Pakistan J. Sci. Ind. Res., Vol. 14, No. 6, December 1971

STREPTOMYCES SPECIES PRODUCING ACTINOMYCIN C

ABOU-ZEID A. ABOU-ZEID and YOUSEF M. SHEHATA

Microbiological and Enzyme Chemistry Research Unit, National Research Centre, Dokki, Cairo, U.A.R.

(Received November 9, 1970)

Taxonomic studies on S. sp. NRC-152 which was isolated from Egyptian soil were carried out. Its morphological and physiological characteristic features were identical with *S. chrysomallus*. The antibiotic produced was extracted and purified from the fermentation cultures and the mycelial mats of the organism. On the basis of physicochemical and biological properties, the antibiotic was identified as actinomycin C.

Streptomyces sp. NRC—152 was isolated from Egyptian soils collected in National Research Centre.[9] Taxonomic studies on Streptomyces sp. NRC—152 was investigated. The antibiotic substance was extracted and purified from the fermentation broth and mycelia. Chemical and biochemical studies on the antibiotic substance revealed that it is actinomycin C.

Identification of the Organism

Streptomyces sp. NRC–152 generally grows at 27–30°C, and the experiments were carried out at 28°C. It shows different colours on different nutritive media as shown in Table 1. The organism produces branching hyphae and the sporophores are thin, straight, long, arranged somewhat in clusters. No spirals are formed It forms aerial mycelia which are variable in colour according to the nature of the constituents of the culture media. The spores are spherical. It produces soluble pigments and the colour of these pigments are pale yellow to deep orange yellow on most of the utilizable media. The organism belongs to the nonchromogenic type. It liquefies gelatin, coagulates and peptonizes milk. It grows well on most of the experimental media except sucrose nitrate agar, starch agar and egg albumen agar. The organism utilizes sucrose, maltose, lactose, glucose, galactose, mannitol, mannose, arabinose, and cannot utilize raffinose and cellulose.[10]

From the published descriptions of actinomycins producing microorganisms, [1,2,5,6,8,11–14] the results of the morphological and physiological characteristics of S. sp. NRC-152 suggest that it may be identical with *S. chrysomallus S. parvus* or S. *citreofluorescens*. S. sp. NRC-152 is not completely identical with *S. citreofluorescens* for the following reasons:

(1) S. sp. NRC-152 produces actinomycin C which consists mainly of actinomycins (C_1,C_2 and C_3), whereas *S. citreofluorescens* produces actinomycin C, colourless antibacterial antibiotic and antiyeast antibiotic.

(2) On starch agar, *S. citreofluorescens* exhibits good growth with weak aerial mycelia and produces yellow soluble pigments, whereas S. sp. NRC-152 shows feeble growth.

(3) On potato agar, *S. citreofluorescens* shows good growth, cottony white aerial mycelia and produces light yellow fluorescent pigments, whereas S. sp. NRC-152 exhibits heavy growth, light yellow aerial mycelia and reddish yellow soluble pigments.

(4) No fluorescent pigments are shown in case of S. sp. NRC-152.

Therefore, the isolated organism was compared with *S. chrysomallus* and *S. parvus*. Their morphological and physiological characteristics are shown in Table 1 and their utilization of carbon source in Table 2, which shows that S. sp. NRC-152 exhibits identical features like *S. chrysomallus*.

Fermentation of Actinomycin C

For the production of the antibiotic actinomycin C, S. sp. NRC-152 was cultured statically at 28°C for 120 hr in penicillin fermentation flasks.

The medium employed for the static flasks contained the following ingredients in g/l tap water: glucose 30.0; $NaNO_3$ 2.0; KH_2PO_4 1.0; $MgSO_4.7H_2O$ 0.5; and $FeSO_4.7H_2O$ 0.005. The initial pH value of the fermentation cultures was adjusted to 7.0 before sterilization. The optimal activity of the microbial broth was obtained at the end of 120 hr on *Bacillus subtilis* NRRL-B-543, while the final pH value of the fermentation broth reached 6.5 at the end of the incubation period.

Isolation of Actinomycin C

The fermentation cultures were collected from penicillin flasks and filtered through Whatman No. 1 filter paper. The antibiotic was extracted with ethyl acetate. The organic layer was washed several times with distilled water and dried under vacuum. The brownish residue was dissolved in chloroform and filtered. The clear chloroform solution of the antibiotic was concentrated under vacuum and n-hexane was added till an orange

TABLE 1.—MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS OF S. sp. NRC-152, *S. chrysomallus* and *S. Parvus*.

Media	S. sp. NRC-152	*S. chrysomallus*	*S. parvus*
Morphology	Branching hyphae, sporophore thin, long, straight. No spirals	Substrate growth soft consisting of long branching hyphae with numerous staining granules	Sporophores straight branched or wavy, no true spirals.
	Spores:spherical or oval 0.4--0.9×1.2--1.5μ Surface of spore: smooth	Sporophores long, straight, no spirals. Spore: oval to elliptical Surface: smooth	Spores oval, 0.9--1.3×1.2--1.8μ
Glucose asparagine agar	G: good, light yellow AM: white SP: citron yellow	G: Smooth, colourless to yellowish AM: powdery, white SP: faint yellow	
Sucrose nitrate agar	weak growth	G: light yellow AM: powdery, white SP: golden yellow	G: yellow, rose or red AM: light yellow to white rose SP: rose-red to bright yellow
Starch agar	Feeble growth	G: Colourless with yellowish AM: powdery, chalk white Strong hydrolysis of starch SP:—ve	G: rose coloured AM: powdery, chalk white Hydrolysis of starch:—ve SP:—ve
Nutrient agar	G: good, pallid yellow AM: white SP: pale yellow	G: poor, shiny, golden yellow AM: white powdery SP: golden yellow (melanin:-ve)	G: yellow AM: light yellow SP: bright yellow
Tyrosine agar (Melanin formation)	—ve	—ve	—ve
Potato agar	G: heavy, deep yellow red AM: light yellow SP: reddish yellow	G: heavy, yellow becoming brownish yellow or orange AM: cottony white to yellowish white	G: Yellow to brown yellow AM: white to yellow
Emerson's agar	G: moderate, colourless AM: cream SP: reddish yellow	G: yellowish with tinge of orange AM: greyish white. SP: light to golden yellow	
Gelatin	G: good, white AM: white SP: orange yellow	G: surface growth heavy, light to dark yellow AM: white SP: yellow-brown only in liquefied portion	G: yellow SP: bright yellow
Liquefaction	+ve	Strong liquefaction	Slow liquefaction
Milk	G: good, light yellow AM: none	G: colourless, with light yellow reverse AM: cottony snow white becoming yellowish slight	
Coagulation	—ve	Slight	No coagulation
Peptonization	+ve	Strong	Rapid peptonization
Antagonistic properties	marked antagonistic effect on bacteria and fungi, produces Actinomycin C	produces actinomycin C. Some strains also produce the anti-fungal cycloheximide	produces actinomycin ×
Cellulose decomposition	None	Very weak growth	G: good, rose colored AM: yellowish grey
H_2S production	—ve	—ve	—ve

yellow precipitate was obtained. The precipitate was dissolved in chloroform and petroleum ether was added to obtain brilliant orange yellow precipitate. The last step was repeated several times to purify the antibiotic substance. The same procedure was carried out for the extraction of the antibiotic from mycelial mats.

TABLE 2.—THE UTILIZATION OF CARBON SOURCES FOR S. SP. NRC-152 *S. chrysomallus* AND *S. parvus*

Carbon source	S.sp. NRC-152	*S. chrysomallus*	*S. parvus*
Sucrose	+ +	+ +	+
Maltose	+ +	+ + +	+ + +
Lactose	+	+ + +	+
Glucose	+ + +	+ + +	+ + +
Galactose	+ + +	+ + +	+ + +
Mannitol	+ +	+ + +	+ + +
Mannose	+ + +	+ + +	+ +
Arabinose	+ +	+ + +	+ +
Raffinose	—	—	—

— = no growth; + = very weak growth; + + = weak growth
+ + + = good growth

Chemical and Physical Properties of Actinomycin C Isolated from S. sp. NRC-152.—The antibiotic is brilliant orange yellow plates and melts at 234–235°C (dec). The elemental analysis of the isolated antibiotic, which was dried on P_2O_5 under vacuum before analysis is as follows: C 56.99%; H 6.86% and N 10.46%.

The optical rotation is $[\alpha]_D^A$ —345 ± 10° in chloroform and UV absorption spectrum shows only two maxima at 260 and 430 nm in methanol. The IR spectrum in KBr tablet shows a close similarity to actinomycin C. In a comparative chromatographic test using benzene saturated with water + 2.0% n-butanol, the spots obtained with the isolated antibiotic and actinomycin C were identical both in R_f, shape and no separation was obtained.

The components of the isolated actinomycin C were revealed by circular chromatography using n-dibutyl ether, n-butanol and 5.0% aqueous β-naphthalene sulphonic acid. The components are mainly VI (C_1), VI (C_2) and VII (C_3).

CHART 1.

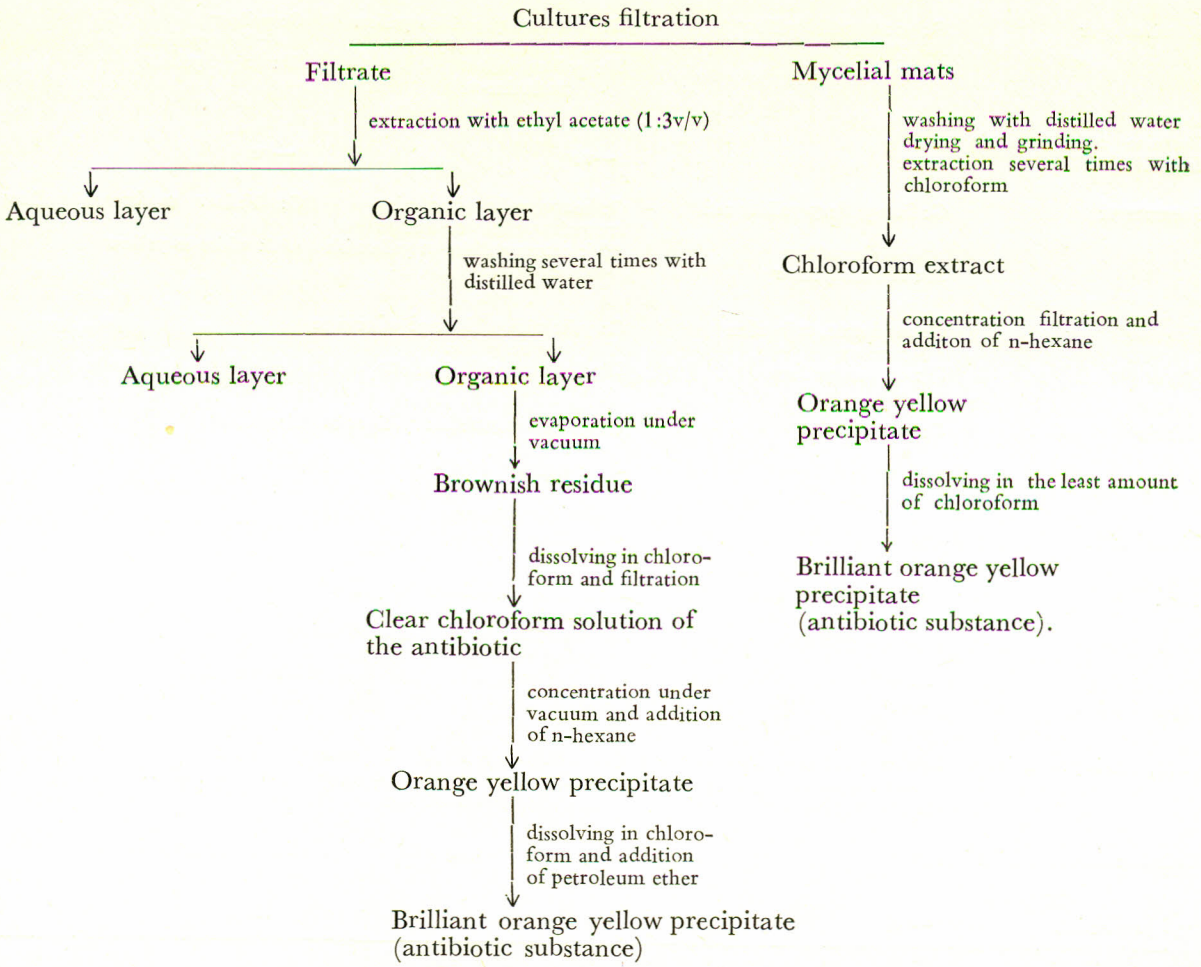

TABLE 3.—MINIMUM INHIBITORY CONCENTRATION OF THE ANTIBIOTIC BY AGAR PLATE METHOD.

Test organisms	M.I.C.* mcg/ml	Test organisms	M.I.C.* mcg/ml
Corynbacterium michiganense B-33	0.064	*Saccharomyces cerivisea* NRRL-Y- 567.	0.064
Agrobacterium tumifaciens NRRL-B-36	80.000	*Salmonella typhi* murum NRC+ **	0.039
Klebsiella pneumonea NRRL B-117	0.128	*Salmonella dublin* NRC **	0.090
Bordetella bronchiseptica NRRL B-140	0.068	*Salmonella pollurum* NRC **	0.060
Escherichia coli NRRL B-210	50.000	*Salmonella euteritides* NRC **	0.170
Staphylococcus aureus NRRL B-313	0.064	*Bacillus cereus* NRC**	0.120
Bacillus subtilis NRRL B-543	0.012	*Staphylococcus aureus* NRC **	0.060
Bacillus cereus NRRL B-569	0.128	*Streptococcus pyogenes* NRC **	0.090
Salmonella typhosa NRRL B-573	0.128	*Salmonella dublin* P 28	–ve
Candida albicans NRRL Y-477	80.000	*Bacillus cereus* (Kana resistant)	0.117
Escherichia coli D 165	–ve	*Bacillus cereus* D 166	–ve
Proteus mirabilis H3	1.000	*Bacillus subtilis* A.A. (chloramphenicol resistant)	0.47
Bacillus subtilis Q 166 (Streptomycin resistant)	0.117	*Bacillus subtilis* D 161 (cathomycin resistant)	0.74
Peseudomonas pyocyaneus	–ve	*Staphylococcus aureus* A 55	0.117
Bacillus subtilis A.A. (ravamycin resistant)	—	*Micrococcus pyogenes*	—
Bacillus cereus (terramycin resistant)	0.117	Haemophilus influenza	0.117

*Minimal inhibitary concentration. **National Research Centre, Dokki, Cairo, U.A.R.

Biological Properties of Actinomycin C.—The minimal inhibitory concentrations of the isolated antibiotic by agar plate method are listed in Table 3.

The isolated antibiotic showed valuable antimicrobial activities. The antibiotic reflected its importance when large varieties of bacteria were destroyed to minimum inhibition concentrations of the antibiotic (Table 3). The same genus of bacterium showed variable minimum inhibition concentrations as in *Bacillus subtilis* NRRL B-543 and *Bacillus cereus* NRRL B-596. The same phenomenon was recorded in the genus *Salmonella typhi*. *Salmonella dublin*, *Salmonella pollurum*, *Salmonella enteritides*, while *Salmonella dublin* P 28 was resistant. The antibiotic showed its antimicrobial activities on bacteria which are resistant to kana, chloramphenicol, streptomycin, ravamycin, terramycin, and cathomycin. *Agrobacterium tumifaciens* NRRL B-36, *Escherichia coli* NRRL-B 210 and *Candida albicans* were resistant to some extent to the antibiotic. The antibiotic showed also an antifungal activity as the minimum inhibition concentration for *Saccharomyces cervisea* NRRL Y-567 was 0.064 mcg/ml. The importance of actinomycin C attracted many researchers due to its antitumor activities.

Actinomycin C is bacteriostatic agent[3,4] active primarly upon Gram-positive bacteria and to a lesser degree upon Gram-negative bacteria. It is also active upon certain neoplasms. It is extremely toxic to animal, a factor which limits its utilization in the therapy of infectious diseases and certain forms of cancer. Kawamata and Imanishi[7] suggested that carcinogenic effect of actinomycin C may be due to its interaction with deoxyribonucleic acid.

Acknowledgement.—Thanks are due to Dr. Joseph Lein, Bristol Laboratories, Division of Bristol-Myers Company, Syracuse, New York, and Dr. J.C. Hoogerheide, Delft, Holland, for their valuable help.

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