CRITERIA SUITABLE FOR SPECIES DIFFERENTIATION
IN CELLULOMONAS AND A REVISION OF THE
GENUS

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Evidence has recently been presented (2) that many of the cellulolytic bacteria of Kellerman and co-workers (10,11,12,13) belong in the Corynebacteriaceae. Accordingly, the genus Cellulomonas has been emended and its transfer to that family has been proposed (3). During the course of the work on Cellulomonas, several of the type cultures in the genus, including that of the type species, were made available to the writer by Dr. Nathan R. Smith, one of the initial co-authors (11). These type cultures² had been maintained in his laboratory for a period of about forty years. Their availability has provided an opportunity both to evaluate the stability and suitability of criteria employed for species differentiation in Cellulomonas and to consider the correctness of the names of the several species that have been assigned to the genus. From the laboratory data accumulated and from examination of published descriptions, recognition or disposition has been made of twenty-seven kinds of bacteria described as species of Cellulomonas.

It is the purpose of this paper to present evidence concerning the legitimacy of these twenty-seven putative species. Of these, ten are retained in Cellulomonas, fourteen names are reduced to synonyms, and three species are excluded from the genus.

CHARACTERISTICS OF THE GENUS CELLULOMONAS

Inasmuch as several organisms placed in Cellulomonas

- 1 U. S. Department of Agriculture and Iowa Agricultural Experiment Station, Ames, Iowa. Journal Paper No. 2259, Project 965, of the Iowa Agricultural Experiment Station.
- 2 These cultures are available from the American Type Culture Collection, 2029 M Street, NW, Washington, D.C.

by earlier workers are herein excluded from the genus, it is appropriate briefly to describe Cellulomonas before evaluating criteria for the separation of species within this group.

An earlier discussion (3) has summarized my reasons for placing Cellulomonas in the family Corynebacteriaceae and for recognizing at least three genera (Corynebacterium, Arthrobacter, and Cellulomonas) within that family. The species in these genera show angular, branched, and irregular cells, and nearly all recent students of them have stated that they should be grouped in the same family. In some instances, new combinations have been proposed in the genus Corynebacterium. Cellulomonas fimi has been designated Corynebacterium fimi by Jensen (8), and Arthrobacter globiforme has been designated Corynebacterium globiforme by Wood (21). Jensen (9) has recently stated that the animal pathogens, the soil saprophytes, and the plant pathogens of the coryneform bacteria "constitute Corynebacterium sensu lato."

The writer believes that various cellulolytic and globiform organisms from soil should not be placed in the genus whose type species is Corynebacterium diphtheriae, even though such organisms are recognized in Corynebacteriaceae. It has been proposed that Arthrobacter Conn and Dimmick (5) be retained. This genus is differentiated from Corynebacterium by its failure to produce acid from fermentable sugars and by its ability to liquefy gelatin and to grow satisfactorily on synthetic media. Jensen (9) speaks of Arthrobacter as follows: "In this group the energy metabolism is almost wholly oxidative, synthetic ability is well developed (growth with inorganic nitrogen and without accessory factors), and proteolytic power is considerable." In addition, it may be pointed out that young cells of Arthrobacter spp. are much more commonly Gram-negative than are those of the animal diphtheroids. On aging, cells of Arthrobacter commonly become Gram-positive, and coccoid or globiform in appearance, as described by Conn and Dimmick (5). Reports of motility in Arthrobacter are sufficiently numerous to show that

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motility must be recognized within this group. Despite scattered reports to the contrary, motility has not yet been demonstrated in organisms that can beyond reasonable doubt be considered as animal diphtheroids. Jensen (9) likewise regards the animal parasitic corynebacteria as genuinely nonmotile, and the soil and plant coryneform bacteria as "incidentally" motile.

Cellulomonas is differentiated both from Corynebacterium and Arthrobacter by its ability to cause disintegration of cellulose. Cellulomonas also is differentiated from Arthrobacter by the ability of the former to produce distinct acidity in dextrose broth. In my experience, Gram-negative staining is more commonly encountered in cells of Cellulomonas than in cells of Arthrobacter. Cellulomonas usually is Gram-negative during the first 24 hours of growth, thereafter Gram-positive or Gram-variable staining is secured on cells grown under favorable conditions. In cultures several days old, the Gram reaction is usually negative.

The ability of Cellulomonas to attack cellulose is apparently a very stable characteristic. It remains demonstrable in cultures maintained in Smith's laboratory for forty years. Siu (19) has remarked that the term "cellulolytic" is used by some workers simply to denote a loosening of the adhesion of the fibers in filter paper strips even though actual cellulose destruction is negligible. The Cellulomonas species recognized below and for which type cultures have been available possess the ability to dissect filter paper strips (0.7 by 6 cm.) standing half immersed in 0.5 per cent peptone broth. The dissection of the filter paper strips in such tubes occurs at the surface of the broth.

CRITERIA FOR SPECIES IDENTIFICATION

The differential criteria most used in the separation of species of Cellulomonas, both by the first students of the genus and in more recent monographs, including the several editions of Bergey's Manual (1), have been the following: motility, gelatin liquefaction, chromogenesis, action on milk, production of ammonia and nitrite, and production of

indole. These criteria are discussed in the following paragraphs.

Motility: Kellerman and co-workers (10, 11, 12, 13) described about two-thirds of their proposed species as motile. Both motile and nonmotile species are represented among the cultures that have been maintained. Cellulomonas biazotea, C. cellasea, and C. gelida, initially described as motile, have been found motile in recent examinations (4). C. flavigena, C. uda, and C. fimi were initially described as nonmotile (10, 11, 13). The first two have been found nonmotile, while the last has shown feeble motility. Flagella have been demonstrated in gold-shadowed cell preparations of C. fimi examined with an electron microscope (4). Inasmuch as the property of motility appears constant, within reasonable limits of errors of observation, it appears desirable to retain this criterion for separation of species in Cellulomonas.

Gelatin liquefaction: Kellerman and co-workers (10, 11, 12, 13) employed gelatin stab cultures for determining liquefaction. For slowly growing and weakly gelatinolytic bacteria, this technique is not entirely reliable. It will be pointed out below that Kellerman and co-workers listed certain species as non-gelatinolytic in some discussions but in others listed the same species as very slowly gelatinolytic. In the current work, cultures of Cellulomonas were grown on gelatin agar plates, and tests for gelatin hydrolysis were made according to the method of Frazier (6). All cultures studied were found capable of gelatin hydrolysis, including Cellulomonas gelida and C. subalba, initially described as non-gelatinolytic. Accordingly, certain species separations that have formerly been made on the basis of gelatin liquefaction no longer appear tenable.

Chromogenesis: Kellerman and co-workers (10, 11, 12, 13) found that approximately one-third of their cellulolytic bacteria showed yellow pigmentation on beef agar. Of their yellow chromogenic and cellulolytic cultures, four have been examined, and all have produced yellow or lemon-yellow growth on nutrient agar. Of four cellulolytic cultures initially described as non-chromogenic, all have shown white,

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ivory, or light cream-colored growth on nutrient agar. Various workers have pointed out the pitfalls in using chromogenesis for taxonomic purposes. Nevertheless, yellow chromogenesis appears to be a relatively stable characteristic within the genus Cellulomonas, and therefore it is retained as an acceptable criterion for the differentiation of species.

Action on milk: With but few exceptions, all species of Cellulomonas noted in the sixth edition of Bergey's Manual (1) are described as capable of producing acid in milk. The excepted species (Cellulomonas rossica, C. folia, and C. ferruginea, to be discussed below) do not meet the generic requirements for Cellulomonas in several respects and should be excluded from the genus. Kellerman and co-workers differentiated certain of their species on the basis of acidity only in milk, in contrast to acidity and coagulation or digestion. Coagulation and digestion when present appeared slowly, usually after several weeks of incubation. Jensen (8), in reporting on the cultural responses of Cellulomonas fimi, noted in addition to an acid reaction in milk, coagulation after 3 weeks at 37°C., but not at 28° to 30°C. McBeth and Scales (13) reported acid production only for C. fimi in milk. Reliance on variations in amount of acidity or extent of digestion in milk for species differentiation in Cellulomonas does not appear to be indicated.

Ammonia, nitrite, and indole production: Kellerman and co-workers (10, 11, 12, 13) noted ammonia, nitrite, and indole production for several of their cellulolytic bacteria. Inasmuch as the carbohydrate fermentation responses of the same bacteria were of little differential value, Kellerman et al. (11), McBeth (12), and the several editions of Bergey's Manual (1) have relied largely on differences in ammonia, nitrite, and indole reactions for species differentiation.

Of the three tests, nitrite production from nitrates has been found to offer the most promise for current use. The type species Cellulomonas biazotea was initially described as nitrite positive, and the type culture still produces nitrites from nitrates. Similarly, the positive nitrite reac-

tions initially reported for C. cellasea, C. flavigena, C. uda, and C. subalba, and the negative nitrite response initially reported for C. gelida, have been confirmed. C. fimi, initially reported nitrite positive, has been found nitrite negative. Jensen (8) found C. fimi to be nitrite positive.

Ammonia production was noted by Kellerman and co-workers for nearly one-half of their species. In our work, no species of Cellulomonas has been found to produce ammonia. Insofar as type cultures available are concerned, ammonia production therefore appears of doubtful value for species separation. Notwithstanding this, discarding the use of the ammonia test is not recommended at the present time. In the case of gelatin liquefaction, some species initially reported negative have been found gelatinolytic. There is reasonable probability that their gelatinolysis was initially overlooked because of slow growth or the test conditions employed, and not that the property has since been acquired. With ammonia production, however, the discrepancy is in the other direction - cultures initially reported positive are now found negative. It is possible that their ability to produce ammonia has been lost. Until such time as the question of ammonia production has been investigated in a number of freshly isolated cultures, it appears advisable to accept certain species of Cellulomonas that would not be recognized if ammonia production were discarded entirely from taxonomic consideration.

Kellerman et al. (11) and McBeth (12) found several of their cultures capable of producing indole. No species of Cellulomonas has been found indole positive in this laboratory. Unfortunately, nearly all the type cultures that have been available for testing represent species that initially were reported as indole negative. An exception has been Cellulomonas fimi, initially described as indole positive. Jensen (8) has studied a culture of C. fimi and has also found it indole negative.

The retention of the indole test for differentiation of species of Cellulomonas must be supported by the same reasoning that supports retention of the ammonia production test.

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But inasmuch as there exists some doubt on the accuracy of the indole tests reported in the earlier literature, the writer has considered it advisable to disregard the indole test for species differentiation in Cellulomonas. The very few occasions in which the indole test determines whether or not a given species reported in the literature is entitled to recognition will be pointed out below.

Carbohydrate fermentation: Taylor (20) summarized his work on the availability of carbon compounds to the soil bacteria in the genus Arthrobacter with the statement that the inconsistent and changeable reactions produced on different sugars only emphasized the futility of any attempt to classify the organisms on the basis of acid production. With but one exception, carbohydrate fermentation responses have not been found informative for species differentiation in Cellulomonas. C. fimi ferments xylose and arabinose, whereas all other species of Cellulomonas that were tested failed to attack these pentoses.

Other tests: All reputable cultures of Cellulomonas available for study hydrolyzed starch. None produced acetylmethylcarbinol. None hydrolyzed sodium hippurate or uric acid. Cellulomonas gelida and C. subalba caused some browning in lead acetate agar, indicating feeble hydrogen sulfide production. Thermal resistance studies revealed no noteworthy differences among the type cultures studied. No serological studies were undertaken.

THE LEGITIMACY OF SPECIES IN CELLULOMONAS

Eighteen species are placed in Cellulomonas in the sixth edition of Bergey's Manual (1) and six nonmotile cellulolytic species previously listed in Cellulomonas are placed in Bacterium. Following Jensen (8), Cellulomonas fimi was transferred to the genus Corynebacterium. The legitimacy of these several species for inclusion in Cellulomonas should be reviewed.

Individual species are discussed in the order in which they appear in the dichotomous key given at the close of this section. Names of species not recognized in the key but considered

synonyms of species names included are discussed immediately following the species with which they are merged. Species excluded from Cellulomonas are discussed last.

1. Cellulomonas biazotea (Kellerman, McBeth, Scales Smith) Bergey et al. 1923.

Synonyms:

Bacillus biazoteus Kellerman et al. 1913.

Proteus cellulomonas var. biazoteus Pribram 1933.

Inasmuch as Cellulomonas biazotea is the type species, it is fortunate both that the type culture has been maintained and that the culture corresponds very closely to its initial description. The culture remains cellulolytic, chromogenic, gelatinolytic, motile, nitrite positive, and ammonia negative.

A minor correction should be made concerning the "optimum temperature 20°C." as given in Bergey's Manual (1). Kellerman et al. (11) stated for all their species: "They grow well from 20° to 37.5°C., and more rapidly at the higher temperature." McBeth (12) stated: "The optimum temperature for most species seems to be between 28° and 33°C."

My observations agree with those of McBeth, and cultural tests have commonly been performed at 30°C. A similar correction, stating the optimum temperature as 28° to 33° C. and not as 20°C, can also be made for the other recognized species of Cellulomonas.

2. Cellulomonas cellasea (Kellerman et al.) Bergey et al. 1923.

Synonyms:

Bacillus cellaseus Kellerman et al. 1913.

Bacillus concitatus McBeth 1916.

Cellulomonas concitata (McBeth) Bergey et al. 1923.

Bacillus desiduus McBeth 1916.

Cellulomonas deciduosa (McBeth) Bergey et al. 1923.

Cellulomonas desidiosa (McBeth) Bergey et al. 1939

Bacillus subalbus Kellerman et al. 1913.

Cellulomonas subalbus (Kellerman et al.) Clark 1951.

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The original description (11) of C. cellasea permits its inclusion in the emended genus Cellulomonas. Such inclusion has been substantiated by examination of the type culture. Culturally and in its biochemical responses, this species resembles C. biazotea, except that C. cellasea produces no yellow pigment. It is considered a legitimate species.

2a. Cellulomonas concitata (McBeth) Bergey et al.

The original description of this organism makes it appear to be correctly placed in Cellulomonas. No culture was available to the writer. Except for the slightly more vigorous growth and the reported production of indole, this organism so closely resembles C. cellasea that its name becomes a synonym of C. cellasea, which has priority of description. If indole-positive cultures otherwise resembling C. cellasea can be isolated and shown to be culturally stable, then recognition of C. concitata might be justified.

2b. Cellulomonas desidiosa (McBeth) Bergey et al.

No culture was available, but this organism likewise appears to be correctly placed in Cellulomonas. Disregarding its reported negative gelatinolysis, C. desidiosa does not differ from C. cellasea, and the name C. desidiosa becomes a synonym of C. cellasea which has priority. Both C. desidiosa and C. concitata (q.v.) were reported by McBeth (12) to be indole-positive.

2c. Cellulomonas subalba (Kellerman et al.) Clark.

Kellerman et al. (11) differentiated Bacillus subalbus from B. pusilus (Cellulomonas pusilla) by the more luxuriant growth of the former on beef agar and by its failure to liquefy gelatin. Discounting its initially reported negative gelatinolysis and ignoring the slight difference reported (11) between its growth on agar ("moderate, flat, white or faintly yellowish white") and that of C. pusilla ("moderate, flat, almost colorless semi-transparent"), there appears justification for uniting these two species. But the

available type culture of Cellulomonas subalba, No. 489 in the American Type Culture Collection, in addition to being weakly gelatinolytic, fails to produce ammonia, and in other respects resembles C. cellasea. The name C. subalba is reduced to a synonym of C. cellasea.

3. Cellulomonas aurogena (Kellerman et al.) Bergey et al. 1923.

Synonyms:

Cellulomonas aurogenes (sic) (Kellerman et al.) Bergey et al. 1923.

Bacillus aurogenus Kellerman et al. 1913.

Cellulomonas flava Sack, 1924.

Cellulomonas flavus (sic) Sack, 1924.

Bacillus gilvus McBeth, 1916.

Cellulomonas gilva (McBeth) Bergey et al. 1923.

No culture of C. aurogena has been available, but the original description appears to place it in Cellulomonas. Until such time as cultures conforming to the description are known, the possibility of identity with C. biazotea is not excluded. Meanwhile, C. aurogena may be recognized as a legitimate species, differentiated from C. biazotea by the reported ability of C. aurogena to produce ammonia.

In their initial description, Kellerman et al. (11) employed the binomial Bacillus aurogenus. In all six editions of Bergey's Manual (1), the name has been cited as B. aurogenes.

3a. Cellulomonas flava Sack, 1924.

No culture has been available. The initial description of this organism placed emphasis on its cellulolytic ability, but presented somewhat limited cultural observations. Its reported motility, yellow chromogenesis, and nitrite and ammonia production seem to identify this bacterium with Cellulomonas aurogena, of which the name C. flava becomes a synonym. Its reported pellicle formation in broth and light brown growth on potato are not typical of C. aurogena.

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3b. Cellulomonas gilva (McBeth) Bergey et al. 1923.

No culture has been available, but the original description of this bacterium makes it appear to be correctly placed in Cellulomonas. McBeth (12) differentiated it from C. aurogena largely on the basis of its failure to liquefy gelatin. With its reportedly negative gelatinolysis ignored, its yellow chromogenesis and production of both nitrite and ammonia seem to identify it with C. aurogena. The name C. gilva is reduced to a synonym of C. aurogena.

4. Cellulomonas pusilla (Kellerman et al.) Bergey et al. 1923.

Synonyms:

Cellulomonas pusila (sic) (Kellerman et al.) Bergey et al. 1923.

Bacillus pusilus (sic) Kellerman et al. 1913.

Bacillus caesius Kellerman et al. 1923; Cellulomonas caseia (Kellerman et al.) Bergey et al. 1923. Cellulomonas casei (Kellerman et al.) Bergey et al. 1934.

Cellulomonas caesia (Kellerman et al.) Bergey et al. 1939.

Bacillus iugis McBeth 1916.

Cellulomonas iugis (McBeth) Bergey et al. 1923.

No culture of Cellulomonas pusilla has been available, but the species is recognized as legitimate on the basis of its reported ability to produce ammonia and failure to show yellow chromogenesis. Its lack of chromogenesis differentiates it from C. aurogena, and its production of ammonia from C. cellulasea.

4a. Cellulomonas caesia (Kellerman et al.) Bergey et al. 1923.

Kellerman et al. (11) differentiated C. caesia from C. pusilla by the ability of the former to cause coagulation and digestion in litmus milk and also by growth on beef agar. C. caesia produced "moderate, flat, thin, slightly bluish fluorescent at angle of 45°" and C. pusilla "moderate flat, almost colorless semi-transparent growth." The lack of other differences makes the recognition of C. caesia as a species appear inadvisable, and the name C.

caesia becomes a synonym of C. pusilla. No cultures have been examined.

4b. Cellulomonas iugis (McBeth) Bergey et al. 1923.

An initial revision of a generic key primarily to correct for gelatin liquefaction brought Cellulomonas iugis and C. pusilla into juxtaposition. Their positive and negative properties as tabulated by McBeth (11) in his summarizing tables 2 and 3 were tabulated as follows:

<u>C. iugis</u> :	----	+	*	+	*	+	--	+	+	-	+	-
<u>C. pusilla</u> :	----	+	-	-	-	+	--	+	+	-	+	-

The two disagreements (marked by asterisks) represented positive gelatin liquefaction and growth on potato for C. iugis and negative gelatin liquefaction and no growth on potato for C. pusilla.

The first disagreement appears to be a typographical error which both McBeth (12) and Bergey's Manual (1) perpetuated in their taxonomic keys. The original description (12) of gelatin liquefaction by C. iugis stated: "Moderate growth at surface and along track of needle at 5 days; napiform liquefaction at 30 days." That (11) for C. pusilla stated: "No surface growth, liquefaction slow, usually becoming infundibuliform or saccate after 15 days."

McBeth (12) described the growth of C. iugis on potato as: "Scant, glistening colorless growth when very heavily inoculated; light inoculation produces no growth." Kellerman et al. (11) described that of C. pusilla as: "No apparent growth after 20 days, but potato is bleached along the track of the inoculum."

Portions of the initial descriptions of these species have been cited in some detail partly to furnish an example of the extremely minor cultural variations on which McBeth (12) based descriptions of new species. Surely, species distinctions must be more tangible than light or heavy inoculation of potato. The writer does not recognize the two species as distinct, even though no cultures have been examined. The

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name C. iugis is made a synonym of C. pusilla.

5. Cellulomonas fimi (McBeth and Scales) Bergey et al. 1923. 1930.

Synonyms:

Bacterium fimi McBeth and Scales 1913.

Cellulomonas fima (McBeth and Scales) Bergey et al. 1923.

Corynebacterium fimi (McBeth and Scales) Jensen 1934.

Bacterium liquatum McBeth and Scales 1913.

Cellulomonas liquata (McBeth and Scales) Bergey et al. 1923.

Jensen (8) found Cellulomonas fimi to show the morphology of the corynebacteria. He concluded that it should not be included in the genus Cellulomonas. At the time of his work he did not have type cultures of other species of Cellulomonas available for study. Subsequent work (2) has shown that the type species C. biazotea as well as others in the genus also show the irregular morphology and staining responses that Jensen encountered in Cellulomonas fimi. With emendation of the genus Cellulomonas, C. fimi is recognized as a legitimate species.

- 5a. Cellulomonas liquata (McBeth and Scales) Bergey et al. 1923.

McBeth and Scales (13) reported that C. liquata produced a yellow chromogenesis more readily than did C. fimi. Jensen (8) considered the two species as closely related, and the sixth edition of Bergey's Manual (1) stated that the two should be regarded as identical. In our laboratory, comparison of type cultures has shown them to be culturally identical. The synonymy of the name C. liquata with C. fimi suggested by other workers is substantiated.

6. Cellulomonas galba (Kellerman et al.) Bergey et al. 1923.

Synonym:

Bacillus galbus Kellerman et al. 1913.

No culture of this species has been examined, but the original description (11) appears to place the organism in Cellulomonas. C. galba is recognized as a legitimate species. It is differentiated from the yellow chromogens C. biazotea and C. aurogena by the reported failure of C. galba to reduce nitrates to nitrites.

7. Cellulomonas gelida (Kellerman et al.) Bergey et al. 1923.

Synonyms:

Bacillus gelidus Kellerman et al. 1913.

Bacillus albidus McBeth 1916.

Cellulomonas albida (McBeth) Bergey et al. 1923.

Bacillus almus McBeth 1916.

Cellulomonas alma (McBeth) Bergey et al. 1923.

Bacillus bibulus McBeth and Scales 1913.

Cellulomonas bibula (McBeth and Scales) Bergey et al. 1923.

Examination of the type culture of C. gelida has shown it is correctly placed in Cellulomonas. The species is gelatinolytic, contrary to the initial description (11). C. gelida is differentiated from C. cellasea, also non-chromogenic and motile, by the failure of C. gelida to reduce nitrates to nitrites.

7a. Cellulomonas albida (McBeth) Bergey et al. 1923.

No culture has been examined. McBeth (12) differentiated this organism from C. gelida by the less vigorous growth of C. albida and its failure to produce ammonia. C. gelida may also fail to produce ammonia. The older species, i.e., C. gelida, is recognized, and the name C. albida is reduced to synonymy.

7b. Cellulomonas alma (McBeth) Bergey et al. 1923.

Although no culture has been examined, the very slight difference in growth on beef agar which McBeth (12) considered sufficient to differentiate C. albida from C. alma is regarded as insufficient for species separation. The name C. alma is regarded as a synonym of C. gelida.

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- 7c. Cellulomonas bibula (McBeth and Scales) Bergey et al. 1923.

The type culture was found to be non-cellulolytic and to differ in several other respects from the initial description (13). As originally described, C. bibula can be regarded as an indole-positive variant of C. gelida. Otherwise, its reported cultural properties suggest its identity with C. gelida. If indole-positive cultures resembling C. gelida otherwise can be isolated and shown to be culturally stable, then recognition of C. bibula as a separate species could be justified. The name C. bibula is regarded as a synonym of C. gelida.

8. Cellulomonas flavigena (Kellerman and McBeth) Bergey et al. 1923.

Synonyms:

Bacillus flavigena Kellerman and McBeth 1912.

Bacterium flavigena McBeth and Scales 1913.

Bacterium flavigenum Kellerman et al. 1913.

Cellulomonas idonea (McBeth) Bergey et al. 1923.

The type culture of C. flavigena has been found in general agreement with the original description (10), and the species is entitled to recognition in the genus Cellulomonas. Its nonmotility, yellow chromogenesis, and ability to reduce nitrates distinguish it from other recognized species.

- 8a. Cellulomonas idonea (McBeth) Bergey et al. 1923

No culture is currently available, therefore species recognition or disposition can be made only on the basis of the original description (12). The yellow chromogenesis, production of nitrite, acidity in milk, failure to produce ammonia, and finally its reported nonmotility would identify it with C. flavigena and reduce the name C. idonea to synonymy.

9. Cellulomonas uda (Kellerman et al.) Bergey et al. 1923.

Synonyms:

Bacterium udum Kellerman et al. 1913.

Proteus cellulomonas var. udus Pribram 1933.

C. uda is recognized as a nonmotile, nonchromogenic species of Cellulomonas. The type culture currently available has been found to agree in its characteristics with the original description (11).

10. Cellulomonas acidula (Kellerman et al.) Bergey et al. 1923.

Synonyms:

Bacterium acidulum Kellerman et al. 1913.

Bacterium castigatum McBeth 1916.

Cellulomonas costigata (sic) (McBeth) Bergey et al. 1923.

Cellulomonas castigata (McBeth) Bergey et al. 1939.

Bacterium lucrosum McBeth 1916.

Cellulomonas lucrosa (McBeth) Bergey et al. 1923.

No culture of C. acidula was available, but the reported failure of the species to produce nitrites from nitrates is considered sufficient basis to differentiate C. acidula from C. uda.

- 10a. Cellulomonas castigata (McBeth) Bergey et al. 1923.

No culture has been available. C. castigata was described by McBeth (12) as producing more acidity from fermentable carbohydrates than was produced by C. acidula; otherwise, the organisms appear to have been culturally identical. The name C. castigata is made a synonym of C. acidula.

- 10b. Cellulomonas lucrosa (McBeth) Bergey et al. 1923.

No culture has been available. C. lucrosa was described by McBeth (12) as showing slightly better growth on agar and more turbidity in broth than is shown by C. acidula; otherwise, the organisms appear to have been culturally identical. The name C. lucrosa is made a synonym of

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11. Cellulomonas (?) ferruginea (Rullmann) Bergey et al. 1923.

No culture is available. The original description of Bacillus ferrugineus by Rullmann (15) fails to mention any cellulolytic activity. Rullmann's bacterium produced alkalinity in milk, failed to ferment sugars, produced a rusty brown color on several cultural media, and on nutrient glycerol agar at 37°C., "nach mehreren Tagen durch die ganze Masse grün fluorescierend." Later, van Itersen (7) reported cellulose degradation by a very small bacillus associated with a large coccus. The former he named Bacillus ferrugineus. This name is considered an illegitimate later homonym of B. ferrugineus Rullmann. It is quite probable that the cellulolytic activity observed by van Itersen was caused by a species of Sporocytophaga. There appears no sufficient reason to recognize Bacillus ferrugineus of Rullmann as a species of Cellulomonas.

12. Cellulomonas folia Sanborn 1926.

No culture was studied by the writer. Published descriptions of this species are too fragmentary to permit its inclusion in Cellulomonas. Sanborn and Hamilton (18) stated that carbohydrates were not fermented. According to a Bergey Manual citation of Sanborn's unpublished notes, both acid and gas are slowly produced from carbohydrates. Inasmuch as gas production is not recognized in Cellulomonas, C. folia is excluded from the genus. Its production of alkalinity in milk is also not typical of Cellulomonas.

13. Cellulomonas rossica (Kellerman and McBeth) Bergey et al. 1923.

The initial description of this species does not support its inclusion in Cellulomonas. It was described (10) as causing rapid liquefaction of gelatin, producing alkalinity in milk, and failing to produce acidity in carbohydrate media. The stock culture currently available agrees with the initial

description in several respects but there is one important difference in that it is not cellulolytic. Identification was not made. Morphologically, the organism does not appear to belong to the Corynebacteriaceae.

KEY TO THE SPECIES OF THE GENUS CELLULOMONAS

- I. Motile with one or few peritrichous flagella.
 - A. Nitrites produced from nitrates.
 - 1. Xylose and arabinose not fermented.
 - a. Ammonia not produced.
 - b. Yellow chromogenesis on nutrient agar.
 - 1. Cellulomonas biazotea.
 - bb. White, greyish, or ivory growth on agar.
 - 2. Cellulomonas cellasea.
 - aa. Ammonia produced.
 - b. Yellow chromogenesis on nutrient agar.
 - 3. Cellulomonas aurogena.
 - bb. White, greyish, or ivory growth on agar.
 - 4. Cellulomonas pusilla.
 - 2. Xylose and arabinose fermented.
 - 5. Cellulomonas fimi.
 - B. Nitrites not produced from nitrates.
 - 1. Yellow chromogenesis on nutrient agar.
 - 6. Cellulomonas galba.
 - 2. White, greyish, or ivory growth on agar.
 - 7. Cellulomonas gelida.
- II. Nonmotile.
 - A. Nitrites produced from nitrates.
 - 1. Yellow chromogenesis on nutrient agar.
 - 8. Cellulomonas flavigena.
 - 2. White, greyish, or ivory growth on agar.
 - 9. Cellulomonas uda.
 - B. Nitrites not produced from nitrates.
 - 10. Cellulomonas acidula.

SUMMARY

From an examination of initial descriptions and in certain instances of type cultures, recognition or disposition has been made of twenty-seven species of bacteria placed in

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Cellulomonas by various authors.

Cellulomonas biazotea, C. cellasea, C. aurogena, C. pusilla, C. galba, C. gelida, C. fimi, C. flavigena, C. uda, and C. acidula are recognized as good species. A dichotomous key for their identification has been prepared.

The names of fourteen species previously recognized in Cellulomonas are made synonyms of the above species. Cellulomonas albida, C. alma, and C. bibula are made synonyms of C. gelida. C. concitata, C. desidiosa, and C. subalba become synonyms of C. cellasea. C. flava and C. gilva are made synonyms of C. aurogena, and C. caesia and C. iugis, of C. pusilla. The opinion of other workers that C. liquata is a synonym of C. fimi is substantiated. C. idonea becomes a synonym of C. flavigena, and C. castigata and C. lucrosa, of C. acidula.

Three bacteria heretofore assigned to Cellulomonas fail to meet the requirements for recognition in the genus and are excluded. These species are: Cellulomonas ferruginea, C. folia, and C. rossica.

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