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EXPERIMENTAL ARTICLES

Taxonomic Investigation of Five Streptomycete Cultures Belonging to ISP Standards

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Abstract—The taxonomic investigation of five streptomycete cultures belonging to the International Streptomyces Project (ISP) standards was carried out using the methods of population analysis, DNA–DNA hybridization, and multilocus DNA fingerprinting. Two species with names considered to be synonymous, *S. alboviridis* ISP 5326 and *S. oligocarbophilus* ISP 5589, were found to be actually identical. Three other species investigated, *S. krainskii* ISP 5321, *S. craterifer* ISP 5296, and *S. anulatus* ISP 5361, whose names are usually referred to as synonymous, were shown to be different species.

Key words: streptomycetes, International Streptomycete Project (ISP), systematics, homologous variability series, molecular genetic analysis

The differentiation of *Streptomyces* species is one of the urgent problems of the modern systematics of actinomycetes. As was emphasized by Kuznetsov [1], the systematics of this group of microorganisms should be revised with account for their diversity in nature. The genetic instability of streptomycetes, which follows from their extremely high frequency of mutation [2], increases the role of a population approach to the systematics of these microorganisms. There is increasing evidence that population analysis, which is based on the population concept of a microbial species [1, 8], makes it possible to elucidate nominal species (i.e., not genuine biological species) in order to make a biologically substantiated choice between synonymous specific names [3–5] and to experimentally demonstrate the necessity of amendments in bacterial systematics [6]. The tendency to reduce the species composition of the genus *Streptomyces* and to form taxonomic clusters with a species status [9] is not always justified; nor has it sufficient experimental underpinning. The artificially created name synonymy among streptomycetes does not reflect their actual diversity and hinders the taxonomic identification of cultures isolated from nature.

In order to illustrate the situation with the systematics of streptomycetes, this paper presents the results of a revision of the taxonomic status of five streptomycete species usually referred to as having synonymous names.

MATERIALS AND METHODS

Five streptomycete species used in this work, *S. alboviridis* ISP 5326, *S. oligocarbophilus* ISP 5589, *S. krainskii* ISP 5321, *S. craterifer* ISP 5296, and *S. anulatus* ISP 5361, were chosen from the International Streptomyces Project (ISP) collection, which is considered a fund of standard streptomycete species. The pairs *S. oligocarbophilus–S. krainskii* and *S. alboviridis–S. anulatus* are referred to in the literature as having synonymous names [9, 10] (Table 1). Furthermore, according to the degree of DNA–DNA hybridization between *S. alboviridis* and *S. craterifer* (86%) and between *S. alboviridis* and *S. oligocarbophilus* (79%) [11], these three species should be considered identical [12].

Comparative population analysis [13] was carried out using synthetic Gauze medium no. 1, on which the variability of streptomycetes was maximal as compared with the other eleven media tested. The structure of sporophores and spore surface was studied in a JEOL JSM-T300 scanning electron microscope, using 10-day-old cultures grown on synthetic CP1 medium with glucose [13]. The antimicrobial activity of 7-day-old streptomycete cultures grown on medium no. 2 with corn extract [13] was evaluated by the method of agar blocks placed on lawns of the following test cultures: *Staphylococcus aureus* 209P, *Micrococcus luteus, Bacillus mycoides, Escherichia coli, Serratia marcescens, Mycobacterium* sp. 607, *M. phlei, Chainia ochracea, Streptomyces levoris, Saccharomyces cerevisiae, Monilia* sp., *Thielaviopsis basicola*, and *Fusarium vasinfectum*. Sensitivity to antibiotics was determined using paper disks impregnated with streptomycin, neomycin, monomycin, gentamicin, tetracycline, erythromycin, chloramphenicol, polymyxin, penicillin, and methicillin.

DNA was isolated by the method of Marmur [14]. The G+C content of DNA was determined from thermal renaturation rates [15]. The degree of DNA–DNA hybridization was evaluated as described by Lysenko and Zhilina [16].

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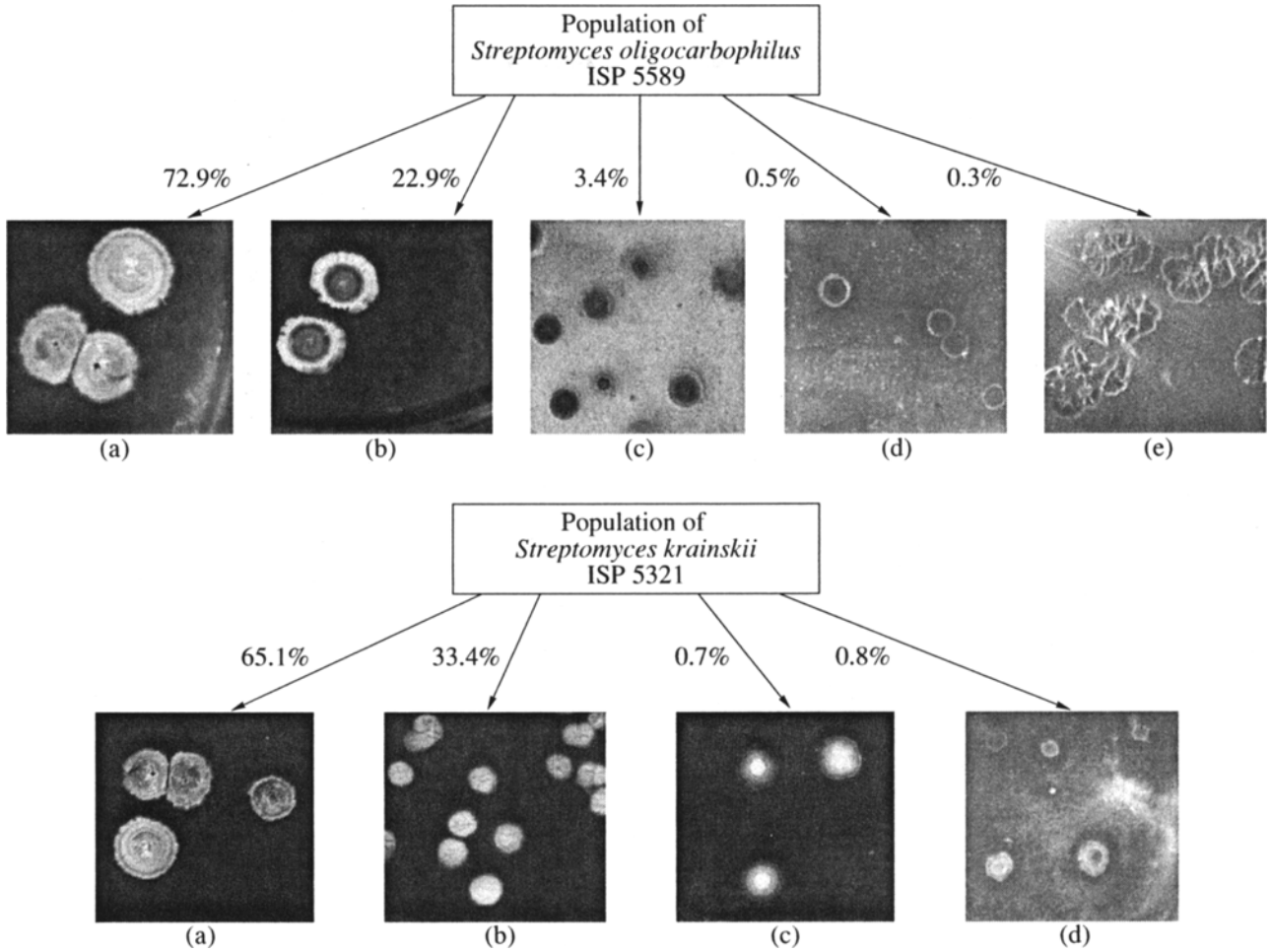

Fig. 1. Spontaneous morphological variants in streptomycete populations. *S. oligocarbophilus* ISP 5589: (a) major, (b) oligosporous white, (c) oligosporous dark, (d) asporogenous, and (e) nocardioform. *S. krainskii* ISP 5321: (a) major, (b) white, (c) oligosporous radiant, and (d) asporogenous.

RESULTS AND DISCUSSION

The taxonomic analysis of *S. oligocarbophilus* ISP 5589 and *S. krainskii* ISP 5321 by the population method showed that both cultures contained four analogous (but not identical) spontaneous variants: major, oligosporous white, oligosporous dark, and asporogenous. *S. oligocarbophilus* ISP 5589 contained, in addition, a nocardioform variant (Fig. 1). Both cultures produced short straight sporophores. Unlike the mycelium of *S. oligocarbophilus*, the mycelium of *S. krainskii* tended to break up. As follows from DNA–DNA hybridization data (Table 2), *S. oligocarbophilus* ISP 5589 and *S. krainskii* ISP 5321 cannot be considered identical species: their genomic similarity is as low as 15%, whereas the typical intraspecies value of this

Table 1. Characterization of the streptomycete species used in this research

Species name	Strains	Notes explaining the choice of given species pairs for comparative analysis
S. oligocarbophilus *S. krainskii*	ISP 5589 ISP 5321	Identity of given species by the data of Gauze *et al.* [10]
S. alboviridis *S. craterifer*	ISP 5326 ISP 5296	High DNA homology of given species (86%) by the data of Mordarski *et al.* [11]
S. alboviridis *S. oligocarbophilus*	ISP 5326 ISP 5589	High DNA homology of given species (79%) [11]
S. alboviridis *S. anulatus*	ISP 5326 ISP 5589	Synonymous names of given species in *Bergey's Manual of Systematic Bacteriology* [9]

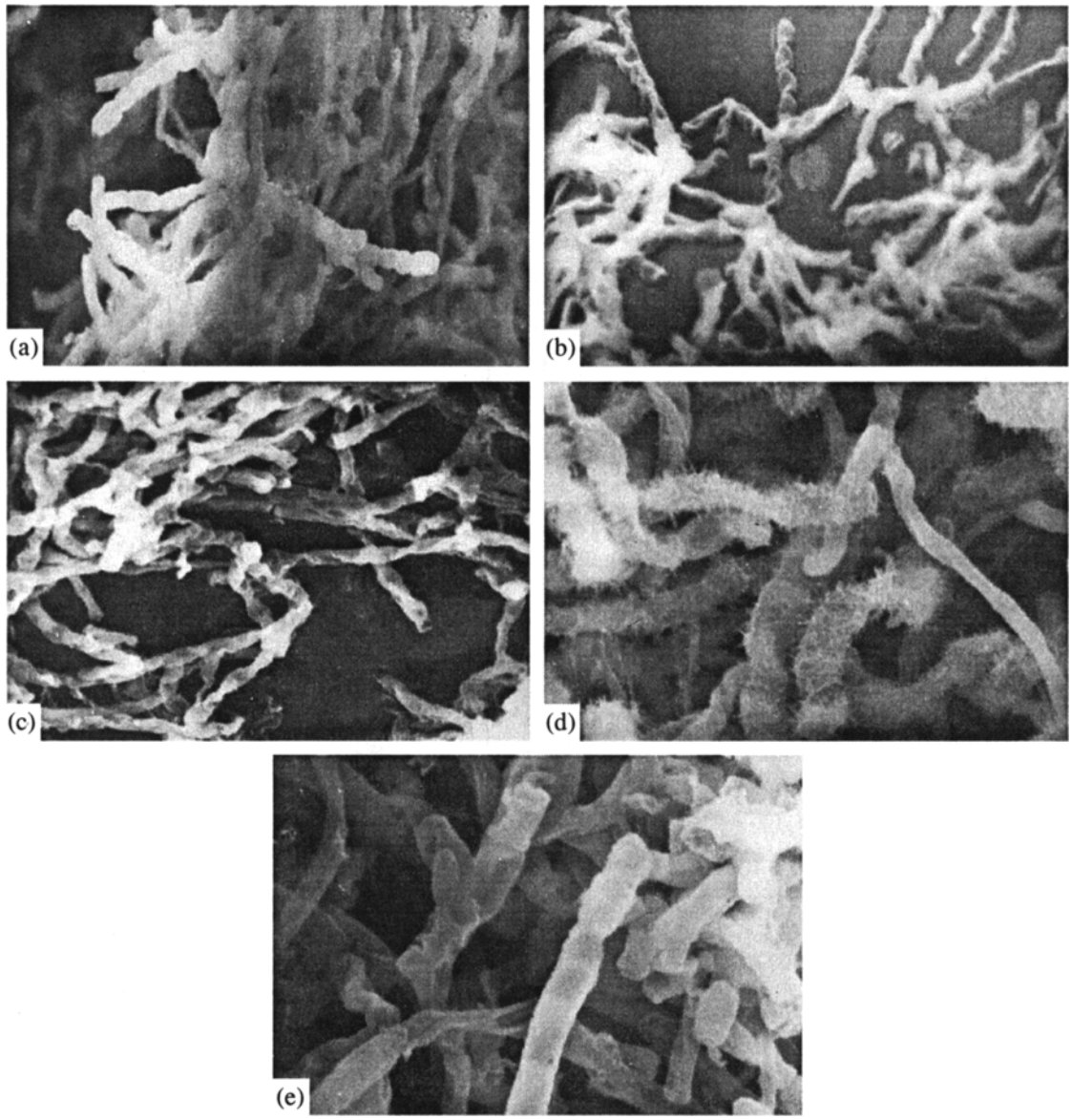

Fig. 2. Aerial mycelium of (a) *S. oligocarbophilus* ISP 5589 (magnification 4800×), (b) *S. krainskii* ISP 5321 (magnification 4800×), (c) *S. alboviridis* ISP 5326 (magnification 4800×), (d) *S. craterifer* ISP 5296 (magnification 7500×), and (e) *S. anulatus* ISP 5361 (magnification 7500×).

parameter is 70–100% [12]. The absence of spontaneous cross-variants in the populations of these two species, the low degree of DNA hybridization between them, and some other taxonomic differences not mentioned here suggest that speculations about the identity of *S. oligocarbophilus* ISP 5589 and *S. krainskii* ISP 5321 [10] are erroneous.

Further taxonomic studies were performed with *S. alboviridis* ISP 5326, *S. craterifer* ISP 5296, and *S. oligocarbophilus* ISP 5589, which, according to

Table 2. DNA–DNA hybridization values for *S. alboviridis*, *S. anulatus*, *S. craterifer*, *S. krainskii*, and *S. oligocarbophilus*

Strain	G+C content, mol %	DNA–DNA homology (%) with				
		ISP 5326	ISP 5361	ISP 5296	ISP 5321	ISP 5589
S. alboviridis ISP 5326	70.4	100				
S. anulatus ISP 5361	69.8	39	100			
S. craterifer ISP 5296	44.4	17	25	100		
S. krainskii ISP 5321	71.0	–	–	–	100	
S. oligocarbophilus USP 5589	71.4	80	23	21	15	100

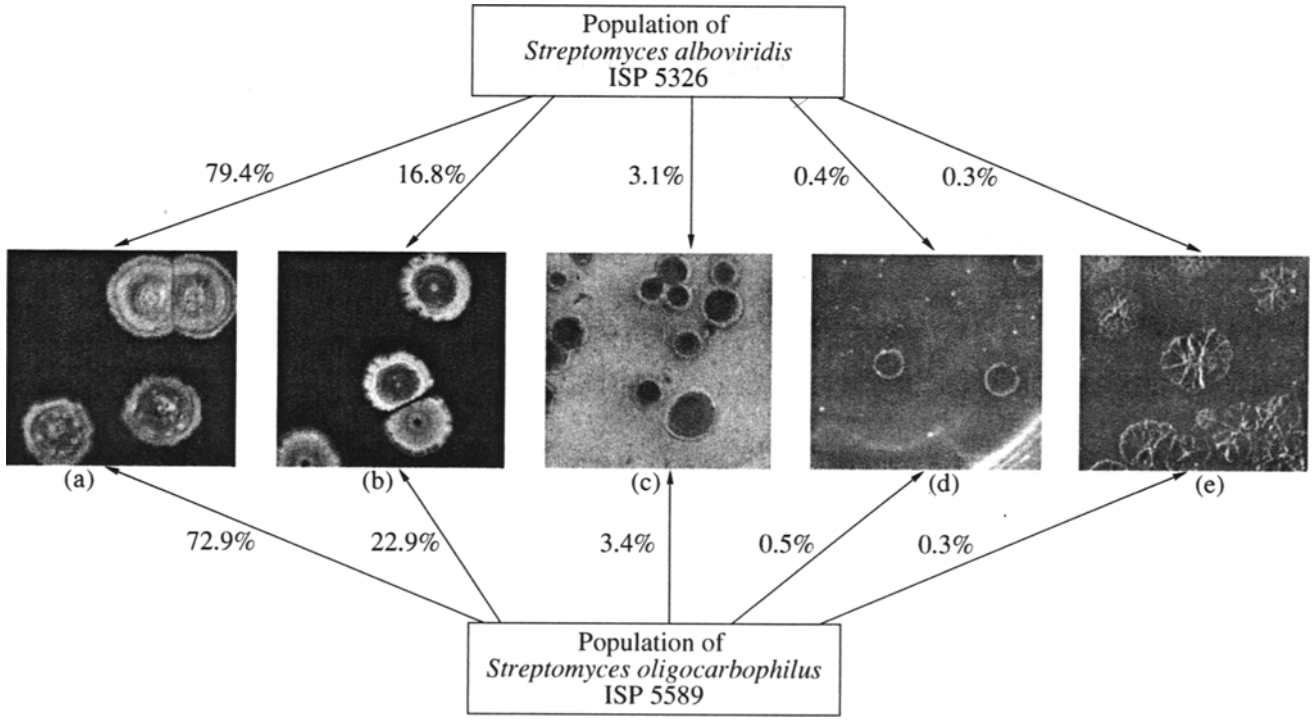

Fig. 3. Spontaneous morphological variants in the populations of ISP 5326 and *S. oligocarbophilus* ISP 5589: (a) major, (b) oligosporous white, (c) oligosporous dark, (d) asporogenous, and (e) nocardioform.

some data in the literature [11], show more than 70% DNA homology (Table 1) and, hence, should be considered identical species. Our studies confirmed a high degree of genomic similarity (about 80%) only between *S. oligocarbophilus* ISP 5589 and *S. alboviridis* ISP 5326 (Table 2). At the same time, the degree of DNA–DNA hybridization between *S. alboviridis* ISP 5326 and *S. craterifer* ISP 5296 was only 17%. Moreover, the G+C content of the DNA of *S. craterifer* ISP 5296 was found to be 44.4 mol %, which is much lower than the G+C content of other representatives of the genus *Streptomyces* (about 70%). In Bergey's 8th edition, the generic affiliation of *S. craterifer* is also called in question [18]. Morphological studies showed that, unlike *S. alboviridis* ISP 5326 (Fig. 2c) and *S. oligocarbophilus* ISP 5589 (Fig. 2a), *S. craterifer* ISP 5296 (Fig. 2d) produces spinous spores. Such an appearance of *S. craterifer* ISP 5296 spores coincides with that contained in the description of this species given by Shirling and Gottlieb [19] but contradicts the description of the species given by Mordarski *et al.* [11]. Thus, the data available in the literature and those presented in this paper testify against the identity of *S. alboviridis* ISP 5326 and *S. craterifer* ISP 5296 proclaimed by Mordarski *et al.* [11]. Moreover, the low G+C content of the DNA of *S. craterifer* ISP 5296 casts doubt on the affiliation of this species to the genus *Streptomyces*.

On the other hand, *S. alboviridis* ISP 5326 and *S. oligocarbophilus* ISP 5589 exhibited a high degree of DNA homology (80%) (Table 2), indicating the intraspecies relatedness of these cultures. The results of multilocus DNA fingerprinting analysis also showed a high degree intraspecies relatedness (about 80%) between *S. alboviridis* ISP 5326 and *S. oligocarbophilus* ISP 5589 [17]. The antimicrobial activities of these species were found to be similar: they both inhibited the growth of the gram-positive bacteria *Micrococcus luteus* and *Mycobacterium* sp. 607, the actinomycete *Chainia ochracea*, and the fungus *Thielaviopsis basicola*, but failed to inhibit the growth of gram-negative bacteria. The antibiotic sensitivity patterns of the species discussed also proved to be similar: they were sensitive to streptomycin, neomycin, gentamicin, monomycin, and erythromycin and resistant to chloramphenicol, polymyxin, penicillin, and methicillin. All these data indicate that *S. alboviridis* ISP 5326 and *S. oligocarbophilus* ISP 5589 are identical species.

In order to choose a proper taxonomic name for these species, it was necessary to decide which of them is a parent species. However, the population analysis of *S. alboviridis* ISP 5326 and *S. oligocarbophilus* ISP 5589 showed that either species contains five spontaneous cross-variants comprising homologous variability series [1], whose relative contents in the populations were almost the same (Fig. 3). Therefore, it is difficult to decide which of the two species is parent. We believe that the name *S. alboviridis* should be of higher priority, since it is included in the Approved list of bacterial names [20], whereas the name *S. oligocarbophilus* must be used as a synonym.

The third set of experiments was devoted to the taxonomic study of *S. alboviridis* ISP 5326 and *S. anulatus* ISP 5361, whose names are referred to as synonymous in Bergey's Manual of Systematic Bacteriology [9].

The degree of DNA homology between these species (39%) (Table 2) turned out to be less than the typical intraspecies value. The dendrogram, constructed from multilocus DNA fingerprinting data [17], confirmed a slight (17.4%) genetic relatedness of these species on the one hand and their general independence on the other hand. Therefore, the aforementioned reference to *S. alboviridis* and *S. anulatus* as synonymous names in Bergey's Manual [9] is erroneous.

Thus, the complex taxonomic analysis of five streptomycete species from the ISP collection, whose names are often referred to as synonymous (*S. oligocarbophilus* ISP 5589, *S. krainskii* ISP 5321, *S. alboviridis* ISP 5326, *S. craterifer* ISP 5296, and *S. anulatus* ISP 5589), showed that only the species *S. oligocarbophilus* ISP 5589 and *S. alboviridis* ISP 5326 are identical. The name *S. oligocarbophilus*, which is not present in the approved list of bacterial names [20], should be used as a synonym of the approved name *S. alboviridis*. In our opinion, *S. oligocarbophilus* ISP 5589 should be withdrawn from the International Streptomyces Project collection of standard streptomycete cultures. On the other hand, the results of our studies do not confirm the announced identity of the following species pairs: *S. alboviridis* ISP 5326 and *S. anulatus* ISP 5361 [9], *S. oligocarbophilus* ISP 5589 and *S. krainskii* ISP 5321 [10], *S. alboviridis* ISP 5326 and *S. craterifer* ISP 5296 [11]. Moreover, the low G+C content of the DNA of *S. craterifer* ISP 5296 (44.4 mol %) casts doubt on the affiliation of this species to the genus *Streptomyces*.

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