Taxonomic and Serological Studies on *Micropolyspora faeni* and *Micropolyspora* Strains from Soil Bearing the Specific Epithet *rectivirgula*

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(Received 8 March 1979)

The results of serological studies on six strains of *Micropolyspora faeni* from hay, sputum and plant debris, and five strains of *Mip. rectivirgula* from soil indicated no significant differences between the two species. Antisera raised in rabbits against purified antigens of the type strains were used to compare the 11 strains by immunoelectrophoresis. The detailed antigenic composition of the type strains was also determined by two-dimensional immunoelectrophoresis against specific rabbit antisera and pooled serum samples from patients suffering from farmer's lung. Cross-reacting antigens were identified by intermediate gel immunoelectrophoresis.

The close similarity of the two species was confirmed by the results of 60 morphological, physiological and biochemical tests applied to the 11 strains. We consider that the strains belong to a single species and propose that the specific epithet *faeni* be conserved for the taxon.

INTRODUCTION

It has recently become apparent that the taxonomy of the genus *Micropolyspora* is in need of further investigation. The generic name was originally proposed for those actinomycetes which produced short chains of spores on both aerial and substrate mycelium (Lechevalier *et al.*, 1961). However, recent chemotaxonomic studies have revealed significant differences between the type species, *Micropolyspora brevicatena*, and other species of the genus. Menaquinone composition and the presence of nocardomycolic acids (Minnikin *et al.*, 1976; Collins *et al.*, 1977) would suggest that *Mip. brevicatena* should be transferred to the genus *Nocardia*. As a prelude to an impending redefinition of the genus *Micropolyspora*, the relationships and status of species within the genus must also be considered.

Micropolyspora faeni was the name proposed by Cross *et al.* (1968) for the thermophilic actinomycete common in mouldy fodder and chiefly responsible for farmer's lung, a hypersensitivity disease of agricultural workers (Pepys *et al.*, 1963; Wenzel *et al.*, 1964). *Thermopolyspora polyspora* was an earlier name given to these organisms by Corbaz *et al.* (1963) but was considered illegitimate because the original species description (Henssen, 1957) was based on contaminated cultures. Henssen (personal communication) has also concluded from a study of *Micropolyspora faeni* strains that they do not resemble her original isolates of *Thp. polyspora*.

Three thermophilic actinomycetes isolated from soil collected in the Pamirs (strains 683 and 1325) and Spitzbergen (strain 1414) and studied in the U.S.S.R. were also placed in the genus *Thermopolyspora* (Krassilnikov & Agre, 1964). The results of subsequent studies

on the morphology and wall composition of these strains, named *Thp. rectivirgula*, suggested that the organism should be regarded as a member of the genus *Micropolyspora* (Lechevalier & Lechevalier, 1967; Kalakoutskii *et al.*, 1968). *Micropolyspora rectivirgula* has never been implicated as a causative agent of respiratory disease.

Comparative studies on the morphology (Dorokhova *et al.*, 1970), physiology (Kalakoutskii *et al.*, 1968) and phage sensitivities (Prauser & Momirova, 1970) of *Mip. faeni* and *Mip. rectivirgula* have indicated a close similarity between the two species. Although the antigenic composition of *Mip. faeni* has been elucidated in relation to its role in hypersensitivity pneumonitis, serological studies on *Mip. rectivirgula* have never been reported. In an attempt to determine whether the two species are synonymous, the serological relationships of five recently acquired strains of *Mip. rectivirgula* and six strains of *Mip. faeni* were studied. Morphological, physiological and biochemical tests were also applied to the 11 strains to confirm the results of the serological comparison.

METHODS

The origins of the 11 strains included in this study are listed in Table 1. They were freeze-dried and working cultures were maintained on a medium (MYA) which contained: malt extract (Oxoid), 3.0 g; yeast extract (Oxoid), 3.0 g; Bacto-peptone (Difco), 5.0 g; glucose, 10.0 g; agar 20.0 g; distilled water, 1 l; pH 6.8 to 7.0. Agar slopes of the strains were stored at 4 °C and subcultured every 14 d.

Serological tests

Preparation of antigenic extracts. Antigenic extracts of the type strains of *Mip. rectivirgula* (CUB 977) and *Mip. faeni* (CUB 55) were prepared in 3 l Erlenmeyer flasks by the double dialysis method of Edwards (1972a). The method was slightly modified as follows. The internal phase consisted of 1 l nutrient broth (Oxoid CM1) contained in a length of Visking dialysis tubing (36/32 in diam.) and the 1 l external phase contained 10 g glucose in an aqueous solution of 0.1 M-glycine and 0.075 M-NaCl, pH 7.2. Following 12 d incubation at 50 °C the antigen-containing external phase was dialysed in cold water, concentrated against pH 7.5, 10% (w/v) polyethylene glycol 400 (BDH), centrifuged at 17000 g for 30 min and the supernatant was freeze-dried. The double dialysis (DD) antigens so produced were used to raise antisera in rabbits and to compare the antigen profiles of the two type strains by two-dimensional immunoelectrophoresis.

Antigenic extracts of all 11 strains were prepared by an alternative and more rapid method. Cultures grown on slopes of MYA for 72 h at 50 °C were used to prepare a heavy suspension of the growth in 5 ml quarter-strength Ringer's solution containing 0.01% (w/v) gelatin. This suspension was inoculated into 80 ml nutrient broth (Oxoid) with added glucose (1.0%, w/v) and incubated on a rotary shaker for 24 h at 50 °C. The resulting growth was centrifuged at 500 g for 5 min, the pellet was washed once in 20 ml 0.1 M-NaCl and resuspended in 20 ml 1.0 M-glycine, pH 7.5. After incubation for 24 h at 50 °C the suspension was sonicated with an MSE ultrasonic probe for 2 min, centrifuged at 1000 g for 10 min and the supernatant was transferred to Visking 18/32 dialysis tubing. Extracts were dialysed for 48 h against chilled running tap water and a further 24 h against two changes of distilled water at 4 °C. The dialysis sacs were then transferred to 10% (w/v) polyethylene glycol and the extracts were allowed to concentrate overnight at 4 °C. Antigens were washed from the tubing with distilled water, adjusted to pH 7.5, centrifuged at 17000 g for 30 min and the supernatants were freeze-dried.

An extract of the type strain of *Mip. caesia* (CUB 448) was also prepared to permit analysis of the cross-reactions of another species of *Micropolyspora* with antisera to *Mip. faeni* and *Mip. rectivirgula*. The extraction procedure was essentially similar to the above but since *Mip. caesia* was mesophilic and slow-growing, the growth was harvested after 4 d incubation at 30 °C and extracted for 5 d with glycine at 30 °C.

These glycine extracts were used to compare the antigen profiles of the 12 strains by immunoelectrophoresis.

Production of antisera. New Zealand white rabbits were used to prepare antisera to the double dialysis antigens of the two type strains. Seven intramuscular injections of increasing doses from 2 to 15 mg of the antigen were administered over a period of 7 weeks. Immediately before injection, the freeze-dried antigenic extract was dissolved in saline and emulsified with an equal volume of Freund's complete adjuvant. Development of the antibodies was monitored by frequent blood sampling from the ear vein and testing by immunoelectrophoresis. Sera were prepared from blood collected by cardiac puncture after 9 weeks.

Rabbit antiserum was used unconcentrated for the development of immunoelectrophoresis plates, but for two-dimensional immunoelectrophoresis the anti-*Mip. rectivirgula* serum was concentrated twofold with lyphogel, used according to the manufacturer's directions (Gelman, Michigan, U.S.A.).

Table 1. Sources of *Micropolyspora* strains used in this study

Collection no.	Name	Origin and reference no. as received
CUB 55	<i>Mip. faeni</i>	Hay. J. Lacey, Rothamsted (A94). Type strain
CUB 58	<i>Mip. faeni</i>	Hay. University of Bradford
CUB 124	<i>Mip. faeni</i>	Bagasse. J. Lacey, Rothamsted
CUB 127	<i>Mip. faeni</i>	Mushroom compost. J. Lacey, Rothamsted, from C. L. Fergus
CUB 131	<i>Mip. faeni</i>	Sputum. J. Lacey, Rothamsted (A415), from B. Moore
CUB 407	<i>Mip. faeni</i>	Sugar beet pulp. University of Bradford
CUB 977	<i>Mip. rectivirgula</i>	Soil. J. Lacey, Rothamsted, from L. V. Kalakoutskii, Moscow (INMI 683). Type strain
CUB 978	<i>Mip. rectivirgula</i>	Soil. J. Lacey, Rothamsted, from L. V. Kalakoutskii, Moscow (INMI 1325)
CUB 979	<i>Mip. rectivirgula</i>	Soil. J. Lacey, Rothamsted, from H. Prauser (IMET 9553)
CUB 981	<i>Mip. rectivirgula</i>	Soil. L. V. Kalakoutskii, Moscow (683). Type strain
CUB 982	<i>Mip. rectivirgula</i>	Soil. M. Goodfellow, Newcastle, from H. Prauser (IMET 49553)

Rabbit antiserum raised against the double dialysis extract of *Mip. faeni* (CUB 55) was absorbed with the freeze-dried extract of *Mip. rectivirgula* (CUB 977), and vice versa. Ten mg aliquots of the antigen were added to the antiserum and allowed to precipitate for at least 4 h. The precipitates were removed by centrifuging and the process was repeated until no further visible precipitates appeared. The absorbed sera were used in two-dimensional immunoelectrophoresis against their respective antigens.

Serum samples from patients with farmer's lung were kindly supplied by Mr F. B. Greatorex (Public Health Laboratory, Taunton). The sera were examined for precipitins against *Mip. faeni*, *Thermoactinomyces thalophilus*, *Tha. vulgaris*, *Tha. putidus* and *Tha. sacchari* by the Ouchterlony double-diffusion technique. Eleven sera with precipitins to *Mip. faeni* only were pooled to provide a suitable quantity of standard antiserum. Double dialysis antigens of *Mip. faeni* and *Mip. rectivirgula* were tested against this pooled antiserum by two-dimensional immunoelectrophoresis.

Immunoelectrophoresis. Antigen profiles of *Micropolyspora* spp. were compared by immunoelectrophoresis on glass plates (82 × 202 mm) coated with 29 ml 1.0% (w/v) agarose (Sigma) in veronal acetate buffer (pH 8.6, $I = 0.05$) containing 0.01% (w/v) sodium azide. Glycine-extracted antigens (20 or 30 mg freeze-dried extract ml⁻¹ in distilled water) were subjected to a 60 min electrophoresis in tanks containing veronal acetate buffer (pH 8.6, $I = 0.1$), at approximately 5 mA/8 V per longitudinal cm. After electrophoresis, 2 mm wide troughs were cut in the gel and filled with rabbit antisera. The plates were incubated for 24 h in a humid container at 4 °C.

Two-dimensional immunoelectrophoresis. The method of Laurell (*ad modum* Clarke & Freeman, 1968) was adapted and performed on glass plates (82 × 82 mm) using the buffered gel described above. DD antigen (5 µl, 150 mg ml⁻¹) was electrophoresed under the conditions described above to provide the first dimension of separation. The second dimension was performed at approximately 1 mA/2 V per longitudinal cm for 18 h in a gel which contained antiserum. Concentrated anti-*Mip. rectivirgula* serum or anti-*Mip. faeni* serum was incorporated into the agarose gel at a concentration of 10% (v/v). Pooled farmer's lung serum was used at 7% (v/v).

Intermediate gel electrophoresis. Cross-reacting antigens present in the DD antigenic extracts were detected by the intermediate gel method of Svendsen & Axelsen (1972). Antigens of one type strain were migrated through an intermediate gel containing heterologous antiserum into a reference gel containing the homologous antiserum.

Examination of precipitin patterns. After development of precipitates, immunoelectrophoresis plates and two-dimensional immunoelectrophoresis plates were washed in 0.1 M-saline, pH 7.0, pressed under

absorbent paper, then washed for 2 d in several changes of saline before being pressed again and dried in a vacuum oven at 50 °C. The plates were then stained for 1 h in 0.5% (w/v) Coomassie Brilliant Blue in 95% ethanol/glacial acetic acid/water (450:100:450, by vol.) and examined for lines of antigen-antibody precipitation.

Colony morphology

Agar plates of the following media were inoculated and incubated at 50 °C: (i) MYA; (ii) nutrient agar (Oxoid CM3) plus 1.0% (w/v) glucose; (iii) modified Umezawa's medium which contained: corn meal agar powder (Oxoid CM103), 17 g; soluble starch (BDH), 10 g; yeast extract (Oxoid), 10 g; NaCl, 2 g; distilled water, 1 l; pH 7.2 to 7.4. Cultures were examined directly on the surface of agar plates using a 40× long working distance objective. Each strain was examined for the presence of chains of spores on both the aerial and substrate mycelium. The pigmentation of the aerial and substrate mycelium was also recorded to provide four morphological characters.

Physiological and biochemical tests

Growth temperature. Slopes of nutrient agar plus 1.0% (w/v) glucose were inoculated and incubated in a polythermostat which was adjusted to provide a temperature range of 20 to 66 °C. Cultures were examined for growth at daily intervals up to 7 d.

Tolerance to pH. Trypticase soy agar (BBL) was used as the basal medium and the pH was adjusted with buffer solutions to give a pH range of 4.0 to 9.0. Replidishes containing the media were inoculated and incubated at 50 °C. Cultures were examined for growth at daily intervals up to 10 d.

Carbon utilization tests. Bacto-Carbon utilization agar (Difco) plus 0.1% (w/v) vitamin-free Casamino acids (Difco) was used as the basal medium. Carbon sources (see Table 3) were sterilized by membrane filtration and added to the basal medium, after autoclaving, to give a final concentration of 1.0% (w/v). Replidishes containing the media were inoculated and incubated at 50 °C. Growth was scored at daily intervals up to 10 d and compared with growth on the basal medium (negative control) and on the basal medium plus 1.0% (w/v) glucose (positive control).

Acid from glucose. The method of Hugh & Leifson (1953) was used. Cultures were incubated at 50 °C and examined for growth and acid production after 7 d.

Tolerance to chemical inhibitors. Trypticase soy agar (TSA) was used as the basal medium. The ability of strains to grow in the presence of NaCl (10, 15 and 20%, w/v), bile salts (0.1 and 0.25%, w/v), sodium azide (0.03%, w/v), potassium tellurite (0.05 and 0.1%, w/v) and tetrazolium chloride (0.005%, w/v) was tested. Cultures were incubated at 50 °C and growth was scored daily up to 14 d.

Degradation of organic compounds. Adenine, tyrosine, hypoxanthine (each 0.5%, w/v), xanthine (0.4%, w/v) and elastin (0.3%, w/v) were added to TSA. The media were poured as agar plates and cultures were incubated at 50 °C. Plates were examined daily up to 14 d for disappearance of the insoluble compounds around the area of growth.

To test for starch hydrolysis, plates of TSA plus 1.0% (w/v) soluble starch (BDH) were inoculated and incubated at 50 °C for 10 d. The agar surface was flooded with Gram's iodine and a clear zone around the growth indicated starch hydrolysis.

Plates of TSA plus 0.1% (w/v) cellulose powder (MN300; Macherey & Nagel, Duren, FRG) were inoculated and incubated at 50 °C for 10 d. The cultures were examined for clearing of cellulose around the areas of growth.

Tweens 20 and 80 were added to TSA plus 0.01% (w/v) CaCl₂ to give a final concentration of 1% (w/v). Plates of the medium were inoculated and incubated at 50 °C for 10 d. Hydrolysis of Tween was indicated by a zone of opacity, due to precipitation of calcium salt, around the area of growth.

Casein hydrolysis was detected by the method of Gordon (1967). Cultures were incubated for 10 d at 50 °C and examined for a clear zone around the area of growth.

The degradation of arbutin and aesculin were detected by the method of Kurup & Fink (1975). Plates were incubated at 50 °C for 10 d and blackening of the medium around the area of growth indicated a positive result.

Urease activity was detected using the medium of Christensen (1946). Slopes of the test medium were inoculated and incubated at 50 °C for 7 d. Slopes of medium without urea were also incubated as a control. Development of a pink colour indicated the production of urease.

Allantoin (0.34%, w/v) and phenol red (0.001%, w/v) were added to Trypticase soy broth (BBL) and test tubes of the medium were inoculated and incubated at 50 °C for 7 d. The medium without allantoin was also inoculated and incubated as a control. Allantoinase activity was indicated by a colour change to pink.

Deoxyribonuclease activity was detected using the medium and method of Kurup & Fink (1975).

Catalase. Cultures were grown on MYA, incubated at 50 °C for 24 h. A loopful of growth was taken

from the agar surface and mixed with a drop of H₂O₂ (20 vol.) on a glass slide. Effervescence indicated the presence of catalase.

Oxidase. Cultures were grown on MYA, incubated at 50 °C for 24 h. A loopful of growth was taken from the agar surface and smeared on a piece of filter paper which had been soaked in a 1.0% (w/v) solution of tetramethyl-*p*-phenylenediamine dihydrochloride. The production of a deep purple colour within 60 s indicated the presence of oxidase.

Phosphatase. A solution of phenolphthalein diphosphthate (sodium salt) was sterilized by membrane filtration and added to sterile TSA, to give a final concentration of 0.0001% (w/v). After inoculation and incubation at 50 °C for 11 d, a drop of concentrated ammonia was placed in the lid of the inverted Petri dish. The development of a pink coloration around the area of growth indicated the production of phosphatase.

Nitrite from nitrate. The medium and method of Gordon (1967) were used to detect nitrate reduction. Cultures were incubated at 50 °C for 12 d.

Analysis of results

The physiological and biochemical tests were repeated and those tests which gave results that were not reproducible were rejected. The results of the morphological, physiological and biochemical tests selected were used to provide 60 unit characters. The percentage similarity was calculated for each pair of strains using the simple matching coefficient (S_{SM}) and a dendrogram was constructed according to the single linkage method of Sneath (1957).

RESULTS

Serological tests

Immunoelectrophoresis. No significant differences in the antigenic composition of six *Mip. faeni* and five *Mip. rectivirgula* strains were demonstrated by immunoelectrophoresis (Fig. 1) that could be used to distinguish them as separate species. Quantitative differences in the antigenicity of preparations could be attributed to vigour of growth in the culture system and were partially eliminated by increasing the concentration of appropriate antigen solutions.

Very few cross-reacting antigens were precipitated from the glycine extract of *Mip. caesia* (Fig. 1, antigens 1 and 13) by anti-*Mip. faeni* and anti-*Mip. rectivirgula* sera.

Two-dimensional immunoelectrophoresis. The precipitation patterns produced by *Mip. faeni* and *Mip. rectivirgula* antigens when run against pooled farmer's lung serum (Fig. 2) demonstrated 34 and 27 peaks, respectively. The presence of seven major antigens common to both extracts in each of the plates was immediately apparent. The various heights of these precipitate peaks reflect the concentration of individual antigens in the extracts.

The total complement of antigens present in the extracts of the two type strains, detected with homologous rabbit antisera, is shown in Fig. 3. The complexity of these patterns effectively prohibits the identification of all antigens common to both extracts. However, certain peaks of characteristic position and shape are visible in both patterns. These plates served as a reference when assessing the cross-reactivity of the extracts by intermediate gel electrophoresis.

Cross-reactivity of *Mip. faeni* and *Mip. rectivirgula* antigens. The antigens in the extracts of the type strains which cross-reacted with heterologous antisera incorporated in the intermediate gel layer are shown in Fig. 4. In classical interpretations of two-dimensional immunoelectrophoresis patterns with intermediate gel, peaks that appear entirely within the intermediate gel, or whose height is depressed, represent cross-reacting antigens. Specific antigens appear as bell-shaped peaks of similar size in both reference and intermediate gel patterns with their bases or legs just beneath the reference gel interface. In the intermediate gel plates of *Micropolyspora* extracts (Fig. 4) all the antigens were either precipitated within the intermediate gel or produced peaks whose legs were elongated and extended into the intermediate gel layer, indicating total cross-reactivity.

More antigens were detected in *Mip. rectivirgula* extracts when tested by this technique against heterologous antiserum than in the reference plates. Conversely anti-*Mip. rectivirgula* serum detected fewer *Mip. faeni* antigens than the homologous antiserum (Table 2).

Fig. 1. Immunoelectrophoresis patterns of glycine extracts of *Micropolyspora* strains against rabbit antisera: (a) anti-*Mip. faeni* serum (0.1 ml per trough); (b) anti-*Mip. rectivirgula* serum (0.2 ml per trough).

Antigens. *Mip. faeni*: 2, CUB 124; 4, CUB 55; 6, CUB 407*; 7, CUB 127; 9, CUB 58; 11, CUB 131*. *Mip. rectivirgula*: 3, CUB 977; 5, CUB 979; 8, CUB 982; 10, CUB 981; 12, CUB 978*. *Mip. caesia*: 1 and 13, CUB 448. (Antigens at 20 mg ml⁻¹ except those asterisked which were at 30 mg ml⁻¹.)

Absorption of rabbit antisera. Absorption of 1 ml anti-*Mip. rectivirgula* serum with 50 mg freeze-dried double dialysis extract of *Mip. faeni* completely removed all antibodies to *Mip. rectivirgula*.

Absorption of anti-*Mip. faeni* serum with 70 mg *Mip. rectivirgula* antigen failed to remove all the antibodies to *Mip. faeni*. On analysis of the absorbed antiserum by two-dimensional immunoelectrophoresis against *Mip. faeni* antigens, 10 of the more electrophoretically mobile antigens appeared as stunted peaks. This indicated that these antigens were present in the *Mip. rectivirgula* extract in only limited amounts. The paucity of these antigens is also indicated by their absence in the two-dimensional immunoelectrophoresis patterns of *Mip. rectivirgula* against its homologous antiserum (Fig. 3b) and against anti-*Mip. faeni* serum (Fig. 4b). A corresponding low concentration of antibodies to these antigens in the anti-*Mip. rectivirgula* serum was seen in the intermediate gel plate (Fig. 4a), where precipitate peaks at the anode end of the pattern appeared mainly in the reference gel and only cross-reacted weakly with the serum in the intermediate gel.

Antisera raised against the type strains showed considerable differences even though the double dialysis antigen preparations showed qualitative and quantitative similarities when tested against pooled farmer's lung serum. The anti-*Mip. rectivirgula* serum required a twofold concentration to enable a comparison of the extracts against their homologous

Fig. 2 (a)

Fig. 2 (b)

Fig. 2. Two-dimensional immunoelectrophoresis patterns produced by precipitating double dialysis extracts of *Micropolyspora faeni* (a) and *Micropolyspora rectivirgula* (b) with pooled farmer's lung serum (FLS). [Antigens at 150 mg ml⁻¹; serum in gel at 7% (v/v).] Stained plates were projected so that peaks could be traced to provide the diagrams below the photographs; projected patterns showed more peaks than were visible in photographs.

and heterologous sera to be made. The anti-*Mip. faeni* serum was also qualitatively superior and could precipitate antigens of *Mip. rectivirgula* which remained undetected by anti-*Mip. rectivirgula* serum. These differences are attributed to differences in immunological response of the rabbits.

Colony morphology

The morphology and pigmentation of all 11 strains were very similar. Colonies were raised and pale orange-yellow to yellow-brown. The substrate mycelium bore short straight chains of spores and the length of the spore chains was increased when the organisms were grown on modified Umezawa's medium. Short, straight chains of spores were borne on the white aerial mycelium but this was rarely abundant. Aerial mycelium production could be enhanced significantly by the addition of sodium chloride (5%, w/v) to the medium.

Physiological and biochemical tests

All 11 strains gave the same result for 41 of the 56 tests used (Table 3). Of the remaining tests, only two separated the strains received as *Mip. faeni* from the strains received as *Mip. rectivirgula*. Only those strains received as *Mip. faeni* hydrolysed casein but some of the strains produced a very weak reaction. Only the strains received as *Mip. faeni* could grow in the presence of 0.1% (w/v) potassium tellurite.

The upper and lower temperature limits for growth on the specified medium varied but all of the strains grew in the range 33 to 55 °C. None of the strains grew outside a pH range of 6.0 to 8.3.

Fig. 3

Fig. 4

Fig. 3. Two-dimensional immunoelectrophoresis of double dialysis extracts of *Micropolyspora faeni* (a) and *Micropolyspora rectivirgula* (b) against their homologous rabbit antisera (RS). [Antigens at 150 mg ml^{-1} ; anti-*Mip. faeni* serum at 10% (v/v); anti-*Mip. rectivirgula* serum concentrated ($\times 2$) and incorporated at 10% (v/v).]

Fig. 4. Two-dimensional immunoelectrophoresis with intermediate gels containing heterologous rabbit antisera. (a) *Micropolyspora faeni* double dialysis extract against anti-*Mip. rectivirgula* serum (II) and anti-*Mip. faeni* serum (I). (b) *Micropolyspora rectivirgula* double dialysis extract against anti-*Mip. faeni* serum (I) and anti-*Mip. rectivirgula* serum (II). [Concentrations of antigens and antisera as in Fig. 3.]

Table 2. Total numbers of antigens detected in extracts of *Micropolyspora faeni* (CUB 55) and *Micropolyspora rectivirgula* (CUB 977) when tested against various antisera by two-dimensional immunoelectrophoresis

Antiserum	Antigens*	
	<i>Mip. faeni</i>	<i>Mip. rectivirgula</i>
Pooled farmer's lung serum	34	27
Anti- <i>Mip. faeni</i> serum	57	43†
Anti- <i>Mip. rectivirgula</i> serum	49†	35

* Double dialysis extracts (150 mg ml^{-1}).

† As determined by two-dimensional immunoelectrophoresis with intermediate gel.

Table 3. *Physiological and biochemical properties of Micropolyspora faeni and Micropolyspora rectivirgula strains*

Tests for which all 11 strains gave a positive result

Utilization, as sole carbon source, of:	Growth in the presence of:
D-Glucose	10% (w/v) sodium chloride
D-Galactose	0.1% (w/v) bile salts
D-Fructose	0.03% (w/v) sodium azide
D-Mannose	0.05% (w/v) potassium tellurite
D-Xylose	Decomposition of:
Lactose	Xanthine
Glycerol	Hypoxanthine
Mannitol	Production of:
Hydrolysis of:	Catalase
Tween 20	Urease
Tween 80	
Aesculin	

Tests for which all 11 strains gave a negative result

Utilization, as sole carbon source, of:	Acid from:
L-Rhamnose	D-Glucose
L-Sorbose	Growth in the presence of:
L-Arabinose	0.25% (w/v) bile salts
Maltose	0.005% (w/v) tetrazolium chloride
Dulcitol	Decomposition of:
Sorbitol	Adenine
Salicin	Tyrosine
Hydrolysis of:	Elastin
Starch	Arbutin
Cellulose	Production of:
Growth at:	Oxidase
pH 6.0	Phosphatase
pH 8.3	Deoxyribonuclease
	Allantoinase

Tests which showed variation between strains*

Utilization, as sole carbon source, of:	Growth in the presence of:
Sucrose (3)	15% (w/v) sodium chloride (8)
Raffinose (3)	20% (w/v) sodium chloride (4)
Cellobiose (5)	0.1% (w/v) potassium tellurite (6)
Inulin (3)	Growth at:
meso-Inositol (8)	30 °C (5)
Hydrolysis of:	57 °C (9)
Casein, weak (3)	60 °C (4)
Casein, strong (3)	63 °C (1)
Reduction of nitrate (9)	

* Numbers in parentheses indicate the number of strains which gave a positive result.

Fig. 5. Dendrogram showing the percentage similarity of strains of *Micropolyspora rectivirgula* and *Micropolyspora faeni*.

All 11 strains clustered at 91% similarity (Fig. 5), with the strains received as *Mip. rectivirgula* grouping at 97% similarity. The closer grouping of the strains received as *Mip. rectivirgula* is probably a reflection of the fact that they were all isolated from soil and that very few strains were represented. The strains received as *Mip. faeni* were isolated from a variety of substrates (Table 1).

DISCUSSION

Serological studies of *Mip. faeni* are usually performed in relation to the organism's role as a causative agent of farmer's lung disease. In this study, the antigenic composition of *Mip. faeni* has been compared with that of *Mip. rectivirgula*, in order to establish whether or not the two species are serologically distinct and recognizable.

The antigenic analysis of the 11 strains of *Mip. faeni* and *Mip. rectivirgula* by immunoelectrophoresis against rabbit antisera revealed only a few differences between their antigen profiles. Such homogeneity of antigenic composition has been recorded for seven strains of *Mip. faeni* (Edwards, 1972*b*), when tested by immunoelectrophoresis against pooled farmer's lung serum. Edwards detected a total of 14 antigens by this method and found that only one minor antigen was absent in each of two strains. The sensitivity of the immunoelectrophoresis method has been increased by using a longer electrophoretic separation on large glass slides and up to 18 antigens were detected in extracts of *Mip. faeni* by Walbaum *et al.* (1969).

The application of two-dimensional immunoelectrophoresis has enabled many more antigens to be recognized in extracts of *Mip. faeni* by Walbaum *et al.* (1973). In their study, 48 antigens were detected in a freeze-thaw extract of *Mip. faeni* and 75 antigens when purified immunoglobulins were incorporated in the gel. The technique of two-dimensional immunoelectrophoresis is therefore more suitable than simple immunoelectrophoresis for the detailed comparison of antigenic extracts.

The difficulty encountered when comparing complex two-dimensional immunoelectrophoresis patterns can be greatly simplified by the incorporation of an intermediate gel to absorb cross-reacting antigens. This modification has been used to identify antigens of clinical importance in *Mip. faeni* extracts (Moller *et al.*, 1976) and was used in our study to recognize antigens common to both *Mip. faeni* and *Mip. rectivirgula*.

The double dialysis extract of *Mip. faeni* (CUB 55) contained 57 antigens, and that of *Mip. rectivirgula* (CUB 977) 49 antigens as analysed by two-dimensional immunoelectrophoresis with and without intermediate gel containing rabbit antisera. Differences in the number and intensity of precipitin peaks have also been detected when extracts of three strains of *Mip. faeni* were tested by two-dimensional immunoelectrophoresis (Kurup & Fink, 1977).

The discrepancy in the total number of antigens detected in the two species could be due partly to differences in the immunological responses of the rabbits used to prepare the antisera, and also to the low concentration of certain antigens in the *Mip. rectivirgula* extract. This apparent difference in the quality of the preparations was also noted in the extracts of the 11 strains tested by simple immunoelectrophoresis, but could generally be eliminated by increasing the concentration of the extract. This demonstrated that certain antigens were present but at low, undetectable concentrations.

The patterns obtained when *Mip. faeni* and *Mip. rectivirgula* extracts were tested for cross-reactions showed no species-specific antigens (Fig. 4). Similarly all antibodies to the antigens of *Mip. rectivirgula* were absorbed by adding 50 mg of the freeze-dried extract of *Mip. faeni* (CUB 55). Further evidence for the very close serological similarity between the two species was provided when extracts of type strains were tested against pooled farmer's lung serum by two-dimensional immunoelectrophoresis (Fig. 2).

The strains of *Mip. rectivirgula* and *Mip. faeni* used in this study could not be separated by morphology or serology and could be separated by only two physiological characters – casein hydrolysis and resistance to 0.1% (w/v) potassium tellurite. Clearly, this suggests that all 11 strains belong to a single species and only one of the specific epithets should be used in future. The final decision as to which epithet is retained rests with the Judicial Commission; in reaching this decision, certain facts should be considered.

According to Rule 23a of the *International Code of Nomenclature of Bacteria* (Lapage *et al.*, 1975), the specific epithet *rectivirgula* has priority since it was published before the epithet *faeni*. However, when *Mip. faeni* was originally proposed, *Mip. rectivirgula* was still known by the binomial *Thermopolyspora rectivirgula* (Krassilnikov & Agre, 1964) and, indeed, it has not yet been formally transferred to the genus *Micropolyspora*. Furthermore, the original description of *Mip. rectivirgula* stated that colonies were colourless to slightly yellow with abundant yellowish aerial mycelium (Krassilnikov & Agre, 1964). *Micropolyspora faeni* produced orange–yellow to yellow–brown colonies with white aerial mycelium (Cross *et al.*, 1968). In this study, we have shown that *Mip. rectivirgula* does produce white aerial mycelium and it seems likely that Krassilnikov & Agre (1964) mistakenly applied the term ‘aerial mycelium’ to the yellowish substrate mycelium, formed on the surface of the agar plate, which also carries abundant spore chains. The previous unavailability of a type strain of *Mip. rectivirgula* and the inaccurate original description of this species have therefore contributed to this nomenclatural problem.

We therefore propose that the name *Micropolyspora faeni* be retained as a *nomen conservandum* (*Bacteriological Code*, Rule 56b; Lapage *et al.*, 1975). The implication of *Mip. faeni* as the main causative agent of farmer’s lung disease has resulted in this name appearing in over 200 separate publications. In contrast, the name *Micropolyspora rectivirgula* has rarely appeared in print and has never been associated with a hypersensitivity disease. If the name *Micropolyspora faeni* were to be rejected, confusion, particularly among medical microbiologists, would be widespread.

This investigation was supported by research studentships provided by the University of Bradford (M.P.A.J.) and the Natural Environment Research Council (A.J.Mc.C.).

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