**Proposal for Classifying
Organisms Related to *Mycoplasma laidlawii* in a Family
Sapromycetaceae, Genus *Sapromyces*,
within the Mycoplasmatales**

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SUMMARY

It is proposed that a second family be re-established within the Mycoplasmatales for those strains not requiring sterol. Following the nomenclature of Sabin, who first made this proposal, the sewage strains of Laidlaw and Elford are renamed *Sapromyces laidlawii* and assigned to a family Sapromycetaceae. The status of strains not requiring sterol is discussed, including that of sewage A and B. In the light of recent evidence the pig isolates, originally named *Mycoplasma granularum*, seem to belong to the genus *Sapromyces*. Recently described tissue-culture isolates, as yet unnamed, represent another species within the Sapromycetaceae. It is questionable whether the organisms classified as *M. laidlawii* var. *inocuum* deserve the status of a named variety of *S. laidlawii*.

In the first three decades of this century two organisms with unique properties were recognized, namely those causing contagious bovine pleuropneumonia and contagious agalactia. Ledingham (1933) suggested assigning them to the family Actinomycetaceae, but Turner (1935) proposed a new order, since he did not consider their unique properties permitted their inclusion in any existing order of the Schizomycetes. Subsequently other similar organisms were isolated from a variety of sources, leading to the recognition of a group of organisms which came to be known as pleuropneumonia-like organisms (PPLO) because of their similarity to the organism causing contagious bovine pleuropneumonia. Isolations included those made by Laidlaw & Elford (1936) from sewage and by Seiffert (1937*a, b*) from soil, compost and manure, and which were regarded as saprophytes.

Sabin (1941*a*) proposed a classification and nomenclature for those organisms of the pleuropneumonia group known at that time. Regarding them as distinct from the Schizomycetes, he suggested placing them in a new class with two families, one containing those species parasitic for animals and the other with one species represented by the sewage isolates of Laidlaw and Elford. Believing the sewage organisms to be saprophytic, the species was named *Sapromyces laidlawi* and the family Saprophytaceae, later amended to Sapromycetaceae to conform to the Rules of Bacteriological Nomenclature (Sabin, 1941*b*).

Edward & Freundt (1956) in their classification recognized only a number of species

of one genus *Mycoplasma*, belonging to an order Mycoplasmatales. They discussed whether the saprophytic strains should be placed in a separate genus, but did not consider that at that time there was sufficient evidence to justify it. At its first meeting in 1966 the Subcommittee on Taxonomy of Mycoplasmatales, while approving the system of classification and nomenclature proposed by Edward and Freundt and recommending its extension and also proposing that the Mycoplasmatales be assigned to a new class, took the view that it was still premature to establish a more elaborate taxonomic scheme for the mycoplasmas, such as placing *M. laidlawii* in a higher taxon (*Minutes*, 1967; Edward *et al.* 1967).

Proposal for the establishment of a second family

A classification in which only a number of species of one genus in a whole biological class is recognized is obviously unsatisfactory and can only be regarded as a temporary measure (see Edward & Freundt, 1969). Moreover, there have recently been numerous isolations of strains similar to *Mycoplasma laidlawii* from mammals, birds, man and tissue cultures and their investigation has revealed certain differences from the classic sewage strains. We therefore consider that the time has now arrived to propose that *M. laidlawii* should be placed in a higher taxon to permit further differentiation and classification of the strains related to it.

It has long been recognized that the 'saprophytic' sewage strains do not require media enriched with serum for growth, whereas other mycoplasmas are serum-dependent. It is now known that the latter need sterol for growth, but sterol is not required by *Mycoplasma laidlawii* and related strains. The requirement of most *Mycoplasma* species for sterol, all of which is incorporated in the membrane lipids, indicates that they differ in membrane composition from *M. laidlawii*. Differences in membrane structure are also suggested by the finding of different locations of nicotinamide adenine dinucleotide phosphate oxidase activity between *M. laidlawii* and six other species examined (Pollack, Razin & Cleverdon, 1965). We have pointed out elsewhere that sterol dependence is a character of fundamental importance. It is one shared by most mycoplasmas and thus helps to distinguish them from other micro-organisms and to justify the Mycoplasmatales being placed in a separate class from the Schizomycetes (Edward & Freundt, 1969). Since *M. laidlawii* and strains like it do not share this property it would seem justifiable to assign them to a taxon of at least as high a rank as a separate family within the Mycoplasmatales.

It has already been pointed out that Sabin (1941*a, b*) had proposed assigning the sewage strains of Laidlaw and Elford to a separate family and genus, named Sapro-mycetaceae and *Sapromyces* respectively. Since many of the strains that now require to be placed in this genus have been isolated from animals and it is even possible that some are pathogenic, a name suggesting a saprophytic role is unfortunate. However, as the name of the genus was validly published, it cannot be rejected as inappropriate under Rule 23 of the International Code of Nomenclature of Bacteria (1966).

It is therefore proposed that a family Sapro-mycetaceae be re-established in the order Mycoplasmatales with a genus *Sapromyces*, the type species being *Sapromyces laidlawii*.

Classification within the second family

Placing the non-sterol requiring strains in a separate family permits their further classification into other genera and species; investigation of such strains is therefore to be encouraged. A beginning has already been made by Tully and Razin. Some 24 strains, mostly isolated from animal and human materials and from tissue cultures, but including the classic strains from sewage and soil, had with few exceptions the same biological properties and behaved similarly when tested serologically by the indirect fluorescent antibody technique (Tully & Razin, 1968). These strains can therefore be regarded as representing the type species *Sapromyces laidlawii*.

The sewage isolates of Laidlaw & Elford (1936) fell into three serological groups. Sewage C appeared distinct by the agglutination reaction, but is no longer available for study. Sewage B was agglutinated only to low titre by an antiserum to Sewage A. In more recent tests of Sewage A and B strains by growth inhibition contradictory results have been obtained. Whereas Morton (1966) and Tully & Razin (1969) obtained equally sized zones of inhibition in reciprocal tests, Clyde (1964) and Argaman & Razin (1969) found only partial inhibition with the heterologous antisera. J. Fabricant (personal communication) was unable to distinguish between Sewage A and B by the metabolic inhibition test. The patterns of cell proteins of Sewage A and B produced by polyacrylamide-gel electrophoresis, although showing a basic similarity, differed in detail, and the many strains of *S. laidlawii* studied by Tully & Razin (1969) exhibited identical or very similar patterns to either Sewage A and B. In view of these findings, more particularly the contradictory serological results, it is not clear whether Sewage A and B strains should be established as distinct varieties or types of *S. laidlawii*.

Tully & Razin (1968) also examined four strains previously classified as *Mycoplasma granularum*. They found them indistinguishable from Laidlaw's sewage strains in their ability to grow on serum-free media and in their fermentation patterns. Like the sewage strains they produced carotenoids, whereas those serum-requiring *Mycoplasma* species tested did not. The electrophoretic patterns of the sewage strains and the *M. granularum* strains showed a basic similarity. When colonies of the two groups of organisms were tested with specific antisera by the direct fluorescent antibody technique there were no cross-reactions. However, there was a partial one-way cross between the *M. granularum* strains and a *S. laidlawii* antiserum in the indirect fluorescent antibody test. There was a similar one-way cross in the growth-inhibition test using one antiserum to a sewage strain, although no inhibition was found later using three other antisera (Tully & Razin, 1969). Similar crossing between the *M. granularum* strains and a *S. laidlawii* antiserum was noted in the complement fixation test without there being any fixation with *S. laidlawii* antigen and a high titred *M. granularum* antiserum (J. G. Tully, personal communication). These findings provide confirmation that the *M. granularum* strains represent a distinct species. This species must be reclassified as belonging to the genus *Sapromyces* and will be renamed *Sapromyces granularum*.

Tully & Razin (1969) investigated two tissue-culture isolates, serologically identical with each other but distinct from the 43 other species and serotypes of Mycoplasmales tested. Both were independent of sterol for growth, but unlike the sewage and *S. granularum* strains did not form carotenoids. It is clear that these two strains can be regarded as belonging to a distinct new species within the Sapromycetaceae, al-

though no name has yet been proposed. Only in the light of further work on the non-sterol requiring Mycoplasmatales will it be possible to evaluate the taxonomic importance of ability to synthesize carotenoids and to determine whether it is justifiable to place this species in a separate genus.

Consideration must also be given to the status of *Mycoplasma laidlawii* var. *inocuum* (Adler & Shifrine, 1964), isolated from chickens and capable of growth in serum-free media. Although originally designated as a separate species, *Mycoplasma inocuum* (Adler, Shifrine & Ortmyer, 1961) it was later recognized to be serologically similar to *M. laidlawii*. On the basis that it differed in regard to a biochemical property, namely an ability to metabolize glucose through a hexose monophosphate shunt, this shunt being absent from the representative strain of *M. laidlawii* (Castrejon-Diez, Fisher & Fisher, 1963), it was regarded as a variety of *M. laidlawii* and renamed *M. laidlawii* var. *inocuum* (Adler & Shifrine, 1964). This organism was included in the investigations of Tully & Razin (1968, 1969), where it behaved similarly to other strains of *S. laidlawii* in regard to carbohydrate fermentation, serology and electrophoretic pattern of cell proteins. At this stage, in the absence of detailed biochemical studies of the metabolism of other Sapromyces strains, it is questionable whether the chicken organism deserves the status of a named variety of *S. laidlawii*.

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