SHORT COMMUNICATIONS

The Selective Isolation of *Nocardia* from Soil Using Antibiotics

By VALERIE A. ORCHARD AND M. GOODFELLOW

*Department of Microbiology, The Medical School, The University of
Newcastle upon Tyne, NE1 7RU*

(Received 25 July 1974)

INTRODUCTION

The primary reservoir for actinomycetes classified in the genus *Nocardia* is thought to be the soil. The classical method of isolating nocardiae from this habitat is the paraffin baiting technique (Gordon & Hagan, 1936). This method, employing glass rods coated with paraffin wax which dip into a carbon-free medium inoculated with soil suspensions, has been used to demonstrate the presence of *Nocardia asteroides*, *N. brasiliensis* and *N. caviae* in soils of the U.S.A. and India (McClung, 1960; Kurup & Sandhu, 1965; Kurup, Randhawa & Sandhu, 1968; Kumar & Mohapatra, 1968) and to isolate *N. asteroides* from clinical material (Mishra & Randhawa, 1969; Kurup, Randhawa & Mishra, 1970). Nocardiae have also been isolated using a technique that involves the inoculation of guinea pig and hamster testicles with soil suspensions amended with penicillin and streptomycin (Conti-Díaz, Gezuele, Civila & Mackinnon, 1971). Neither the inoculation nor the paraffin baiting technique is of value in quantitative studies, and the latter is of limited use for isolating strains because of the difficulties of separating nocardiae from contaminating bacteria and fungi.

Laboratory strains of *Nocardia* were tested for their *in vitro* susceptibility to 52 antimicrobial agents (Goodfellow & Orchard, 1974) in an attempt to highlight those compounds which could be useful for the selective isolation of nocardiae from natural habitats. The present study reports the use of media containing antibiotics capable of supporting high numbers of nocardiae with few bacterial contaminants.

METHODS

Media. The basal medium was Diagnostic Sensitivity Test Agar (DST; Oxoid, CM261) supplemented with the antifungal antibiotics Actidione (Upjohn) and Mycostatin (Squibb), each at a concentration of 50 µg/ml. Sterile aqueous solutions of chlortetracycline hydrochloride (Lederle), demethylchlortetracycline hydrochloride (Lederle) and methacycline hydrochloride (Pfizer) were added to the basal medium to give final concentrations of 45, 5 and 10 µg/ml, respectively. The antibacterial antibiotics were used singly or in combination to determine which supported the highest number of *Nocardia*-like bacteria (Table 1).

Soils. Six different soil samples were collected and stored at 4 °C before testing for the presence of nocardiae.

Preparation of soil suspensions. For each sample the initial dilution was prepared by adding 1 g of soil to 10 ml of sterile 25 % (v/v) strength Ringer's solution. The 10⁻¹ dilutions were shaken for 30 min on a Griffin flask shaker (Griffin & George Ltd, Manchester) at setting 8. After agitation, 1 ml of each soil suspension was pipetted into 9 ml of diluent to

Table 1. Dilution plate counts of *Nocardiae* in five garden soils and one sandy soil

Source of soil sample	Growth of <i>Nocardiae</i> ($10^{-3} \times \text{No./g dry wt}$) on DST plus:			
	Demethylchlor-tetracycline (5 $\mu\text{g/ml}$)	Methacycline (10 $\mu\text{g/ml}$)	Demethylchlor-tetracycline (5 $\mu\text{g/ml}$) + chlortetracycline (45 $\mu\text{g/ml}$)	Methacycline (10 $\mu\text{g/ml}$) + chlortetracycline (45 $\mu\text{g/ml}$)
Ghana	1.0	1.0	0.1	0.2
Ghana (sandy soil)	2.0	0.5	1.0	0.4
Mexico	4.6	0.9	3.6	1.8
Mexico	2.1	0.5	1.0	0.1
Thailand	3.0	5.0	4.3	4.6
Venezuela	4.1	32.0	4.5	2.7

give a 10^{-2} dilution. Surface spread plates were made by spreading 0.1 ml of the 10^{-1} and 10^{-2} soil suspensions over dried DST plates containing the various antibacterial antibiotics, and over control plates containing the antifungal agents only. The plates were incubated at 25 °C and examined after 7, 14, and 21 days.

RESULTS AND DISCUSSION

Colonies with a pink to red stroma, covered to a greater or lesser extent with white aerial hyphae, were recovered on all but the control plates. Purified isolates were found to be Gram-positive, partially acid-fast, non-motile and produced a primary mycelium which fragmented into pleomorphic elements. Ten randomly selected strains were found to contain lipid LCN-A and had a type IV wall. All of these properties are consistent with the classification of the isolates in the genus *Nocardia* (Cross & Goodfellow, 1973). The number of nocardiae occurring in the different soils is shown in Table 1.

Over 100 randomly selected isolates were examined for properties considered to be diagnostic for nocardiae. With a few minor exceptions strains were resistant to lysozyme, reduced nitrate to nitrite and degraded allantoin and urea, but were unable to hydrolyse casein, hypoxanthine, keratin, tyrosine or xanthine. This pattern of reactions is consistent with their classification in the *Nocardia asteroides* complex (Cross & Goodfellow, 1973). Although strains of *N. brasiliensis* and *N. caviae* have yet to be recognized, laboratory cultures of these bacteria grow well on all of the media used in this study.

It is now generally recognized that most of the media recommended for the selective isolation of actinomycetes favour streptomycetes, and are probably quite unsuitable for the detection of other taxa. The media used in this study support the growth of nocardiae but inhibit other actinomycetes.

We are grateful to Dr G. Bowden and his colleagues for doing the cell wall analyses.

REFERENCES

- CONTI-DÍAZ, I. A., GEZUELE, E., CIVILA, E. & MACKINNON, J. E. (1971). Termotolerancia y accion patogena de cepas de *Nocardia asteroides* aisladas de fuentes naturales. *Revista Uruguaya de Patologia Clinica y Microbiologia* 9, 232-241.
- CROSS, T. & GOODFELLOW, M. (1973). Taxonomy and classification of the actinomycetes. In *Actinomycetes: Characteristics and Practical Importance*, pp. 11-112. Edited by F. A. Skinner and G. Sykes. London: Academic Press.

- GOODFELLOW, M. & ORCHARD, V. A. (1974). Antibiotic sensitivity of some nocardioform bacteria and its value as a criterion for taxonomy. *Journal of General Microbiology* **83**, 1-13.
- GORDON, R. E. & HAGAN, W. A. (1936). A study of some acid-fast actinomycetes from soil with special reference to pathogenicity to animals. *Journal of Infectious Diseases* **59**, 200-206.
- KUMAR, R. & MOHAPATRA, L. N. (1968). Studies on aerobic actinomycetes isolated from soil. I. Isolation and identification of strains. *Sabouraudia* **6**, 140-146.
- KURUP, P. V. & SANDHU, R. S. (1965). Isolation of *Nocardia caviae* from soil and its pathogenicity for laboratory animals. *Journal of Bacteriology* **90**, 822-823.
- KURUP, P. V., RANDHAWA, H. S. & MISHRA, S. K. (1970). Use of paraffin bait technique in the isolation of *Nocardia asteroides* from sputum. *Mycopathologia et mycologia applicata* **40**, 363-365.
- KURUP, P. V., RANDHAWA, H. S. & SANDHU, R. S. (1968). A survey of *Nocardia asteroides*, *N. caviae*, and *N. brasiliensis* occurring in soil in India. *Sabouraudia* **6**, 260-266.
- MCCLUNG, N. M. (1960). Isolation of *Nocardia asteroides* from soils. *Mycologia* **52**, 154-156.
- MISHRA, S. K. & RANDHAWA, H. S. (1969). Application of paraffin bait technique to the isolation of *Nocardia asteroides* from clinical specimens. *Applied Microbiology* **18**, 686-687.