CHLOROPOLYSPORINS A, B AND C, NOVEL GLYCOPEPTIDE ANTIBIOTICS FROM *FAENIA INTERJECTA* SP. NOV.†

I. TAXONOMY OF PRODUCING ORGANISM

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Strain SANK 60983, an actinomycete isolated from a soil sample, was found to produce the new glycopeptide antibiotics, chloropolysporins A, B and C. Short chains of spores occur in the both aerial and substrate hyphae. *meso*-Diaminopimelic acid is present in the cell wall and galactose and arabinose in the whole-cell hydrolysate. Mycolic acid is absent. On the basis of the morphological, cultural and physiological characteristics, this strain was determined to be a new species of *Faenia* designated *Faenia interjecta* sp. nov. The type strain of *F. interjecta* Okazaki and Enokita is SANK 60983.

In the course of an extensive screening program for new antibiotics produced by rare actinomycetes, new antibiotics, chloropolysporins, were discovered in our laboratories. This paper deals with the taxonomy of the producing organism. Fermentation, isolation, physico-chemical characterization and structure elucidation as well as biological properties of the antibiotics will be described in subsequent papers.

Materials and Methods

Actinomycete Strain

Strain SANK 60983 was isolated from a soil sample collected at Kuroiso, Tochigi Prefecture, Japan. One drop of the water suspension of the soil sample was plated on the surface of NH_4Cl-glycerol agar medium consisting of glycerol 1.5%, NH_4Cl 0.2%, $CaCO_3$ 0.1%, K_2HPO_4 0.05%, $MgSO_4 \cdot 7H_2O$ 0.5%, $FeSO_4 \cdot 7H_2O$ 0.001% and agar 2.0% at pH 7.0 before sterilization. The plate was incubated at 28°C for 10 days.

The strain SANK 60983 was inoculated into International Streptomyces Project (ISP) medium 1 in a Sakaguchi flask and grown for 3 days at 28°C on a reciprocal shaker. After harvesting by centrifugation, the culture was washed twice with sterile distilled water by centrifugation, and then used as inoculum for various studies. In addition to the ISP media described by SHIRLING and GOTTLIEB[1], several agar media recommended by WAKSMAN[2] were also used in this study.

Morphological Characterization

The spore chain morphology and the hyphae of the strain grown on various agar media at 28°C for 14~21 days were determined with a light microscope. A sample, treated with a critical point dryer (HCP-1, Hitachi Co., Ltd.) after stepwise dehydration by ethanol, was observed under an MSM-6 scanning electron microscope (Akashi Seisakusho Co., Ltd.).

Cultural Characterization

Observation of the growth on various agar media was made after incubation at 28°C for 14 days unless otherwise mentioned. The mass colors of the growth were assigned in common terminology.

† *Micropolyspora interjecta* SANK 60983 (FERM BP-538).

Exact colors were determined by comparing the mycelial color with color chips from the "Guide to Color Standard" (Nippon Shikisai Kenkyusho, Tokyo, Japan).

Physiological Tests

Physiological tests were carried out with each medium as follows: ISP media 1, 6 and 7 for melanin formation, ISP medium 4 for starch hydrolysis, gelatin stab for gelatin liquefaction, dehydrated skim milk (Difco) for milk coagulation and peptonization, nitrate broth (Difco) for nitrate reduction. The media used for determination of casein, tyrosine and xanthine decomposition were prepared by dissolving 10 g of dehydrated skim milk in 100 ml of distilled water and by dissolving 0.5 g of tyrosine or 0.4 g of xanthine in 100 ml of nutrient agar, respectively. The cultures on all of the media tested were incubated at 28°C for 14 days except on milk (37°C, 10 days) and gelatin (26°C, 21 days) media. Carbohydrate utilization was studied by the procedure described by SHIRLING and GOTTLIEB. The effect of temperature on growth was determined by streaking the inoculum over the surface of ISP medium 2 with a temperature gradient incubator TN-3 (Toyo Kagaku Sangyo Co., Ltd.). The tolerance against sodium chloride of the culture was investigated by streaking the inoculum onto the same medium as used for the temperature study, except that it contained sodium chloride at 1.0, 2.0, 3.0, 5.0, 7.0, 10.0 or 14.0% and incubating at 28°C for 21 days. The susceptibility of the strain to antibiotics was determined by the agar dilution method (ISP medium 2) at pH 7.0. Growth under anaerobic condition was performed by the Gas Pak system (BBL) using the same method and medium as those for the sodium chloride tolerance and temperature studies.

Chemotaxonomy

Purified cell wall and whole-cell hydrolysates were analyzed by the methods of BECKER *et al.*[3], LECHEVALIER and LECHEVALIER[4]. Phospholipid, acyl type in cell wall, menaquinones and mycolic acid were performed by the methods of LECHEVALIER and LECHEVALIER[5], UCHIDA and AIDA[6], COLLINS *et al.*[7] and HECHT and CAUSEY[8], respectively.

Results and Discussion

Morphological Characterization

Strain SANK 60983 grows relatively well on various media. The aerial mycelium is hardly visible on most media but may occasionally be visible on glycerol - asparagine agar or on potato extract - carrot extract agar. The aerial mycelium arises from the substrate mycelium. The aerial hyphae are 0.6~1.2 μm in diameter and vegetative hyphae are 0.5~0.8 μm in diameter. Both the aerial and the vegeta-

Plate 1. Photomicrograph of strain SANK 60983 (on potato extract - carrot extract agar, 28°C, 21 days ×300).

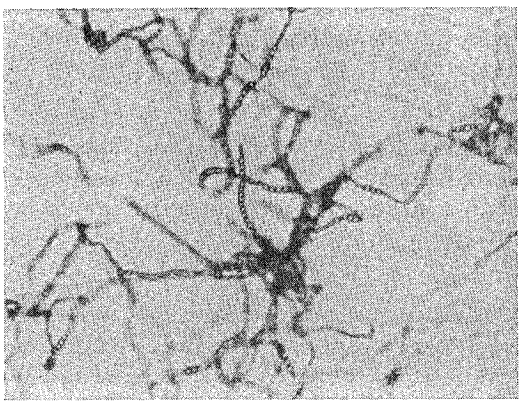
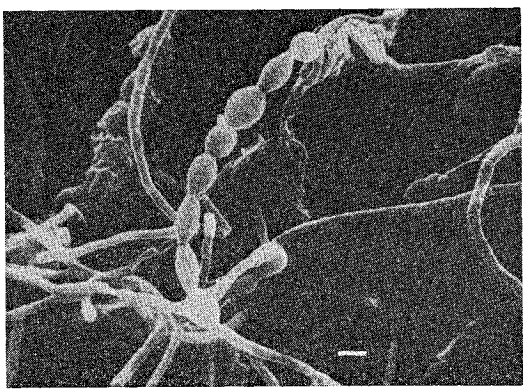


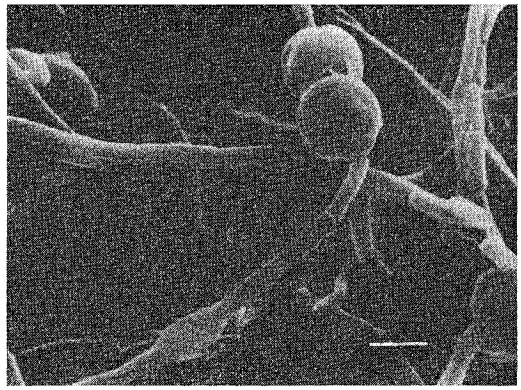
Plate 2. Scanning electron micrograph of spores of strain SANK 60983 (on potato extract - carrot extract agar, 28°C, 14 days).
Bar represents 1 μm.



tive hyphae bear short chains of intercalary and terminal spores, normally from 1 to 10, although occasionally there are more than 10 spores (Plate 1).

The spores were spherical to oval with a smooth surface and 0.4~1.5 by 0.5~1.2 μm in size as revealed by scanning electron microscopy and shown in Plates 2 and 3. No distinctive fragmentation of the hyphae was observed although it may be seen during later stages of the culture. Development of whirls, sporangia, or other special organs was not observed on the media employed.

Plate 3. Scanning electron micrograph of spores of strain SANK 60983 (on potato extract - carrot extract agar, 28°C, 14 days).
Bar represents 1 μm.



Cultural Characteristics

The vegetative growth colors of strain SANK 60983 are pale yellow, yellowish brown or yellowish gray. Aerial hyphae are not produced on most media, although white aerial hyphae are produced on some media. No soluble pigment is produced. Table 1 shows the results obtained after cultivation for 14 days at 28°C on various culture media.

Physiological Properties

The physiological properties of strain SANK 60983 are shown in Table 2. Although coagulation and peptonization of skim milk are both shown as negative, they may occasionally turn positive after long-term incubation. The organism did not grown under anaerobic conditions. It was able to survive after incubation at 50°C for 8 hours. The temperature range for growth was 20~37°C. Growth in 5 to 7% NaCl was inconsistent. No growth occurred at 10%. The strain was resistant to 100 μg/ml of tetracycline, kanamycin, carbenicillin, novobiocin, nystatin, cycloheximide and amphotericin B. Minimal inhibitory concentrations (μg/ml) of streptomycin, ampicillin, chloramphenicol, gentamicin, cephaloridine, erythromycin, benzylpenicillin and rifamycin were 1.5, 6.25, 12.5, 12.5, 12.5, 25.0, 50.0 and 50.0, respectively.

Chemotaxonomy

meso-Diaminopimelic acid, arabinose and galactose were found to be present in the cell walls, which are thus of Type IV, and the whole cell sugar pattern was Type A. The types of acyl group of the cell wall and major menaquinone were acetyl type and MK-9 (H_4). Phospholipid type was probably PIII, as a small amount of phosphatidylcholine was detected. Mycolic acid was not detected.

Identification and Classification

A comparison of the description of strain SANK 60983 with the actinomycetes listed in the Approved lists of bacterial names[9], BERGEY's Manual of Systematic Bacteriology[10] and recent taxonomic literature indicate that this organism is morphologically and chemotaxonomically related to the micropolysporas group; *Actinopolyspora*, *Saccharopolyspora*, *Pseudonocardia* and *Faenia* (Table 3).

However, the genera *Actinopolyspora* and *Saccharopolyspora* have spores growing only on the tips of aerial hyphae; the former is as highly halophilic genus, while the acyl type of the cell wall of the

Table 1. Cultural characteristics of strain SANK 60983.

Yeast extract - malt extract agar (ISP 2)	G:	Abundant, raised, wrinkled, yellowish brown
	AM:	None
	R:	Yellowish brown
	SP:	None
Oatmeal agar (ISP 3)	G:	Good, smooth, dull yellow
	AM:	None
	R:	Dull yellow
	SP:	None
Inorganic salts - starch agar (ISP 4)	G:	Abundant, smooth, yellowish gray to pale yellowish brown
	AM:	None
	R:	Yellowish gray to pale yellowish brown
	SP:	None
Glycerol - asparagine agar (ISP 5)	G:	Good, wrinkled, yellowish brown
	AM:	Poor, white
	R:	Yellowish brown
	SP:	None
Peptone - yeast extract - iron agar (ISP 6)	G:	Moderate, smooth, pale yellowish brown
	AM:	None
	R:	Pale yellowish brown
	SP:	None
Tyrosine agar (ISP 7)	G:	Abundant, raised, wrinkled, pale yellowish brown
	AM:	None
	R:	Dull yellow
	SP:	None
Sucrose - nitrate agar	G:	Abundant, raised, wrinkled, pale yellow
	AM:	None
	R:	Pale yellowish brown
	SP:	None
Glucose - asparagine agar	G:	Moderate, smooth, yellowish gray
	AM:	None
	R:	Yellowish gray
	SP:	None
Nutrient agar (Difco)	G:	Moderate, smooth, pale yellowish brown
	AM:	None
	R:	Pale yellowish brown
	SP:	None
Water agar	G:	Poor, smooth, yellowish gray
	AM:	None
	R:	Yellowish gray
	SP:	None
Potato extract - carrot extract agar	G:	Moderate, smooth, yellowish gray
	AM:	Poor, white
	R:	Yellowish gray
	SP:	None

G: Growth, AM: aerial mycelium, R: reverse color, SP: soluble pigment.

latter is the glycolyl type. For these reasons, strain SANK 60983 can not be assigned to either of these genera. Although the genus *Pseudonocardia* bears spores both on aerial and substrate mycelia, as does strain SANK 60983, it forms acropetally long and cylindrical spores. Thus, strain SANK 60983 can not be assigned to the genus *Pseudonocardia*. The difference between the genus *Faenia* and strain SANK 60983 is the growth temperature. The former grows on most complex media at 35 to 60°C, while the latter at 20 to 42°C, although it is thermoduric. However, it seems correct to regard strain SANK 60983 as a member of the genus *Faenia*.

Table 2. Physiological properties of strain SANK 60983.

Decomposition:	Adenine	−		D-Mannitol	+
	Casein	+		D-Fructose	+
	Xanthine	−		L-Rhamnose	+
	Hypoxanthine	+		Sucrose	+
	Urea	+		Raffinose	±
Starch hydrolysis		±	Utilization of	D-Glucose	+
Gelatin liquefaction		+	carbon sources:	L-Arabinose	+
Milk coagulation		−		D-Xylose	+
Milk peptonization		−		Inositol	+
Nitrate reduction		+		D-Mannitol	+
Deoxyribonuclease secretion		+		D-Fructose	+
Melanin formation:	ISP 1	−		L-Rhamnose	+
	ISP 6	−		Sucrose	+
	ISP 7	−		Raffinose	±
Acid production from:			Growth in NaCl:	3% w/v	+
	Sodium acetate	−		5% w/v	±
	Sodium succinate	−		7% w/v	±
	Sodium citrate	−		10% w/v	−
	Sodium pyruvate	−	Range of growth	10°C	−
	Sodium tartrate	−		20°C	+
	D-Glucose	+		28°C	+
	L-Arabinose	+		37°C	+
	D-Xylose	+		42°C	±
	Inositol	+		45°C	−

+: Positive, −: negative, ±: slightly positive.

Table 3. Comparison of strain SANK 60983 with known micropolysporas.

	Actino-polyspora	*Saccharo-polyspora*	*Pseudo-nocardia*	*Faenia*	SANK 60983
Aerial mycelium	+	+	+	+	+
Fragmentation of SM*	+	+	+	±	±
Sporophores	AM(T)*	AM(T)	AMSM(T)	AMSM(IT)	AMSM(IT)
Motility	−	−	−	−	−
Cell wall type	IV	IV	IV	IV	IV
Whole cell sugar pattern	A	A	A	A	A
Mycolic acid	NT	—	—	—	—
Acyl type	Ac	Gly	Ac	Ac	Ac
Menaquinone (MK)	MK-9	MK-9	MK-8/9	MK-9	MK-9

* SM: Substrate mycelium, AM: aerial mycelium, T: tip of hyphae, I: intercalary.
NT: Not tested.

Faenia rectivirgula, the type species of the genus *Faenia* is the only species of the genus and the strain grows at 33 to 60°C with the best growth occurring between 50 and 55°C.

Although the genus *Faenia* has thermophilic character, it seems to be reasonable to give the genus *Faenia* for actinomycetes having spore chains both on the substrate and on the aerial mycelia, and with type IV cell wall. Therefore, we propose, tentatively, strain SANK 60983 as a new species of the genus *Faenia* and designate it as *F. interjecta* Okazaki and Enokita sp. nov. Etymology; derived from L. interjectus, referring to spores in the middle of the hyphae. The strain of *F. interjecta* has been deposited in the Fermentation Research Institute, Agency of Industrial Science and Technology, Ibaraki Prefecture, Japan, with the accession number of FERM BP-538.

Discussion

Strain SANK 60983 was originally proposed as a new species of the genus *Micropolyspora* which includes the following five species; *Micropolyspora brevicatena*, *Micropolyspora internatus*, *Micropolyspora angiospora*, *Micropolyspora rectivirgula* and *Micropolyspora faeni* (Approved lists of bacterial names, 1980[9]). However, *M. brevicatena* has been transferred to the genus *Nocardia*[11] because it possesses mycolic acid in its cell wall. *M. internatus* and *M. angiospora* can not also be accommodated in the genus *Micropolyspora*. Because the former has mostly single spores similar to the genus *Saccharomonospora* and the latter shows the cell wall type III similar to the genus *Actinomadura* or *Excellospora*. Besides, there is little doubt that *M. rectivirgula* and *M. faeni* are synonymous despite considerable differences between the original descriptions of these two species.

Under such confusing status of the genus *Micropolyspora*, KURUP and AGRE[12] proposed the name *Faenia* gen. nov. for the genus *Micropolyspora*. Because, *M. brevicatena*, the type species of the genus *Micropolyspora* has been transferred from *Micropolyspora* to the genus *Nocardia*, whereas MCCARTHY *et al.*[13] and LACEY *et al.*[14] proposed to conserve the genus name *Micropolyspora* with *M. faeni* Cross, Maciver, and Lacey as the type species.

The Judicidal Commission recently announced that the genus *Faenia*, with type species *Faenia rectivirgula*, as validly published by Kurup and Agre remains legitimate[15]. Accordingly, we decided to designate strain SANK 60983 as *Faenia interjecta*.

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