Coryneform Bacteria

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PREFACE

Coryneform bacteria are widely distributed in nature and are found in high numbers in some habitats. They are found in soil and on plants, in the sea and on fish, in dairy products and other foods, in activated sludge, in poultry deep litter and as part of the normal flora of man and other animals.

Coryneforms are important to our well-being in several ways. Apart from the human pathogens (the ravages in the past of *Corynebacterium diphtheriae* are well known), serious economical losses are caused by coryneform diseases of animals (including salmonid fish) and food crops. On the other hand some coryneforms are of considerable economic benefit, for instance in the industrial production of amino-acids, the microbial conversion of steroids and the ripening of certain cheeses.

The classification and identification of coryneform bacteria have long been difficult and confused. Because of their distinctive morphology, the coryneforms were traditionally regarded as a group of related organisms and were usually placed in the genus *Corynebacterium*. Attempts to split what became a very unwieldly genus were difficult and at best were only partly successful. Much of the problem was that with a few exceptions, "classical" features were of little value in separating taxa within the coryneform group and genera were thus ill-defined. As a result, new isolates could be placed with equal justification in any of several genera and new species were defined on very flimsy criteria.

However, the application of modern taxonomic methods such as numerical taxonomy, cell-wall analysis, lipid analysis and nucleic acid base composition and hybridisation determinations has had a dramatic effect on the systematics of the coryneform bacteria. Our thinking about the taxonomy of these organisms is changing radically and a reliable classification seems to be emerging at last.

I.J.B.

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This book, the first in the series of "Special Publications of the Society for General Microbiology", arose as a result of a symposium, entitled "Coryneform Bacteria", organised by the Systematics Group of the Society for General Microbiology and held in September, 1975. Because of the importance of and development in this topic, the contributors to this book agreed to up-date the material they presented at that symposium, so that the majority of the chapters now include work published up until about mid-1977.

We would like to express our appreciation firstly to all the contributors for the help that they have given in the preparation of this book, secondly to the Systematics Group of the Society for General Microbiology, in particular Dr. Dorothy Jones who was convener of this group at that time, and lastly to Mrs. M. Adams of the Cardiff University Industry Centre who had to type and set out all these pages.

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CONTENTS

WHAT DO WE MEAN BY CORYNEFORM BACTERIA?

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INTRODUCTION

In a sense the title of this chapter is somewhat misleading because it rather implies that there is general agreement as to which organisms should qualify for the description 'coryneform bacteria'. This is not so. For example, in the section entitled 'Coryneform Group of Bacteria' in the 8th edition of *Bergey's Manual* [1] the following genera are considered: *Corynebacterium, Arthrobacter* and *Cellulomonas,* with *Brevibacterium* and *Microbacterium* as *genera incertae sedis,* and 'tentatively' *Kurthia.* Yet in the introduction to that section, reference is made to a study by Davis and Newton [2] in which representatives of no less than eleven genera were referred to as coryneform bacteria; in addition to those already mentioned, they included *Mycobacterium, Nocardia, Jensenia, Listeria* and *Erysipelothrix.* Indeed this list could be extended further by including those genera which have been referred to elsewhere in the literature as anaerobic coryneform bacteria. It is therefore appropriate at the beginning of a book dealing with 'The Coryneform Bacteria' to re-examine the origin and meaning of that expression and to consider which taxa it embraces.

The word 'coryneform' was introduced into the general literature by Jensen [3] although he stated later [4] that the word was coined by Ørskov [5]. However in order to try to understand his reasons for doing so we must go back rather further in time. The genus *Corynebacterium* was created by Lehmann and Neumann in 1896 [6] to accommodate the diphtheria bacillus, and a few similar animal parasitic species were included later. The genus was defined mainly on the basis of morphological characters and staining reactions, features which at that time were considered very characteristic of these organisms. Also, the species included in *Corynebacterium* had a primarily respiratory mode of metabolism and their

lack of acid-fastness distinguished them from those of the morphologically rather similar genus *Mycobacterium*. However, in the course of time, it was realised that bacteria which had these general characteristics occurred in habitats other than the animal body. Because morphological similarity was then generally believed to indicate relatedness, they too were placed in *Corynebacterium*. For similar reasons several species of plant pathogenic bacteria, as well as a number of saprophytic species, were transferred to the genus. Thus eventually the name *Corynebacterium* was applied not only to human and animal parasitic species but also to a wide range of saprophytes from, for example, soil, water, milk, dairy products and fish, as well as to a number of plant pathogenic species [3,4,7]. All that this diverse assemblage of bacteria had in common were certain more or less distinctive morphological features and staining reactions. In the main the organisms were aerobes and facultative anaerobes but a few anaerobes were also included.

When Jensen wrote his extensive review in 1952 [3], it was obvious that the situation had got out of hand, a view forcibly expressed by Conn [8], and there was a general feeling that the genus should once more be restricted to the human and animal parasitic species. However, with the paucity of differential features then available there was no obvious way in which this could be achieved without resorting to habitat relationships. Admittedly, certain of the saprophytic corynebacteria could be accommodated in genera which already existed such as *Arthrobacter* and *Cellulomonas*, a course of action advocated by Clark [7]; but because of their poor characterisation Jensen considered that it would be premature to recognise such genera, a view later endorsed by Gibson [9]. In any case their acceptance would still have left many species unplaced, not least the plant pathogenic species.

In an attempt to meet these conflicting points of view Jensen proposed the following compromise: he referred to the animal parasitic and pathogenic species as *Corynebacterium sensu stricto* and to *Corynebacterium* as it had become in the wide sense as *Corynebacterium sensu lato*. It was to the latter grouping that he applied the expression 'coryneform bacteria'. It may be mentioned in passing that he considered that the "small group of thermoduric bacteria .... usually given generic rank as *Microbacterium*" were "closely allied" to *Corynebacterium sensu stricto*.

Thus the coryneform bacteria comprised *Corynebacterium sensu stricto* (in Jensen's sense) together with the saprophytic and plant pathogenic species of similar morphology and staining reactions. The grouping was therefore based on the

possession of certain distinctive morphological features; but because of the importance attached to morphology at that time, it was believed that the coryneform bacteria were a group of related organisms. This view was firmly held by Jensen and persisted for a number of years.

It should be noted that the morphology of coryneform bacteria changes during the growth cycle, sometimes quite extensively, and that it is also markedly influenced by the cultural conditions used [10,11]. Nevertheless, although the microscopical appearance varies quite widely in different coryneform bacteria, there are certain features which occur more or less constantly.

Using Jensen's description [3] as a guide Cure and Keddie [11] described these characteristic features in the following way. "In exponential phase cultures in complex media, irregular rods occur which vary considerably in size and shape and include straight, bent and curved, wedge-shaped and club-shaped forms. A proportion of the rods are arranged at an angle to each other to give V-formations but other angular arrangements may be seen. Rudimentary branching may occur, especially in richer media, but definite mycelia are not formed. In stationary phase cultures the cells are generally much shorter and less irregular and a variable proportion is coccoid in shape. The rods may be either motile or non-motile; endospores are not formed. They are Gram-positive but may be readily decolourised and thus may show only Gram-positive granules in otherwise Gram-negative cells. They are not acid-fast".

One of the more characteristic features of coryneform bacteria, the angular arrangements often referred to as V-formations, is frequently, but sometimes wrongly, attributed to "snapping" post-fission movements. According to Starr and Kuhn [12] this phenomenon was first described in *Corynebacterium diphtheriae* by Kurth in 1898 but the term "snapping" was applied to it by Hill in 1902. It was later shown to occur in a number of 'diphtheroid organisms' by Graham-Smith [13]. However, at least in some coryneform bacteria, V-formations may arise in a number of ways other than by snapping post-fission movements [12,14].

Komagata, Yamada and Ogawa [15] considered that "snapping division" was a characteristic of *Corynebacterium sensu stricto* whereas in other coryneform bacteria V-formations arose by "bending division". However "snapping" post-fission movements have been shown convincingly in at least one legitimate *Arthrobacter* sp. [12] and a second was used as a model for an ultrastructural explanation of the phenomenon [48].

THE GENERA

If only the bacteria which conform to the above description are considered, and which, in addition, have an entirely or primarily respiratory mode of metabolism, then we may refer to the following genera as the 'aerobic coryneform bacteria': *Corynebacterium*, *Arthrobacter*, *Cellulomonas*, *Curtobacterium*, *Microbacterium* (not *Microbacterium thermosphactum*) and *Brevibacterium* (in part). Most of these genera are heterogeneous in composition but progress is being made in delineating them more clearly.

Corynebacterium

There is a growing opinion that the genus should be restricted to those species with *meso*-diaminopimelic acid (DAP), arabinose and galactose in the cell-wall and which contain corynomycolic acids [16,17]. Most species with these characteristics are facultatively anaerobic [17,18] and have a relatively narrow range of DNA base ratios. This concept of the genus is generally supported by recent numerical taxonomic studies [19,20].

Corynebacterium in this sense includes *Corynebacterium diphtheriae* and its close relatives but also some saprophytic species, for example, *Corynebacterium glutamicum* and some very similar glutamic acid-producing nomenspecies, and *Microbacterium flavum*. However, it excludes some animal parasitic species, most saprophytes and all plant pathogenic species [18,21,22].

Arthrobacter

The genus comprises aerobes whose most characteristic feature is the possession of a growth cycle in which the irregular rods observed in young cultures are replaced by coccoid forms in older cultures [23]. However this feature is not exclusive to *Arthrobacter* [19,24] and moreover the species currently included are heterogeneous in cell-wall composition and in some other respects [23]. Nevertheless, the genus contains a large core of differently named strains which closely resemble the type species *Arthrobacter globiformis* in cell-wall composition and in vitamin, nitrogen and carbon nutrition [18,23]. An obvious way of making *Arthrobacter* more homogeneous would be to take the somewhat arbitrary step of limiting the genus to those species which, like the type, contain lysine in the cell-wall. Such a proposal has been made by Yamada and Komagata [25]. Desirable as such a move undoubtedly is, it does not reduce the dependence on morphology in the circumscription; many coryneform bacteria other

than legitimate arthrobacters contain lysine in the wall [18, 24,26].

Cellulomonas

Once distinguished from other coryneform bacteria mainly by the property of cellulolysis, it now seems that the genus, as revised by Clark [7,27], was well founded. Apart from *Cellulomonas fimi*, the available authentic named strains form a fairly homogeneous group with respect to morphology, cell-wall composition, vitamin, nitrogen and carbon nutrition, and DNA base ratios [24,25,28,29,30,31]. Some numerical studies have suggested that *Cellulomonas fimi* is misclassified [19,20], but the authentic strain usually examined has the cell-wall composition [18] and distinctive peptidoglycan structure [30] characteristic of the genus. (The strain referred to in references 18,19 and 20 was NCIB 8980 which purportedly is derived from ATCC 484, while that referred to in reference 30 was ATCC 484 (USDA 133), an authentic cotype strain. However, evidence obtained since this chapter was written (I.J. Bousfield, personal communication) has indicated that whereas NCIB 8980 does not attack cellulose, ATCC 484 is cellulolytic and also differs from NCIB 8980 in some other respects. These findings may explain the differences in opinion about the taxonomic position of this species).

Curtobacterium

This genus was created by Yamada and Komagata [25] with *Curtobacterium (Brevibacterium) citreum* as type species and contains some former *Brevibacterium* spp. and the plant pathogens *Corynebacterium flaccumfaciens* and *Corynebacterium poinsettiae*. Other work indicates that *Corynebacterium betae* should also be included in the genus [18,20]. The main distinguishing features of its members are: the presence of ornithine in the cell-wall, DNA base ratios in the range 66-71% and slow and weak acid production from sugars. Further work is necessary to determine whether or not *Curtobacterium* is a good genus but it is worthy of note that Schleifer and Kandler [17] independently grouped together the same species on the basis of peptidoglycan structure. This differed from that found in *Cellulomonas*, the genus that *Curtobacterium* appears to resemble most closely.

Microbacterium

Of the principal species bearing this name, *Microbacterium lacticum* seems to be a distinct and recognisable entity on the basis of numerical [20], non-numerical [32] and chemotaxonomic evidence [24,26,33]. Therefore, although the genus *Microbact-*

erium was considered *incertae sedis* in *Bergey's Manual* [1], *Microbacterium lacticum* could form the nucleus of a redefined genus, although the circumscription of such a genus would pose considerable problems. Certainly it could not be based on the exceptional heat resistance so long considered to be a distinctive characteristic of this species [34], because it does not appear to be a constant feature of all the strains [32].

There is now a great deal of evidence, both numerical taxonomic [20] and chemotaxonomic [18,22,24,26,33,35], supporting the transfer of *Microbacterium flavum* to *Corynebacterium*. This species has the characteristics of the restricted concept of *Corynebacterium* described above. Strains bearing the name *Microbacterium thermosphactum* do not have a coryneform morphology and represent a distinct taxon for which Sneath and Jones [36] have proposed the genus *Brocothrix* with *Brochothrix thermosphacta* as the only species.

Brevibacterium

This genus was proposed by Breed in 1953 [37,38], with *Brevibacterium linens* as type species, for a number of Gram-positive rods formerly classified as *Bacterium* spp. Because of the poor circumscription it became a repository for a large and varied collection of species of non-spore-forming Gram-positive rods which could not be accommodated elsewhere. However *Brevibacterium linens* has a coryneform morphology [11,39], a feature not noted in the 7th edition of *Bergey's Manual* [34], and chemotaxonomic [17,18] and numerical phenetic [20] evidence indicate that it is a good species. *Brevibacterium linens* could thus form the nucleus of a redefined genus *Brevibacterium* as suggested by Yamada and Komagata [25], but the genus would at present contain only one species. Many other *Brevibacterium* spp. can now be allocated to various other coryneform genera, such as *Corynebacterium*, *Arthrobacter* and *Curtobacterium*, and to the '*rhodochrous*' taxon [18,22,25], but at least one is a Gram-negative rod [40].

CONCLUSION

The dependence on morphological features and staining reactions for recognising coryneform bacteria does create problems, and although the genera described above would generally be accepted as coryneform bacteria, in other cases the situation is by no means as clear-cut. The large complex of strains usually referred to as the '*rhodochrous*' taxon, which may in part be overlapped by the genus *Gordona*, highlights

such problems. The '*rhodochrous*' complex is a recognisable but heterogeneous taxon equivalent in rank to the genera *Mycobacterium* and *Nocardia* [41,42,43]. However, whereas some '*rhodochrous*' strains have a coryneform morphology [18], others are mycelial and some are slightly acid-fast [20,44]. Thus in morphology and staining reactions this group cuts across the boundaries of the coryneform and nocardioform bacteria, and of the mycobacteria.

Also both numerical taxonomic [19,20] and chemotaxonomic [17,18,22] studies have shown that, contrary to earlier opinion, the various fractions of the coryneform group are taxonomically unrelated. As has been shown in many other bacterial groups, morphology is a poor criterion of relatedness. Indeed, *Corynebacterium* in the restricted sense is considered to be more closely related to *Mycobacterium* and *Nocardia* (the CMN group, ref.16) than to other coryneform bacteria. Also *Bacterionema matruchotii*, presently classified in the Actinomycetaceae because of its mycelial growth and filamentous morphology [45], has been shown to resemble *Corynebacterium diphtheriae* in cell-wall composition [46], DNA base ratio and glucose metabolism [45], and in the structure and size of the mycolic acids which it contains [47].

For reasons such as those mentioned, it has been suggested that the word 'coryneform' be dropped [16]. However the working concept of a large complex, the coryneform bacteria, is a useful one if it is accepted for what it is: it delineates a broad morphological group, sometimes imperfectly, but does not imply relatedness within it.

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