain B 211 F.I.

Anthracyclines have so far mainly been isolated from *Streptomyces* but also from *Actinomadura*[20] and *Streptosporangium*[21,22], whereas polyene antibiotics have been found in the genera *Streptomyces* and *Streptoverticillium*. To our knowledge, the present report is the first on the production of anthracyclines as well as of polyene antibiotics by a microorganism of the genus *Micromonospora*.

About the origin of strain B 211 F.I. some alternative hypotheses can be set forth. The first one might be that strain B 211 F.I. is not derived from *S. peucetius* but is simply the result of a contamination. Against this possibility however is the fact that the supposed contaminant should have been a strain producing the very same antibiotics as found in *S. peucetius* cultures, to say nothing about the particular type of anthracyclines produced which, as it is known, are very rare in microorganisms.

Another possibility might be that the new strain would be the result of a contamination followed by some kind of genetic transformation. In this latter case it could be supposed that an air-borne *Micromonospora* contaminated the culture of *S. peucetius* and that transferred to the *Micromonospora* some genetic determinant, like a plasmid, supporting the production of anthracyclines. The possible involvement of episomic factors in antibiotic production has been reported[27,28]. This hypothesis is however unlikely because strain B 211 F.I. produces, as does *S. peucetius*, two different classes of antibiotics, namely anthracyclines and polyenes, and it is hard to believe that their production is supported by a single plasmid or alternatively that a transfer of two kinds of plasmids contemporaneously occurred.

Finally, the hypothesis that strain B 211 F.I. could have originated through mutation from *S. peucetius* might be considered. This hypothesis is strengthened by the knowledge that the genera *Streptomyces* and *Micromonospora* are phylogenetically correlated[23~26]. On the other hand it is also known that morphological characters in the Streptomycetes are frequently codified on extrachromosomal

Table 3. Comparison of major cell wall constituents of *S. peucetius* and *Micromonospora* sp. strain B 211 F. I.

Strains	Cell wall composition						
	Diamino acid pattern		Sugar pattern		Fatty acid pattern		
	DAP	Type	Constituents	Type	Iso and ante-iso branched	Unsaturated	10-methyl branched
Streptomyces peucetius var. *caesius*	LL	I	only traces of different sugars	C	+	−	−
Micromonospora sp. strain B 211 F. I.	DL (*meso*) and hydroxy	II	xylose, ribose, glucose, galactose, arabinose	D	(+)	(+)	+

elements[27,28] and that they can be easily removed by heat treatment.

The data presently in hand, are however, not sufficient to decide which of the hypotheses put forward is the right one. Further studies with this aim are in progress in our laboratories and in other scientific institutions.

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