STUDIES ON NEW ANTIBIOTIC LIVIDOMYCINS*

I. TAXONOMIC STUDIES ON THE LIVIDOMYCIN-PRODUCING STRAIN *STREPTOMYCES LIVIDUS* NOV. SP.

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The lividomycins are new aminoglycosidic antibiotics containing 2-amino-2,3-dideoxy-D-glucose and are produced by *Streptomyces lividus* nov. sp., strain No. 2230-N_1. Lividomycins A and B have been isolated, purified and characterized. The lividomycins-producing strain also produces mannosyl paromomycin and paromomycin I. The morphological, cultural and physiological characteristics of strain No. 2230-N_1 were studied and as a result of comparison with known species, the organism was considered a new species.

In the course of antibiotic screening studies, new antibiotics, the lividomycins were isolated from fermented broths of strain No. 2230-N_1. This paper describes morphological, cultural and physiological characteristics of strain No. 2230-N_1. The fermentative production, isolation, purification, physicochemical properties and biological activities of the lividomycins are reported in the succeeding paper[1].

Materials and Methods

Strain No. 2230-N_1: Isolated from a soil sample obtained at Nagoya City in Japan.

Morphological observations: Cultures of strain No. 2230-N_1 incubated on some media at 27°C for about two weeks, were observed with a light microscope. Cultures of strain No. 2230-N_1 incubated on glycerol CZAPEK's agar, also were observed with an electron-microscope.

Cultural characteristics: Each of the media used in this study was prepared according to descriptions in WAKSMAN[4], and SHIRLING and GOTTLIEB[5]. Unless otherwise stated, all cultures were incubated at 27°C for 21 days and observations were carried out every 7 days after inoculation. Color determinations were recorded for mature cultures.

Utilization of carbon sources: Utilization of carbon sources by strain No. 2230-N_1 was investigated with the method of PRIDHAM and GOTTLIEB[6].

Gas chromatographic analysis: The nature of the sugars and fatty acids of strain No. 2230-N_1 were determined by the method of OKAMI, HAMADA and UEDA[7]. The mycelial

* This antibiotic was previously reported as antibiotic No. 2230[2] or quintomycin[3]. However, owing to the similarity of the name quintomycin with the trade name "quintamycin", the name was changed to lividomycin.

Fig. 1. The extraction and separation of sugars and fatty acids

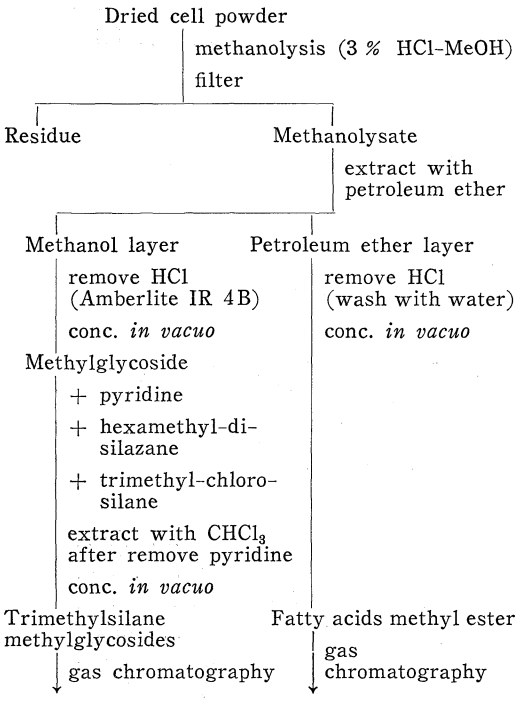

Plate 1. Electronmicrograph of *Streptomyces lividus*, strain No. 2230-N_1 (×5,000)

mass was washed with water and lyophilized. This dried cell powder was extracted and separated by the method shown in Fig. 1.

Results and Discussion

1. Morphological characteristics

The aerial mycelium was sparse or absent on most of the media used. The chains of spores were either simply flexuous or terminated in loops or primitive spirals. Mature spore chains contained from 10 to 15 spores where the aerial mycelium was relatively abundant. The spores were oval to ellipsoidal and $0.2\times0.3\sim0.5\ \mu$ in size by electronmicroscope examination. The spore sheath surfaces were smooth. Plate 1 shows an electronmicrograph of spores of strain No. 2230-N_1.

2. Cultural characteristics

The color of aerial mycelium was white or light gray to dark blue on most media. On glycerol CZAPEK's agar the blue color was enhanced. The organism grew well vegetatively on most of the media except for that containing cellulose. The color of vegetative mycelium on synthetic media generally was light gray to bluish black. The diffusible pigment was faint light rose in glycerol CZAPEK's agar and potato glucose agar. No diffusible pigments were noted with other media. Table 1 shows the cultural characteristics of strain No. 2230-N_1.

Physiological characteristics of strain No. 2230-N_1 are shown in Table 2. Growth of the strain was observed over a wide temperature range, with the optimum temperature between 27°C and 34°C when the organism was grown on glycerol CZAPEK's agar. On that medium, the reverse of colonies changed color from bluish black to dull purplish pink on addition of 0.05 N HCl solution, and this pigmentation was reversed on subsequent addition of 0.05 N NaOH solution. Starch hydrolysis, nitrate reduction, milk peptonization and gelatin liquefaction tests were positive, whereas melanoid pigment, cellulose decomposition and milk coagulation tests were negative. The utilization of carbon sources by strain No. 2230-N_1 is presented in Table 3.

The characteristics cited above indicate that is a *Streptomycete.*

Table 1. Cultural characteristics of *Streptomyces lividus*, strain No. 2230-N_1

Medium	Growth	Aerial mycelium	Soluble pigment
Glycerol CZAPEK's agar	Good, light gray to dark blue or black	Sparse, light gray to dark blue	None, rarely light rose or raspberry
Glucose asparagine agar	Cream to dark or black	Sparse, light gray	None
Calcium malate agar	White to shadow blue	Very scant, white or light gray	None
Starch agar	Good, light gray to dark blue or black	Sparse, white or light gray	None
Peptone glucose agar	Good, dark blue or dark gray	Scant, white to gray	None
Tyrosine agar	Moderate, pale brown to dark gray	Slightly, white	None
Gelatin stab 20°C	Good, white to light gray	None	None
Litmus milk	Moderate, cream color on surface	None	No change
Nutrient agar	Good, wrinkled, moist, Yellowish brown	Very scant, light bluish gray	None
Glucose nutrient agar	Good, wrinkled, pale yelllow	Very scant, light bluish gray	None
Potato glucose agar	Moderate, dark blue to dark gray, slightly penetrating into medium	Scant, light bluish gray	None, rarely light rose
Potato plug	Wrinkled, pale reddish orange	None	None
Blood agar	Good, dark yellowish green	Scant, gray	None
LOEFFLER's serum	Good, lustrous dark blue	None	None
Cellulose medium	No growth		
Yeast extract-malt extract agar, ISP Medium 2	Good, dark bluish gray to bluish black	Scant, white to gray	None
Oatmeal agar, ISP Medium 3	Moderate, bluish black	Scant, gray	None
Inorganic salts starch agar, ISP Medium 4	Moderate, grayish brown to bluish black	None	None
Glycerol asparagine agar, ISP Medium 5	Good, bluish black	White to gray	None
Peptone-yeast extract-iron agar, ISP Medium 6	Moderate, dull yellow to yellowish brown	None	None

3. Gas chromatographic analysis of sugars and fatty acids contained in strain No. 2230-N_1

There were some initial questions as to whether strain No. 2230-N_1 belongs to the genus *Streptomyces* because the aerial mycelium formed sparsely or was absent on many media. Therefore, the sugars and fatty acids in the mycelium of the strain were compared with those of representative strains of *Nocardia* and *Streptoverticillium* using the gas chromatographic analytical method of OKAMI *et al.*[7] As shown in Table 4, arabinose is present in *Nocardia* but not in strain No. 2230-N_1. The latter contain anteiso-, iso- and odd-numbered fatty acids, whereas the former contain unsaturated and 10-methyl-C_{18} fatty acids. These results constitute additional proof that strain No. 2230-N_1 is not a *Nocardia* and is best classified as a *Streptomyces*.

4. Comparison of *Streptomyces* strain No. 2230-N_1 with other species

When compared with the characteristics of those *Streptomyces* species in the Gray

or the Blue color series described in "The Actinomycetes, Vol. 2" by WAKSMAN[4] and in the ISP* reports of SHIRLING and GOTTLIEB[8,9,10] there were several which resemble strain No. 2230-N_1 in some respects. These were *Streptomyces gedanensis*, *S. intermedius* and *S. finlayi*. These species, however, were differentiated from *Streptomyces* strain No. 2230-N_1 as follows:

Streptomyces gedanensis forms cream to brownish colored vegetative growth and produces no diffusible pigment on potato agar medium, forms yellow to cream-colored vegetative growth on starch medium and does not peptonize milk or reduce nitrate. Strain No. 2230-N_1 forms dark blue to dark gray vegetative growth and scant mycelium and usually does not produce a diffusible pigment on potato glucose agar, forms light gray to dark blue or black vegetative growth on starch agar, and gives positive milk peptonization and nitrate reduction tests.

Table 2. Physiological characteristics of *Streptomyces lividus*, strain No. 2230-N_1

Melanoid pigment	negative
Reduction of nitrate	positive
Cellulose decomposition	negative
Coagulation of milk	negative
Peptonization of milk	positive
Hydrolysis of starch	positive
Liquefaction of gelatin	positive
Growth temperature	15~45°C
Optimum temperature	27~34°C

Reversed side of colonies: Addition of 0.05 N HCl changes color from bluish black to dull purplish pink, and this pigmentation is reversed on addition of 0.05 N NaOH.

Table 3. Carbon source utilization of *Streptomyces lividus*, strain No. 2230-N_1

Carbon source	Utilization	Carbon source	Utilization	Carbon source	Utilization
D-Xylose	−	α,α-Trehalose	++	Inulin	−
L-Arabinose	−	Inositol	++	Dulcitol	−
D-Glucose	++	Lactose	−	Glycerol	++
D-Galactose	++	Sorbitol	++	Sodium citrate	+
D-Fructose	+	D-Mannitol	++	Sodium acetate	+
Maltose	++	Dextrin	+	Calcium malate	+
Sucrose	−	Starch	+		
Raffinose	+	L-Rhamnose	−		

Carbon utilization was investigated using the method of PRIDHAM and GOTTLIEB[6].

Streptomyces intermedius forms good olive green vegetative growth and produces an olive green diffusible pigment in cellulose medium, whereas strain No. 2230-N_1 does not.

Streptomyces finlayi forms hairly spores, whereas strain No. 2230-N_1 forms smooth spores.

Strain No. 2230-N_1 also was compared with organisms produced paromomycins, *Streptomyces catenulae*[9], *Streptomyces paucisporogenes*[10], *Streptomyces rimosus* f. *paromomycinus*[11], *Streptomyces chrestomyceticus*[12], *Streptomyces pulveraceus*[13] and *Streptomyces albus* var. *metamycinus*.[14] These organisms were differentiated from strain No. 2230-N_1 as follows:

Streptomyces catenulae does not reduce nitrate and color of the reverse side of colonies does not change with changes in pH. Strain No. 2230-N_1 gives a positive nitrate reduction test and color of the reverse side of colonies changes from bluish black to dull purplish pink with changes in pH.

Streptomyces paucisporogenes is verticillate, forms brown to reddish brown colored vegetative growth, and color of the reverse side of colonies does not change with changes in pH. Although the ratio of C_{14} to C_{16} iso-fatty acids and other gas chromatographic data of strain No. 2230-N_1 is comparable to that of *Streptoverticillia* (Table 4), no evidence of whirls was noted. In addition, orther characteristics suggest the organism is not a *Streptoverticillium*, hence it cannot be identified with *S. paucisporogenes*.

Streptomyces rimosus f. *paromomycinus* forms pale yellow to pale brown colored

* ISP: International *Streptomyces* Project.

Table 4. Gas chromatographic analysis of sugar and fatty acid contents of *Streptomyces lividus*, strain No. 2230-N_1 and other strains

Strain	Sugar content (%)							
	Ribose	Rhamnose	Mannose	Galactose	Glucose	Arabinose	Ribitol	Unknown
Streptomyces lividus, strain No. 2230-N_1	8	6	16	15	55	—	—	1
Nocardia brasiliensis 851	4	—	10	10	58	9	—	6
Streptomyces griseus R4-3475	1	—	6	trace	82	—	9	—
Streptoverticillium baldacii IPV 174	5	3	11	22	54	—	—	1

Strain	Fatty acid content (%)												
	Straight chain					Iso-				Anteiso-		10-Me-	Unsat.	
	14*	15	16	17	18	14	15	16	17	15	17	18	16=1	18=1
Streptomyces lividus, strain No. 2230-N_1	1	2	12	1	—	20	9	35	4	12	4	—	—	—
Nocardia brasiliensis 851	3	—	45	—	3	—	—	—	—	—	—	29	7	14
Streptomyces griseus R4-3475	2	5	8	2	—	10	9	28	4	18	10	—	—	—
Streptoverticillium baldacii IPV 174	5	1	15	—	—	13	7	22	1	27	8	—	—	—

* Carbon number

vegetative growth on most media and dense clusters of spirals occur on synthetic media. Strain No. 2230-N_1 forms bluish gray or bluish black colored vegetative growth on synthetic media and the aerial mycelim is sparse or absent on most media.

Streptomyces chrestomyceticus forms colorless or yellow colored vegetative growth on most media, gives a positive tyrosine decomposition reaction and does not peptonize milk. Strain No. 2230-N_1 does not decompose tyrosine and peptonizes milk.

Streptomyces pulveraceus forms colorless, orange to xanthine orange, and colorless vegetative growth on glycerol CZAPEK's agar, glucose asparagine agar and LOEFFLER's coagulated serum medium respectively. Also, it produces a brown diffusible pigment on glucose nutrient agar. Strain No. 2230-N_1 gives none of the reaction.

Streptomyces albus var. *metamycinus* forms yellowish gray or grayish brown colored vegetative growth on synthetic media, does not reduce nitrate, coagulates milk and produces light rose or reddish yellow diffusible pigment on glucose nutrient agar or milk medium. Strain No. 2230-N_1 does not produce a diffusible pigment, nor coagulates milk, and reduces nitrate.

Therefore, *Streptomyces* strain No. 2230-N_1 was considered to represent a new species for which the name *Stretomyces lividus* nov. sp. is proposed. The epithet refers to the characteristic bluish black colored vegetative growth of the strain on most synthetic media. Progeny of the type strain of *Streptomyces lividus* nov. sp. have been deposited in the Fermentation Research Institute, Agency of Industrial Science and Technology, Chiba, Japan, as FERM-P No. 50 and in the American Type Culture Collection, U.S.A., as ATCC No. 21178.

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