Chemical Analysis of Hydrolysates and Cell Extracts of *Nocardia pellegrino*

HALINA MORDARSKA, MARIAN MORDARSKI, and DALILA PIETKIEWICZ

Department of Biosynthesis, Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, and Department of Microbiology, Pomeranian Medical School, Szczecin, Poland

Hydrolysates and cell extracts of 23 strains of *Nocardia pellegrino* were analyzed chemically. The components of the cell extracts indicated that the strains cannot be regarded as members of the genus *Mycobacterium* but that they belong to the genus *Nocardia*. Moreover, it is concluded that the strains of *N. pellegrino* constitute a uniform taxon and produce an LCN-A (lipid characteristic for *Nocardia*) similar to that produced by *N. calcaraea*.

In a previous paper (13), 23 strains of *Nocardia pellegrino* were characterized by standardized bacteriological tests which revealed a marked similarity of these strains to other nocardiae. The results were entirely consistent with those of Juhasz and Bönicke (7), who questioned the placement of these microorganisms in the genus *Mycobacterium* and proposed their reclassification in the genus *Nocardia*. Strains of *N. pellegrino*, in contrast to those of acid-fast bacilli, are characterized by a triphasic developmental cycle, stain negatively by the Ziehl-Neelsen method, produce reddish-brown pigments, and metabolize meta-nitrophenol. Moreover, they are insensitive to *Mycobacterium* phages but are sensitive to *Nocardia* phages (2). Mycolic acids, *sensu stricto*, have not been found in the cells of *N. pellegrino*, although nocardomycolic acids have been reported (8).

This investigation is concerned with a chemical analysis of the hydrolysates and cell extracts of *N. pellegrino* strains for chemotaxonomic purposes.

Bacterial strains. The designations and sources of the 23 strains of *N. pellegrino* studied are given in Table 1.

Media and methods. Cells were incubated for 3 to 5 days at 37°C in submerged culture in modified Sauton's medium (12) and in glucose-peptone broth 79 (14). After checking the purity of the cultures, 1% formaldehyde was added, and 24 h later the mycelium was washed and dried. LCN-A (lipid characteristic for *Nocardia*) was analyzed by thin-layer chromatography of ethanol-ether cell extracts (10). Sugars and amino acids were demonstrated by paper chromatography of acid hydrolysates of

undefatted and defatted mycelium (1, 9). The standard lipids used as controls were obtained from *N. asteroides* (received from the Mayo Clinic, Rochester, Minn.), *N. brasiliensis* 154, *S. griseus* 22, *S. olivaceus* 2238, and *N. calcaraea* IMET 7177 (12).

Acid hydrolysates of cells of the 23 strains studied contained the following amino acids: alanine, arginine, cystine, cysteine, phenylalanine, glycine, histidine, aspartic acid, meso-diaminopimelic acid, leucine, lysine, methionine, valine, proline, threonine, and tyrosine.

In Table 1 are listed the sugar components of the hydrolysates of the whole cells.

Alcohol-ether extracts of the strains showed the presence of LCN-A with a lower R_f value ("calcaraea type") than that of the reference LCN-A from standard strains of *N. asteroides* ("asteroides type") (6, 11) (Fig. 1).

The results of the qualitative analysis of cells propagated on both of the culture media mentioned above were identical with regard to the amino acid and sugar compositions of defatted and undefatted cells. In spite of the lack of quantitative assays, a higher content of lipids, especially LCN-A of the calcaraea type, was evident in extracts of cells grown in modified Sauton medium than in those from cells grown in glucose-peptone broth 79. On the other hand, the contents of sugars and amino acids were higher in the hydrolysates of cells grown in Sauton's medium.

The lack of unified opinions and of methods for differentiating nocardiae and mycobacteria is a serious obstacle to the classification of these microorganisms. This statement particularly applies to species with an indistinct taxonomic profile: such species are probably

TABLE 1. Sugar composition of whole-cell hydrolysates of *N. pellegrino* strains

Strains	Sources	Sugars				
		Arabinose and galactose	Glucose	Mannose	Ribose	Rhamnose
"Budapest"	Institute of Tuberculosis, Budapest	+	+	+	Trace	Trace
I.G.	Institute of Tuberculosis, Warsaw	+	+	+	+	Trace
Prague II	Institute of Tuberculosis, Prague	+	+	+	+	Trace
Sn 5101	Research Institute, Borstel	+	+	+	Trace	Trace
Sn 5102	Research Institute, Borstel	+	—	—	Trace	—
Sn 5103	Research Institute, Borstel	+	—	—	Trace	—
Sn 5104	Research Institute, Borstel	+	—	—	Trace	—
Sn 5105	Research Institute, Borstel	+	—	—	Trace	—
Sn 5106	Research Institute, Borstel	+	—	—	Trace	—
Sn 5107	Research Institute, Borstel	+	+	+	+	Trace
Sn 5108	Research Institute, Borstel	+	—	—	Trace	—
Sn 5109	Research Institute, Borstel	+	+	—	Trace	—
Sn 5110	Research Institute, Borstel	+	+	—	Trace	—
Sn 5111	Research Institute, Borstel	+	—	—	Trace	—
Sn 5112	Research Institute, Borstel	+	—	—	Trace	—
SO	Pasteur Institute, Lille	+	+	+	Trace	Trace
SR	Pasteur Institute, Lille	+	+	+	Trace	Trace
81	Pasteur Institute, Lille	+	+	+	Trace	Trace
107	Pasteur Institute, Lille	+	+	+	Trace	Trace
327	Pasteur Institute, Lille	+	+	+	Trace	Trace
330	Pasteur Institute, Lille	+	+	+	Trace	Trace
906	Pasteur Institute, Lille	+	+	+	Trace	Trace
906 B	Pasteur Institute, Lille	+	+	+	Trace	Trace

natural links between artificially arranged groups of microorganisms, such as *N. pellegrino*, whose systematic position has only recently been established (3).

Previously (11, 12) it was shown that the type of LCN-A analogue produced by nocardiae may be used to divide these orga-

nisms into two subgroups: "asteroides" and "calcareae." The LCN-As present in members of these subgroups differ markedly with respect to their R_f values. The asteroides subgroup comprises all the nocardiae sensu stricto (type IV cell wall and LCN-A of the "asteroides type") with *N. asteroides* as the representative.

FIG. 1. Triplicate thin-layer chromatography of cell extracts of pellegrino strains. Developing system: petroleum ether (bp 40-60 °C)-diethyl ether-glacial acetic acid (85:15:1); 2-Sn 5101, 4-Sn 5102, 6-Sn 5103; standards: 1-*S. griseus* 22, 3-*N. asteroides* U.S.A., 5-*N. calcaria* IMET 7177.

TABLE 2. Chemotaxonomic characters of *N. pellegrino* and related taxa

Taxa	Meso-DAP	Sugars		LCN-A	
		Arabinose	Galactose	Asteroides type	Calcaria type
<i>N. pellegrino</i>	+	+	+	—	+
<i>N. asteroides</i>	+	+	+	+	—
<i>N. calcaria</i>	+	+	+	—	+
<i>Mycobacterium</i>	+	+	+	—	—

In the calcaria subgroup are placed all strains with a type IV cell wall but with LCN-A of the calcaria type. The representative of this subgroup is *N. calcaria*. The calcaria type lipid was found in strains of *Mycobacterium rhodochrous* (11), the correct generic assignment of which is also uncertain (4, 5). Preliminary data on the structure of nocardomycolic acids suggest that the acids from *M. rhodochrous* may have a different chemical structure from those of nocardiae sensu stricto (6).

The chemotaxonomic characters of the *N. pellegrino* strains studied are summarized in Table 2. All of the strains show the calcaria type of LCN-A and therefore should be included in the calcaria subgroup. This finding definitely undermines the classification of these strains in the genus *Mycobacterium* and at the same time confirms their similarity to members of the genus *Nocardia*.

The number and type of amino acids in the hydrolysates of whole cells of the 23 strains of *N. pellegrino* were identical. Only the sugar composition varied in different strains. Galactose and arabinose were present in all of the strains, but xylose was not detected. This is consistent with sugar type A, which is characteristic for nocardiae, mycobacteria, and most of the corynebacteria. A majority of the strains also contained glucose and mannose and traces of ribose and rhamnose. However, these sugars are not diagnostically significant, occurring also

in many other members of the order *Actinomycetales* (9). The group of strains of French origin had an entirely uniform chemical composition.

The chemotaxonomic characters of the strains studied show that they form a uniform taxon with an LCN-A similar to that of *N. calcaria* and that they are different from mycobacteria.

We thank A. Tacquet for some of the strains used in this study.

REPRINT REQUESTS

Address requests for reprints to: Marian Mordarski, Department of Biosynthesis, Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, ul, Chalubinskiego 4, Poland.

LITERATURE CITED

1. Becker, B., M. P. Lechevalier, R. E. Gordon, and H. A. Lechevalier. 1964. Rapid differentiation between *Nocardia* and *Streptomyces* by paper chromatography of whole-cell hydrolysates. *Appl. Microbiol.* 12:421-423.
2. Castelnovo, G., G. Bellezza, M. E. Duncan, and J. Asselineau. 1964. Etude sur les mycobactéries et les nocardiae. I. Constitution antigénique. II.

- Relations sérologiques entre mycobactéries et nocardiae. III. Sensibilité aux phages. Ann. Inst. Pasteur **107**:828-844.
3. Cross, T., and M. Goodfellow. 1973. Taxonomy and classification of the actinomycetes p. 11. In G. Sykes and F. A. Skinner (ed.), *Actinomycetales*: characteristics and practical importance. Academic Press Inc. New York.
 4. Goodfellow, M. 1971. Numerical taxonomy of some nocardioform bacteria. J. Gen. Microbiol. **69**:33-38.
 5. Goodfellow, M., A. Fleming, and M. J. Sackin. 1972. Numerical classification of "*Mycobacterium*" *rhodochrous* and Runyon's group IV mycobacteria. Int. J. Syst. Bacteriol. **22**:81-98.
 6. Goodfellow, M., D. E. Minnikin, P. V. Patel, and H. Mordarska. 1973. Free nocardomycolic acids in the classification of nocardias and strains of the "rhodochrous" complex. J. Gen. Microbiol. **74**:185-188.
 7. Juhasz, S. E., and R. Bönicke. 1965. *Mycobacterium* oder *Nocardia pellegrino*? Beitr. Klin. Tuberc. **130**:155-169.
 8. Lanéeelle, M. A., J. Asselineau, and G. Castell-nuovo. 1965. Études sur les mycobactéries et les nocardiae. IV. Composition des lipides de *Mycobacterium rhodochrous*, *M. pellegrino*, et de quelques souches de nocardiae. Ann. Inst. Pasteur **108**:62-82.
 9. Lechevalier, M. P. 1968. Identification of aerobic actinomycetes of clinical importance. J. Lab. Clin. Med. **71**:934-944.
 10. Mordarska, H. 1968. A trial of using lipids for the classification of actinomycetes. Arch. Immunol. Ther. Exp. **16**:45-50.
 11. Mordarska, H., M. Mordarski, and M. Goodfellow. 1972. Chemotaxonomic characters and classification of some nocardioform bacteria. J. Gen. Microbiol. **71**:77-86.
 12. Mordarska, H., and M. Mordarski. 1970. Cell lipids of *Nocardia* p. 47. In H. Prauser (ed.), *The Actinomycetales*. Gustav Fischer Verlag. Jena.
 13. Pietkiewicz, D., J. Andrzejewski, and H. Mordarska. 1973. *Nocardia pellegrino*: I. Versuch einer morphologischen und biochemischen Charakteristik der Art. Z. Bakterirol. **13**:171-172.
 14. Prauser, H., and R. Falta. 1968. Phagensensibilität, Zwellwand-Zusammensetzung und Taxonomie von Actinomyceten. Z. Allg. Mikrobiol. **8**:39-46.