Taxonomic Studies of Pentalenolactone-Producing Actinomycetes

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Pentalenolactone is an antibiotic mainly active against gram-positive and gram-negative bacteria, and weakly active against yeasts and fungi. It was primarily discovered by Koe *et al.*¹⁾ in culture broth of a *Streptomyces* sp., later designated as *Streptomyces roseogriseus* by Sobin *et al.*²⁾ Keller-Shierlein *et al.*³⁾ reported that *Streptomyces arenae* produced arenaemycin, which was identical to pentalenolactone (Fig. 1). Subsequently, Okazaki *et al.*⁴⁾ reported that 32 strains belonging to streptomycetes, including three known species: *Streptomyces omiyaensis*, *S. albofaciens* and *S. viridifaciens*, produced the same antibiotic, pentalenolactone. Therefore, pentalenolactone-producing actinomycetes seem to be widely distributed.

On the other hand, the structure of pentalenolactone, which was established by both Takeuchi *et al.*⁵⁾ and Martin *et al.*⁶⁾, is that of a terpenoid compound, and terpenoid compounds are relatively rare metabolites of actinomycetes. It would be very interesting to know how many species have the ability to produce such a terpenoid compound. Therefore, pentalenolactone producers of the culture collection of Sankyo Co., Ltd. (SANK) were chosen and classified at the species level.

It should also be noted that the species concept of actinomycetes, especially the genus *Streptomyces*, has been very confused, and there are many problems in the recognition and definition of species⁷⁾.

Here, we carried out their species distinction by using mainly spore morphology and DNA-

DNA hybridization, which are believed to be the most reliable species criteria of the genus *Streptomyces*.

Twenty-five pentalenolactone-producing strains, isolated from soils collected at various places not only in Japan but also in the foreign countries of Malaysia, Taiwan, the Philippines, the U.S.A., and Australia, were chosen for these taxonomic studies. As all of the strains revealed good to abundant growth on various agar media and had aerial mycelium except strain SANK 64794, which had rudimentary aerial hyphae (Fig. 2-A), and LL-diaminopimelic acid in cell walls, they were all assigned to the genus *Streptomyces*, and they were divided into six groups based on morphologies of spore chain and surface, as shown in Table 1.

Group I: Strain SANK 64794 formed rudimentary aerial hyphae. But it did not form special structures, such as sporangia, zoospores, ball-like bodies, zig-zag formation or sclerotia. In addition, LL-diaminopimelic acid in the cell wall. Therefore, the strain seemed one of the streptomycetes, probably the genus *Streptomyces*.

Group II: Strains of this group formed straight or flexible sporophores in monopodial branching on the aerial mycelia. Mature spore chains were generally long with 10 to 50 or more spores per chain. Spores were cylindrical with smooth surface (Fig. 2-B). Growth was yellowish brown in color and developed brownish white to gray on various media. Melanoid

Fig. 1. The chemical structure of pentalenolactone.

pigments were not produced. Based on traditional classification, both of two strains of this group most resembled *S. omiyaensis*.

Group III: Mature spore chains were moderately long with 10 to 50, or often more than 50 spores per chain. The spore chains were rectina-

culiapti with spiny spore surface (Fig. 2-C). Growth were pale yellowish brown to pale yellowish orange and developed grayish white to brownish white aerial mycelium. Melanoid pigments were produced. Among known species, no species were similar to two strains of

Fig. 2. Scanning electron micrographs of representative strains of each group on potato-carrot extract agar after incubation at 28°C for 14 days. Bar refers to 5 µm.

Table 1. Grouping of pentalenolactone-producing streptomycetes.

Group	Num.	Chain*1	Surface*2	Fig.(SANK)
I	1	—	—	A(64794)
II	2	RF	SM	B(61094)
III	2	RA	SP	C(60295)
IV	5	SRA	SP	D(60395)
V	8	S	SM	E(65694)
VI	7	S	SP	F(60495)

*1: Spore chain morphology. RF; *Rectiflexibilis*, RA; *Retinaculiaperti*, S; *Spirales*, SRA; *Spirales to Retinaculiaperti*

*2: Spore surface. SM; Smooth, SP; Spiny

this group.

Group IV: Mature spore chains were generally 10 to 50 per chain, although shorter chains may also be common. The spore chains were spirales or occasionally rectinaculiaperti with spiny surface (Fig. 2-D). Growth were yellowish brown to dark olive gray and developed brownish gray aerial mycelium. Melanoid pigments were produced. One of five strains of this group, strain SANK 60395 was resembled *S. griseoflavus*.

Group V: The spore chains were categorized in spiral. Open irregular spirals also appeared to arise from an axial hypha, but true whorls were

not formed. Mature spore chains generally possessed 10 to 50, or sometimes more than 50 spores per chain. Spore surfaces were smooth (Fig. 2-E). Growth were pale yellowish brown and developed aerial mycelium of white to brownish gray. Melanoid pigments were not produced. Based on traditional classification, one of eight strains of this group, strains SANK 65694 was most resembled *S. albofaciens*.

Group VI: The spore chains were spirales with spiny surface (Fig. 2-F). Mature spore chains were generally 10 to 50 spores per chain. Growth were pale yellowish brown and developed aerial mycelium of light brownish gray. Melanoid pigments were produced. One of the strains of this group, strain SANK 60681 was resembled *S. griseochromogenes*.

The DNA-DNA hybridization values between representative strains of each group including strain SANK 64794 (group I) having no aerial mycelium were shown in Table 2. The DNA-DNA hybridization values between them were low (less than 40%). The traditional taxonomic data were well agreed with those of genetic ones. Therefore, it is obvious that each of the six groups belongs to different species of the genus *Streptomyces*.

Otherwise, the hybridization values between strains of group V that contain the most number

Table 2. Levels of DNA relatedness among each representative strains of six groups.

Group	% Homology with photobiotin-labeled DNA					
	I	II	III	IV	V	VI
I (SANK 64794)	100	12	8	18	14	28
II (SANK 61094)	27	100	17	27	21	40
III (SANK 60295)	20	11	100	20	18	28
IV (SANK 60395)	21	12	27	100	18	14
V (SANK 65694)	23	17	29	30	100	26
VI (SANK 60495)	21	15	40	26	28	100

Table 3. Levels of DNA relatedness among strains belonging to group V.

SANK	% Homology with photobiotin-labeled DNA			
	60681	65794	61494	65994
65494	96	36	10	19
65594	100	12	8	8
60681	100	6	6	9
65794	21	100	8	9
65894	37	100	12	13
65694	17	7	6	6
61494	21	19	100	19
65994	9	11	10	100

of strains are shown in Table 3. Strains SANK 65494, SANK 65594 and SANK 60681 showed high DNA relatedness to one another. Therefore, these strains belong to the same species.

Strains SANK 65794 and SANK 65894 showed about 100% DNA relatedness to each other. These two strains belong to the same species. Strains SANK 65694, strain SANK 61494 and strain SANK 65994 showed low DNA relatedness with each other. From these results, it is clear that the group IV consist of 5 species.

Therefore, it is certified that 25 pentalenolactone-producing actinomycetes consist of at least ten different species.

It is not unusual that the same antibiotic is produced by different species of actinomycetes, especially streptomycetes. For example, carbomycin, viomycin and streptomycin are produced by more than five species of the genus *Streptomyces*. However, it is a rare case that pentalenolactone is produced by at least ten substantially different species, although their species names have not been determined yet.

Those pentalenolactone-producing strains were chosen in several thousand of actinomycetes containing various rare genera, though the data did not shown. It is very interesting that a terpenoid antibiotic, pentalenolactone distributed in only *Streptomyces*, not in rare genera, and was produced by a wide range of species of the genus *Streptomyces*. On the other hand, Kizuka *et al.* reported that trehazolin-producing strains were distributed not in *Streptomyces* but only in rare genera such as *Micromonospora*, *Saccharothrix* and *Amycolatopsis*⁸⁾. Thus, the same metabolite producing strains seem to be good materials for studies on diversity and application of actinomycete metabolisms. We believe pentalenolactone would be a good candidate for analysis of terpenoid biosynthesis.

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