TRANSFER OF BACTERIUM ANITRATUM
SCHAUB AND HAUBER 1948 TO THE GENUS
LINGELSHEIMIA GEN. NOV.

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ABSTRACT. On the basis of morphological properties Bacterium anitratum Schaub and Hauber 1948 is classified in the family Moraxellaceae Ryter and Piéchaud 1963. Since the species differs in its physiological and biochemical properties from species of the genus Moraxella a new genus is proposed and named Lingelsheimia. It is proposed to transfer Bacterium anitratum Schaub and Hauber 1948 to this genus. Lingelsheimia anitrata comb. nov. is the type-species of the genus (basionym: Bacterium anitratum Schaub and Hauber).

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INTRODUCTION

Schaub and Hauber (1948) described Bacterium anitratum as a morphologically and biochemically homogenous new species but they did not indicate its taxonomic position. They stated: "As the taxonomic position of these Gram-negative bacilli is not clear, it seems advisable at this time to place them in the genus Bacterium, which is reserved for organisms not yet assigned to any of the recognized genera."

During the following years B. anitratum was placed in several bacterial genera, including Achromobacter by Brisou and Morichau-Beauchant (1952) and by Stenzel and Mannheim (1963), Acinetobacter by Brisou and Prévot (1954) and Cytophaga by Lautrop (1961). Other authors suggested that Bacterium anitratum is closely related to members of the genera Mima and Herellea (Ewing 1949) and Moraxella (Piéchaud and Second 1951).

The transfer of Bacterium anitratum to the following genera should therefore be discussed.

Achromobacter Bergey et al. 1923
Acinebacter Brisou and Prévot 1954
Cytophaga Winogradsky 1929
Herellea DeBord 1942
Mima DeBord 1942
Moraxella Lwoff 1939

The respective suggestions for the allocation of Bacterium anitratum to any of the above genera with the exception of Cytophaga were based mainly on the similarity of biochemical properties to species in the genera listed.

i. Relationship of B. anitratum to the Genus Cytophaga

The sole proposal for the transfer to the genus Cytophaga was based on a morphological characteristic of Bacterium anitratum. Lautrop (1961) observed a gliding and creeping motion of the cells. This finding was confirmed by Halvorsen (1963), and in part by Schlieber (1964) who frequently found, besides a seemingly gliding and creeping motion, jerky movements and the production of a garland-like border pattern surrounding the colonies on nutrient- and blood-agar media after 8-10 days incubation at room temperature. In an emendation of the species description these properties are considered of taxonomic importance and significance.

Lautrop (1961) suggested the transfer of Bacterium anitratum to the genus Cytophaga. But there are serious objections to this proposal. The many species thus far placed in the genus Cytophaga differ in their cultural and biochemical properties from those of B. anitratum. Stanier (1957) in Bergey's Manual recognized 11 species, but noted that many other species (not listed)—14 at least according to Jeffers and Holt (1961)—are species incertae sedis. Index Bergeyana (1966) lists 27 as validly published and legitimate.

The type species of the genus Cytophaga (C. hutchinsonii Winogradsky) breaks down cellulose and produces a bright yellow pigment. All the species recognized by Stanier in Bergey's Manual (1957) show optimal growth at temperatures between 20° and 30°C and grow poorly if at all at 37°C. Some of the recognized species are halophiles. There have been 33 species placed in the genus Cytophaga, many of which do not breakdown cellulose nor are they bright yellow. A survey of these species was presented by Jeffers and Holt (1961), and Jeffers (1964) has developed a selection of media to be used preferably in the study of myxobacters.

The desirability of a reevaluation of the complex genus Cytophaga has been strongly advocated by Soriano and Lewin (1965).

The essential characteristics so far recognized for species of the genus Cytophaga are, however, lacking in "anitratum" bacteria with the exception of the "gliding" motility. As pointed out above, Schlieber (1964) observed seemingly gliding or creeping motions in one group of these strains (B. anitratum), but he also noted frequently a jerky and twitching type of locomotion which was not found in the Cytophaga strains he used for comparison. Schlieber (1964) states: "Der Bewegungsablauf, der alle Organismen der zweiten Gruppe kennzeichnet, ist durch ruckhafte, in kleinen Sprüngen ablaufende Ortsveränderungen geprägt, wobei nach jeder dieser kurzen Bewegungsphasen die Zelle für einen Sekundenbruchteil verharzt, ehe der nächste Bewegungsablauf folgt. Die Bewegungsrichtung der Zellen wechselt zumeist nach jeder Phase und verläuft nicht nur in der Zell-Längsachse, sondern häufig auch quer zu ihr. Die Bewegungsenergie scheint bei einzelnen Stämmen verhältnismässig gross zu sein, denn der Bewegung einer Zelle im Wege liegende andere Zellen werden zur Seite gestossen. Dabei vermögen besonders "energiereiche" Zellen ihren Bewegungsraum zu vergrössern. In seltenen Fällen lassen sich bei verhältnismässig frei gelagerten Zellen andeutungsweise kriechende Bewegungen erkennen. Verbiegungen oder Knickungen der Zellkörper konnten bei der beschriebenen zweiten Stämme-Gruppe in keinem Falle beobachtet werden."

This led Seeliger, Schubert and Schlieber (1964) to transfer Bacterium anitratum Schaub and Hauber (1948) into a new genus called Lingelsheimia to be included provisionally in the family Cytophagaceae of the order Myxobacterales. The denomination in honour of W. von Lingelsheim seemed justified as it is very likely that this species was first described by von Lingelsheim, even if the identity can no longer be proved, because none of his original strains has been preserved.

In the meantime, however, new findings have been published, which not only justify the proposal of a new genus Lingelsheimia, but also shed new light on the taxonomic position of this genus within the classification of bacteria.

Lautrop (1965) has confirmed the observation of Schlieber (1964) by finding the jerking and twitching movements. In consequence Lautrop (1965) has changed his conclusion as published in 1961. He states (1965) that upon closer analysis

the type of locomotion of cells of B. anitratum cannot be considered as gliding. The speed of the movement due to short intermittent jerks is only about 1-2 μ per minute as contrasted with 100 μ per minute found in some Cytophaga cultures. Lautrop (1965) now describes the locomotion as twitching or jumping. But Lautrop (1965) also mentions the observation of twitching in other species of Eubacteriales, belonging to the genera Moraxella, and aflagellated strains of Pseudomonas and Flavobacterium. He concludes that it is not possible to decide whether the differences in gliding and twitching locomotion are due to different mechanisms or varied expressions of the same fundamental property. The taxonomic significance of this remains likewise uncertain.

ii. Relationship of B. anitratum to the Genus Moraxella

Piéchaud and Second (1951) recommended the transfer of B. anitratum into the genus Moraxella in its broadest sense. In 1963 Piéchaud reported the gliding motion ("glissement") also in Moraxella strains.

The electron-microscopic investigations of Ryter and Piéchaud (1963) further demonstrated that there are fundamental differences between the Moraxellae and other Eubacteriales. These differences concern the morphology of the cell nucleus as well as the cytoplasm and structure of the cell walls. Ryter and Piéchaud draw the following conclusions:

- "1°) La structure de la paroi des Moraxella ainsi que leur mobilité les distinguent nettement des Eubactéries et les rapprocheraient des Vitreoscilla, qui sont des cyanophycées sans chlorophylle, des myxobactéries et d'autres cyanophycées.
- 2°) La forme de leur noyau offre également une certaine parenté avec celle de certaines cyanophycées.
- 3°) La présence de deux zones cytoplasmiques distinctes révèle chez elle une complexité qui n'a pas encore été observé chez d'autres Schizomycètes.

Les Moraxella présentent donc caractères de mobilité (glissement) connus jusque'ici exclusivement chez les myxobactéries qui sont des Schizomycètes et chez les Schizophycées. Il est difficile pour le moment de rattacher les Moraxella à l'un

ou l'autre de ces groupes. La structure des myxobactéries est d'ailleurs trop mal connue pour que l'on puisse faire une comparaison utile. Une comparaison avec les Chroococcales qui sont des cyanophycées à éléments isolés donnera peut-être des renseignements intéressants.

Quoi qu'il en puisse être, le genre Moraxella, en raison de la structure très particulières des espèces qui le constituent, ne peut-être rattaché à aucune famille connue des Schizomycètes. Une famille nouvelle doit être créée et nous proposons le nom de Moraxellaceae nov. fam. Cette famille ne renferme pour le moment qu'un genre, le genre Moraxella Lwoff qui en constitue le type."

Lwoff (1964) confirmed the concept of Ryter and Piéchaud (1963) when he discussed Steel and Cowan's (1964) recommendations to transfer B. anitratum to the genus Acinetobacter.

The findings of Ryter and Piéchaud are in accordance with our opinion and give additional evidence of the taxonomic importance of the cellular morphology including the modus of locomotion.

Piéchaud (1963) suggested combining the species Moraxella lacunata, M. duplex, M. lwoffii and M. glycidolytica with their variations into one single genus, Moraxella. This in our opinion leads to the inclusion of very heterogeneous species into this genus. We rather support the proposal of Henriksen (1952) to restrict the genus Moraxella to the oxidase-positive species Moraxella lacunata and M. duplex. This appears to be necessary because the oxidase reaction is recognized as an important character to distinguish genera or families in well-analysed bacterial groups. Thus it appears to be logical to contrast the oxidase-positive genus Moraxella with the oxidase-negative species which should be assigned to a new genus Lingelsheimia. The genera Moraxella and Lingelsheimia can then be placed in the family Moraxellaceae suggested by Ryter and Piéchaud (1963). It remains open at this time with which order of Schizomycetes this family should be included.

iii. Relationships of B. anitratum to Other Genera
(Achromobacter, Acinetobacter, Herellea, Mima
and Diplococcus)

In the following, reasons are presented which speak against the transfer of B. anitratum to any one of the following genera: Achromobacter, Acinetobacter, Herellea, Mima and Diplococcus.

a) B. anitratum cannot be included in the genus Achromobacter as the type species Achromobacter liquefaciens has peritrichous flagella. The latter property is an essential character to the inclusion of Achromobacter in the Eubacteriales and would be in contradiction to the mode of locomotion of B. anitratum.

b) The genus Acinetobacter cannot be considered because the type-species Acinetobacter stenohalis as suggested by Prévot (1961) is an obligatory halophilic species, reducing nitrate and unable to break down carbohydrates. Furthermore according to Prévot and Brisou (1954) the genus Acinetobacter belongs into Achromobacteriaceae (peritrichously flagellated).

The legal position of A. stenohalis as type-species of the genus Acinetobacter is, however, uncertain, because Brisou has in 1957 designated Acinetobacter anitratum (Schaub and Hauber) Brisou and Prévot (1954) as type-species of that genus with the basionym Bacterium anitratum Schaub and Hauber 1948. This was followed by the designation of a new type-species for the genus Acinetobacter by Prévot in 1961 who suggested A. stenohalis.

According to Prévot (1961) the definition of the genus Acinetobacter Brisou and Prévot 1954 is as follows:

"Achromobacteraceae immobiles; parfois encapsulées; formes courtes et coccoides fréquentes. Culture facile sur les milieux ordinaires. Quelques espèces pathogènes pour l'homme."

In order to clarify the situation, the authors of this paper got in touch with Prof. Brisou who states (written communication of September 21, 1966) that he prefers to follow the proposal of Prévot (1961) and that he recognizes A. stenohalis Prévot 1961 as the type-species of the genus Acinetobacter.

Since the original author has thus withdrawn his earlier proposal as made in 1957, A. stenohalis becomes the legiti-

mate type-species of the genus Acinetobacter Brisou and Prévot 1954.

On this basis Acinetobacter anitratum can be transferred to the genus Lingelsheimia.

c) Allocation to the genus Mima is likewise excluded because according to the original description by DeBord (1942) species of Mima do not attack carbohydrates.

d) Herellea vaginicola, the only species of the genus Herellea cannot be considered to be identical with B. anitratum because H. vaginicola acidifies mannitol and dulcitol which character has never been observed with any B. anitratum strain. Furthermore DeBord (1942) states in the description of the species H. vaginicola and of the genus Herellea that the organism is Gram-labile and of doubtful motility.

Which kind of motility was observed is not clearly stated. It seems rather doubtful whether motility—as shown under (g) in Table 1 of DeBord's description—"would warrant more than subspecific, at most specific, value." The following reasons have been given by DeBord for his classification:

"The tribe Mimeae should be placed under the Bacteriaceae for the present. The organisms of this tribe which do not ferment carbohydrates would not answer the familial description of the Enterobacteriaceae as defined by Bergey et al. It does not appear desirable to place the organisms in separate tribes upon the basis of a single fermentative test or upon the assumption that the source from which the various organisms were isolated would give a positive or negative correlation with a tribe."

3) In 1906 von Lingelsheim described an organism for which he proposed the name Diplococcus mucosus. His description was incomplete and led to considerable confusion in the literature which was reviewed by Seeliger (1953). There are some good arguments in favor of the identity of von Lingelsheim's organism and Bacterium anitratum (see Seeliger 1953). Others, however, have not shared this view. For instance Cowan (1938) described a nitrate-reducing Neisseria strain which he considered to be identical with D. mucosus. Véron, Thibault and Second (1959) as well as Chassignol (1961) agree that von Lingelsheim's organism should be placed in the genus Neisseria and consequently they transferred Diplococcus mucosus to the genus Neisseria

proposing the name Neisseria mucosa, thus corroborating the opinion of Murray (1939). On the other hand Stenzel and Mannheim (1963) without referring to the work of Véron et al. (1959) and Chassignol (1961) likewise transferred Diplococcus mucosus von Lingelsheim 1906 to the genus Achromobacter proposing the name Achromobacter mucosus (von Lingelsheim 1906) Mannheim and Stenzel 1962. They proposed their strain 3516/60 (NCTC 10303) as the neotype strain. This strain was among those cultures studied by Schlieber (1964), who observed with this culture the same type of motility that led Lautrop (1961) to exclude B. anitratum from the Eubacteriales.

The authors of the present communication hold the view that Diplococcus mucosus is an inadequately described species the various emendations of which have added to the already existing confusion.

The Judicial Commission is therefore asked to place Diplococcus mucosus von Lingelsheim according to rule 24f of the International Code of Nomenclature of Bacteria in the list of nomina rejicienda.

iv. Proposal of a new genus Lingelsheimia nom. nov.
(Seeliger, Schubert and Schlieber)

For reasons presented above we conclude that B. anitratum should be assigned to a new genus, Lingelsheimia. The type species is Lingelsheimia anitrata (Schaub and Hauber) comb. nov. The genus Lingelsheimia should be placed in the family Moraxellaceae Ryter and Piéchaud.

The name Lingelsheimia was proposed first by Schlieber (1964) but it was not effectively published. The name has also been submitted for publication in a special issue of Ann. immunol. hungarica dedicated to Prof. Rauss (Seeliger et al. 1966).

It should further be mentioned that according to rule 24a the specific epithet anitratum as used here for Lingelsheimia anitrata would be illegitimate if an earlier valid name would be found. A careful study of the descriptions of the organisms said to be identical or closely related to Bacterium anitratum is necessary.

Generic description

Lingelsheimia gen. nov. Short, Gram-negative bacteria; no fruiting bodies or microcysts; no flagella. Creeping and jumping motility on appropriate agar media. Heterotrophic,

oxidizing various organic substances. Oxidative breakdown of carbohydrates. No reduction of nitrate to nitrite on media containing peptone. Oxidase reaction negative.

Type-species: Lingelsheimia anitrata (Schaub and Hauber 1948) comb. nov.

Species-description: Lingelsheimia anitrata (Schaub and Hauber 1948) Seeliger, Schubert and Schlieber 1966.

Morphology: Short, coccoid cells, usually with round ends; occasionally highly pleomorphic involution forms are found. Encapsulated cells are often seen in strains isolated from pathologic materials. These capsules are lost only after repeated subculture at room temperature. On appropriate media a slow creeping type of motility is observed. Some strains show a twitching and jumping type of locomotion not to be confused with the fast gliding of Cytophaga.

Growth on nutrient agar media: Colonies 2-3 mm in diameter, whitish, convex, smooth with entire edges. After 8-10 days growth at room temperature, colonies show garland-like borders. No production of pigment either at room temperature or during incubation at 37°C.

Growth on nutrient broth: Homogeneous turbidity with slight sediment and definite surface pellicle.

Gelatin stab: Liquefaction none or very slow.

Indol reaction: Negative.

Voges-Proskauer reaction: Negative.

Methyl-red reaction: Positive.

Growth on Koser's citrate agar: Positive.

Sodium malonate not utilized.

No production of hydrogen sulfide on Kligler's agar.

Litmus milk: Acid after 3-4 days incubation; coagulation after one week.

Nitrate not reduced to nitrite on peptone media.

Oxidative breakdown of glucose, galactose and arabinose.

Acid in media containing 10% lactose.

Lactose, sucrose, mannitol, salicin, maltose, inositol, trehalose, sorbitol, adonitol, raffinose, rhamnose and dulcitol are not attacked in fermentation tubes containing 1% of the carbohydrate.

Oxidase reaction: Negative.

Catalase reaction: Positive.

Growth only under aerobic conditions.

Good growth at 37°C.

Pathogenic for mice and guinea pigs after intraperitoneal injection of broth cultures.

Habitat: Unknown.

Isolated from urine, spinal fluid, and pus of human origin; often from feces, dust and fomites, not seldom from food.

The proposed type strain is B5W 71, identical with ATCC 15149. This strain was used by Ferguson and Roberts (1950) and is maintained in several recognized type culture collections. In case that another type strain would be designated by other workers it is suggested that the final designation of a type strain be postponed until an expert committee has evaluated the various strains.

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ADDENDUM

After submission of the manuscript for publication, R. HUGH and R. REESE (Designation of the type strain for Bacterium anitratum, *Int. J. Syst. Bact.* 1964, 17:245-254) have proposed to use one of the original strains of SCHAUB and HAUBER as type strain and have selected for this purpose SCHAUB'S strain 81, ATCC 18606, RH 2208. The authors are ready to recognize this culture as the type strain for Lingelsheimia anitrata according to Rule 9d(3) of the International Code of Nomenclature of Bacteria.