Biochemical Markers in the Taxonomy of the *Actinomycetales*

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Summary. Useful biochemical markers for the classification of *Actinomycetales* are: 1. DL- or LL-diaminopimelic acid in the peptidoglycan, 2. sugar composition of polysaccharides, 3. fatty acid spectrum of cell lipids. The occurrence of various kinds of branched fatty acids and of unsaturated fatty acids is of special value.

During the last decade several biochemical criteria have found application in the taxonomy of the order *Actinomycetales*[2-4]. 1. Occurrence of lysine or meso- or LL-diaminopimelic acid (DAP) in the peptidoglycan of the cell wall; 2. sugar composition of polysaccharides; 3. fatty acid spectrum of cell lipids. These biochemical markers have brought considerable progress, especially with regard to the recognition of those genera which lack distinctive morphological features, i.e. *Actinomyces*, *Mycobacterium*, *Nocardia* and *Actinomadura*. Further, increasing knowledge of these properties will improve our understanding of the natural relationship among the genera of this biologically heterogenous bacterial order. Some of the methods have become routine in diagnostic laboratories[5,6]; however the determination of the fatty acid spectrum requires equipment and experience in gaschromatographic work which is not as widespread. In this communication, we wish to report the results of our biochemical studies on a selected number of genera and species of *Actinomycetales*. Main emphasis was laid upon the fatty acid spectrum.

Materials and methods. The biochemical markers 1. and 2. mentioned above, were identified by thin layer chromatography of appropriate whole cell hydrolyzates[5]; the fatty acid spectrum was determined by gas chromatography after transesterification with methanol-BCl_3[7-9]. The instrument used was a Hewlett-Packard model 5 700 A, duel-flame ionization gas chromatograph. The columns ($180 \times 0{,}3$ cm) were packed with 15% diethyleneglycol-succinate on Chromosorb W AWDNCS 80/100. The 2 chromatograms shown in the Figure demonstrate the efficiency of the method, as well as the difference between *Nocardia* and *Streptomyces*. The peaks were identified by comparison with a known mixture of esters obtained from E. Merck (Darmstadt) and Science Laboratories (Serva, Heidelberg). A detailed description of the methods em-

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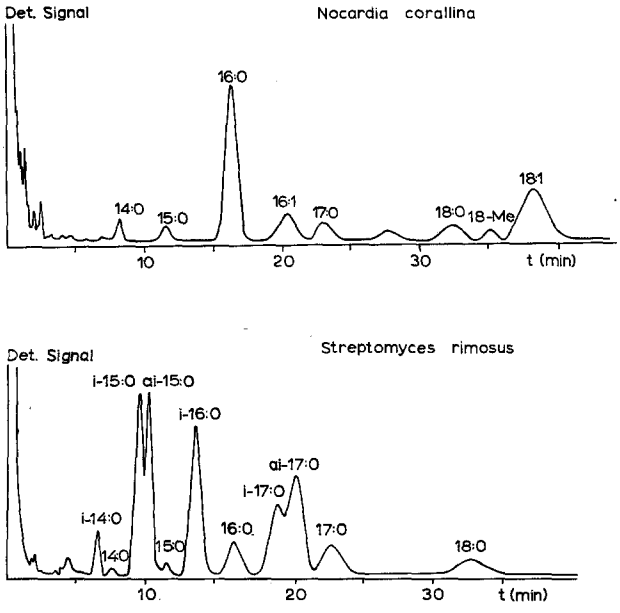
Biochemical characters of genera of *Actinomycetales*

Genus	No. of species	No. of strains	DAP	Sugars				Fatty acids			
				Ara	Gal	Mad	Xyl	sat	iso a-iso	10 meth	unsat
1. *Actinomyces*	3	4	—	—	(v)	—	—	+	—	—	+
2. *Mycobacterium*	4	13	DL	+	+	—	—	+	—	+	+
3. *Nocardia*	6	12	DL	+	+	—	—	+	—	+	+
4. *Micropolyspora*	1	1	DL	+	+	—	—	+	—	+	+
5. *Streptomyces*	9	10	LL	—	—	—	—	+	+	—	—
6. *Streptoverticillium*	2	2	LL	—	—	—	—	+	+	—	—
7. *Microellobosporia*	3	3	LL	—	—	—	—	+	+	—	—
8. *Actinoplanes*	1	1	DL	+	+	—	+	+	+	—	—
9. *Amorphosporangium*	1	1	DL	+	+	—	+	+	+	—	—
10. *Planobispora*	1	1	DL	—	(v)	+	—	+	+	+	—
11. *Streptosporangium*	1	2	DL[a]	—	(v)	+	—	+	+	+	—
12. *Micromonospora*	2	2	DL[a]	v	—	—	+	+	+	+	—
13. *Thermoactinomyces*	1	2	DL	—	(v)	—	—	+	+	—	—
14. *Saccharomonospora*	1	1	DL	+	+	—	—	+	+	—	—
15. *Microbispora*	2	2	DL	—	v	+	—	+	+	+	—
16. '*Nocardia autotrophica*'	1	14	DL	+	+	—	—	+	+	+	+
17. *Actinomadura* (a) *madura*-Typ	1	3	DL	—	v	+	—	+	+	+	+
(b) *dassonvillei*-Typ	1	1	DL	—	—	—	—	+	+	+	+

[a] Contains in addition hydroxy-DAP. – DAP, diaminopimelic acid; Ara, arabinose; Gal, galactose; Mad, madurose; Xyl, xylose; sat, saturated; iso, iso branched; a-iso, anteiso branched; 10 meth, 10 methyl branched; unsat, unsaturated.

ployed, as well as the characterization of all actinomycetes of our collection which have been investigated, will be presented elsewhere.

Results and discussion. The results obtained with 62 strains of 41 species and 16 genera of *Actinomycetales* are listed in the Table. For the purpose of classification, it suffices to indicate the presence or absence of a certain class of fatty acids. Further, only fatty acids with more than 2% of total acids were considered (for quantitative data of the individual acids, see the forthcoming publication). The DAP separates the *Streptomycetaceae* (Table, No. 5, 6 and 7) with LL-DAP from all other *Actinomycetales*, most of which contain DL-DAP; in *Actinomyces* the DAP of the peptidoglycan is replaced by lysine. Numerous actinomycetes with DL-DAP can be differentiated by their sugar and or fatty acid spectrum. However, *Mycobacterium* and the *Nocardiaceae* (No. 3 and 4) exhibit both the sugar type A with arabinose and galactose and the same fatty acid spectrum. These organisms can be differentiated by their mycolic acid type[10,11]. As already shown in the early studies[3,4] the occurrence of xylose and madurose (sugar type D and B respectively) are of diagnostic value. Here we want to add to these well-known features the fatty acid spectrum. If only the 4 classes of acids 1. saturated, 2. iso- and anteisobranched, 3. 10-methyl and 4. unsaturated, are taken into consideration, the following patterns emerge: 1. +−−+: *Actinomyces*, 2. +−++: *Mycobacterium, Nocardiaceae*, 3. ++−−: *Streptomycetaceae, Actinoplanes, Amorphosporangium, Thermoactinomyces, Saccharomonospora*, 4. +++−: *Planobispora, Streptosporangium, Micromonospora, Microbispora*, and 5. ++++: *Actinomadura*. The distribution of these patterns shows that variation exists in some of the present families: Actinoplanaceae and Thermomonosporaceae. The 3 biochemical markers discussed in this paper, as well as the distribution of mycolic acids of various types among *Actinomycetales*[10,11], will be of great help to the actinomycete taxonomist.



Gaschromatographic resolution of fatty acid esters from *Nocardia corallina* and *Streptomyces rimosus*. (Parameters: Inj. port: 250 °C; oven: 155 °C; detector 300 °C; carrier gas N_2: 30 ml/min; hydrogen: 30 ml/min; air 240 ml/min; sample 1 µl.)

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Emericid*, a New Polyether Antibiotic from *Streptomyces hygroscopicus* (DS 24 367)

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Summary. Emericid is a new polyether polycyclic ionophore antibiotic excreted by *Streptomyces hygroscopicus* (DS 24 367). Active in vitro against Gram-positive bacteria, it is ineffective in vivo. At a 0.006–0.02% level in the diet it protects chickens and rabbits against coccidiosis.

Recent research on the production of antimicrobial agents by microorganisms have led to the discovery of emericid (31 559 R.P.), a new antibiotic isolated from the culture broths of *Streptomyces hygroscopicus* DS 24 367.

The strain, which was isolated from a sample of soil collected abroad and selected by classical tests of antibiosis[1], exhibits all the main morphological and biochemical features of the species *Streptomyces hygroscopicus*, as described by several authors[2,3]. It is stored as a dry spores and sterile soil mixture.

Emericid is produced by submerged culture as follows: the strain is first grown from the spores on BENNETT's agar medium[4] in test tubes for 15 days at 26 °C. A suitable inoculum is obtained by successive transfers of the tube culture; first into 250 ml of a liquid medium (composition in g/l: yeast extract 15, anhydrous glucose 10, agar 2) in a 2 l flask incubated for 48 h at 26 °C on a rotary shaker, then into 40 l of another medium (in g/l: peptone 10, yeast extract 5, glucose monohydrate 10, agar 2, pH before sterilization 7,3) contained in a stirred and aerated 75 l fermenter. After 25 h at 27 °C this last

* From a recent paper by N. OTAKE (Tetrahedron Lett. *1970*, 4147) emericid and lonomycin would be identical.

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