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CLASSIFICATION OF THE KLEBSIELLA-AEROBACTER
GROUP WITH SPECIAL REFERENCE TO THE
COLD-TOLERANT MESOPHILIC AEROBACTER TYPES

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SUMMARY: Aërobacter liquefaciens Grimes and Hennerty 1931 is a later illegitimate homonym of A. liquefaciens Beijerinck 1900. A. lipolyticus nom. nov. is proposed in its place. A detailed description is given. A. lipolyticus is widely distributed in nature and is important in dairy science. It usually fails to produce gas from lactose at 37°C, but produces it abundantly at 20-30°C. A. aërogenes with which it may be confused, produces gas from lactose at 37°C. A. lipolyticus differs from A. hibernicus by liquefaction of gelatin.

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Since Escherich (1885) published his paper on "Intestinal Bacteria" and Beijerinck (1900) described and named Aërobacter aërogenes, the classification of the taxa of the family Enterobacteriaceae has been a subject for investigation, discussion and review. The different viewpoints on the interrelationships of the genera Klebsiella and Aërobacter are discussed in the 7th edition (1957) of Bergey's Manual of Determinative Bacteriology, the International Bulletin of Bacteriological Nomenclature and Taxonomy founded in 1951 and in many papers published in the various journals dealing with bacteriology, microbiology and public health.

The Enterobacteriaceae Subcommittee (originally The Salmonella Subcommittee) and the Judicial Commission and their members collectively and individually have expressed their viewpoints in a series of papers in the International Bulletin.

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Kauffmann and Edwards (1952) emended the description of Klebsiella to include nonmotile, nonliquefying, Gram-negative rods, fermenting lactose and mannitol, citrate positive and urea negative, or late and irregularly positive, and named two species, K. pneumoniae and K. rhinoscleromatis. They stated:

"It is realized that no provision is made for the motile forms classified as Aërobacter. It is felt that present knowledge of these forms is insufficient to justify their placement in a definite group."

Edwards and Fife (1955) studied 856 cultures of Klebsiella and Aërobacter and stated that no single biochemical test or combination of tests sufficed clearly to distinguish the genus Klebsiella and A. aërogenes from cultures of A. cloacae, and that many aberrant and intermediate strains occurred. It was suggested that the nonmotile forms which do not liquefy gelatin be placed in the genus Klebsiella, and that Aërobacter be redefined as a motile liquefying group with A. cloacae as the type. With regard to motility, Brooke (1953) in his study of strains of A. cloacae and Klebsiella (all isolated from human sources, most of them from urine) noted that while all the Klebsiellae were nonmotile, a number of A. cloacae strains were also nonmotile.

Cowan (1954) reviewed the names of the coliform bacteria and asked the Judicial Commission to consider whether Aërobacter Beijerinck 1900 should be placed in the list of nomina generica rejicienda. The Commission should also be asked to determine whether a newly defined genus Aërobacter (not Aërobacter Beijerinck) should be conserved with the type A. cloacae Jordan, or with a redefined (motile) species A. aërogenes, or whether the genus Cloaca Castellani and Chalmers 1919 should be conserved?

The Editorial Board (1954) published a preliminary statement on the status of the generic names, Escherichia, Klebsiella, Aërobacter and Cloaca. The Enterobacteriaceae Subcommittee (1954, 1958) reported on these genera. In the 1958 Report, the Subcommittee proposed to define Klebsiella as consisting of nonmotile, encapsulated bacteria that conform to the definition of the family Enterobacteriaceae and to define Cloaca (Aërobacter) as consisting of motile bacteria that conform to the definition of the family Enterobac-

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teriaceae, and give typical biochemical reactions. The Subcommittee pointed out that cultures of Klebsiella which have been typed were derived largely from respiratory infections and urinary infections in man and from human feces.

Winslow et al. (1919), Weldin (1927), Ruchhoft et al. (1931), Ostermann et al. (1941), Olsen (1942), and Borman et al. (1944) all noted the difficulties experienced by workers in trying to classify the Klebsiella-Aërobacter group on biochemical tests, particularly sugar fermentations. Ostermann et al. state that they found no single test or group of tests that would distinguish many of their Klebsiella (Friedländer) cultures from those of the coli-aërogenes group and state that it seems not unlikely that much of the confusion still surrounding the Klebsiella (Friedländer) and coli-aërogenes organisms is due to failure to recognize the full significance of variation.

Edwards (1929) isolated strains of Aërobacter from feces, soil, water, and milk and noted that both the Aërobacter strains and the Friedländer (Klebsiella) strains were rather variable in their fermentative characters.

Parr (1938) noted the difficulty of classifying the coliform bacteria owing to the essentially intergrading nature of the organisms in this group and defined intermediates as coliform organisms which have one or more of the characteristics of Escherichia coli and one or more of those attributed to Aërobacter aërogenes and notes "among the coliform bacteria characters are from time to time lost or those in abeyance are regained." Parr (1939) noted that these intermediate forms either do not conform to the so-called LMViC pattern, or lack power to ferment certain carbohydrates, or produce only acid where the production of acid and gas is considered normal, or display a combination of fermentation characters the reverse of that which normally occurs. It is to be noted that Parr (1936) isolated strains of Aërobacter from feces which had been stored in an ice box for as long as two months.

Stuart et al. (1938) noted that, with some of their 5,200 cultures isolated from human and bovine feces, from dust of cowbarns, and from milk, growth was more luxuriant at room temperature than at 37°C and that cultures (grown in methyl red medium) that produced only acid when grown at 37°C for 36 hours produced acid and gas when grown at room temperature for the same period, and also that the methyl

red reaction varied with temperature of incubation. These irregularities in the methyl red reaction were most noticeable in the strains which either failed to produce gas from lactose at 37°C or produced gas at room temperature only.

Stuart *et al.* (1940) proposed the term "aberrant" coliforms for cultures not fermenting lactose, producing acid only, or requiring more than 48 hours for the production of gas at 37°C. These strains were isolated from water, soils and cereals. Many of the so-called "aberrant" strains grew abundantly at room temperature but sparsely or not at all when incubated at 37°C (in other words they isolated mesophilic strains of Aërobacter).

The Enterobacteriaceae Subcommittee (1958) first gives the biochemical characteristics of the Cloaca (Aërobacter) group and then states "Although these biochemical reactions can be considered as typical of the group, there are aberrant cultures, not fermenting lactose, sucrose, rhamnose, sorbitol, raffinose, methyl red positive, Voges-Proskauer, citrate, or KCN negative. Anaerogenic strains also exist."

My work on the coliform group as a whole has convinced me that a satisfactory classification of the Klebsiella-Aërobacter group cannot be based solely on sugar fermentations. That this fact is generally recognized is shown by the many qualifications found in the literature such as "frequently do not attack"; "may or may not attack"; "many strains ferment"; "usually do not form acid and/or gas." Using a pH meter one can state that fermentation has occurred, but cannot state that a coliform culture is not capable of developing the ability to ferment a given carbohydrate. In carrying out the methyl red and Voges-Proskauer reactions Fouad *et al.* (1953) have shown the necessity for a chemically defined glucose ammonium phosphate medium.

Some strains of Aërobacter do not produce gas in lactose broth at 37°C but do so at 15-30°C. Begue and Lichstein (1958) have shown that in Saccharomyces cerevisiae the required synthesis of pantothenic acid is accomplished at 30°C but in some manner is prevented at 37°C, i.e. an enzyme-temperature system is postulated. Lichstein (1960) notes "it is becoming increasingly apparent that antagonisms and interactions among nutrients as well as the character of the physical environment influence markedly the nutritional demands of an organism" and "genetic expression is affected by the environment and the presence of a particular gene

does not necessarily mean that the corresponding character will be expressed."

The ability of non-lactose-fermenting organisms isolated from feces to ferment lactose after long cultivation in lactose media has been shown by a number of workers, particularly Kriebel (1934) who draws the conclusion that these strains are closely related to the colon group, possibly as variants, since they tend to dissociate into lactose-fermenting coli-like organisms. Tregoning and Poe (1937) found that the production of sucrose-positive variants was readily accomplished in some strains of Escherichia and Aërobacter. Di-Girolamo et al. (1958) have shown that strains of E. coli that did not use arabinose as the sole carbon source did so if seeded on a mixture of rhamnose and arabinose, and noted that the methylpentose is an indicator of the enzyme of arabinose metabolism, which by itself has no inductive property.

Breed (1957) in Bergey's Manual comments on the unsatisfactory position of the Klebsiella-Aërobacter group and notes that no method has been found to differentiate the majority of K. pneumoniae strains from urinary strains commonly classified as A. aërogenes. He concludes that while awaiting a better solution of the problem it was felt advisable to continue to recognize A. aërogenes (as a species distinct from K. pneumoniae) and both genera Aërobacter Beijerinck and Klebsiella. He pointed out that species of Aërobacter that occur in dairy products are frequently derived from grain and that they are found on panicles of the grass family in open fields.

The Enterobacteriaceae Subcommittee (1958) recommended biochemical methods for differentiation of the genera of the Enterobacteriaceae. Except for gelatin liquefaction at 21°C, they suggested that tests be carried out at 37°C for 2 days. If negative, follow with further incubation at 21 to 25°C for 5 days. In our work on the cold-tolerant mesophilic strains of Aërobacter, the above methods have been followed since 1928 at an incubation temperature of 21°C, since these strains grow poorly or not at all at 37°C. It is difficult to compare our data with those of previous workers before 1950, since others usually worked with nonmotile cultures and carried out their tests at 37°C. The methyl-red test particularly is influenced by the temperature of incubation, since temperature influences the rate of production of acid in the medium.

Schafler et al. (1959, 1960) obtained through "training" on lactose media and by selection lactose-positive strains of Salmonella which also became cellobiose-positive. An interesting finding was that the appearance of lactose-positive variants from cellobiose-positive variants of Salmonellae was particularly or totally inhibited by succinate or glutamate. Schema et al. (1943) found increasing tolerance of A. aërogenes to sodium pentachloropentate and noted that in certain cultures gas was no longer formed from arabinose or rhamnose, and that in such cultures the methyl-red test became positive.

That temperature of chemicals can cause gene unstabilization has been shown by Zamenhof and co-workers (1958, 1961). They used a typical strain of E. coli and a lactose nonfermenting mutant of that strain. A similar situation may well apply to strains of Klebsiella and Aërobacter. There is also the question of growth requirements and I suggest that mesophilic (plant) strains of Aërobacter ingested in the food of an animal (given time) would adapt themselves to their habitat and that the ability to ferment lactose or other properties could be lost. Such a strain later isolated from the urinary tract or from feces might well be identified as Klebsiella or as an intermediate strain. Martinec et al. (1961) discussing the taxonomic status of Serratia marcescens Bizio state that relatively great variability of some strains were observed during the fermentation of carbohydrates and add "it is interesting that the majority of these anomalies was observed in strains isolated from different species of insects."

As a dairy bacteriologist, I have been interested since 1925 in strains of Aërobacter that fermented lactose poorly or not at all at 37°C. They were first isolated from creamery water supplies contaminated by soil. Since in a creamery water supply it is as important to eliminate soil contamination as to detect fecal contamination, in routine examination for the coliform group lactose-broth tubes were inoculated in duplicate at 21°C and at 37°C. This procedure, also applied to milk and milk products, led to the isolation from water, milk, cream, poor quality butter, gassy cheese, and ice cream, of strains of Aërobacter whose optimum temperature ranged from 20°C to 30°C, that grew well at 15°C and grew slowly at 4°C (1931).

Grimes (1934) concluded that while it is now usual to

carry out the test for Escherichia and Aërobacter from water and dairy products at 37°C the test should also be carried out also at 21°C, otherwise the Aërobacter group, although present, may not be identified. These cold-tolerant mesophilic strains of Aërobacter have been isolated by many workers from soil, grasses, ears and panicles of cereal crops, milk, cream, poor quality butter, gassy cheese, ice cream, creamery and farm water supplies and dairy farm equipment, chilled meat and crabmeat.

A characteristic of these strains of Aërobacter is their poor growth or lack of growth in lactose broth at 37°C. Thomas and co-workers (1958) state that out of 1040 cultures isolated from raw milk and farm dairy equipment which fermented lactose with the formation of acid and gas to 30°C, 473 (nearly 50%) failed to produce gas from lactose in two days at 37°C.

Although this paper is concerned mainly with mesophilic strains of Aërobacter I have also worked on the strains that grow best at 37°C, likewise strains of Escherichia and various intermediate strains. I am of the opinion that while one can state that a particular strain is capable of a particular fermentation, it cannot be said that it is incapable of fermenting that substance. Whether or not fermentation occurs depends on the original environment, medium used, temperature of incubation and length of time of incubation.

The growth medium and the temperature of incubation may also influence motility. I find peptone broth the best medium for determining motility, the culture to be incubated for 24 hours at its optimum temperature. One does not get as good results with lactose broth, for the strains of Aërobacter grow plumper and larger, and in the motility test they are found to be sluggishly motile or nonmotile.

I am in agreement with Hormaeche and Edwards (1960) that capsules and slime formation are of no value in differentiation since the related strains are not constant in these properties. In our routine work (incubation temperature 21°C), streaking or plating on Levine media from lactose broth cultures showing acid and gas at 21°C in 24 to 48 hours the mucoid strains are readily separated from the nonmucoid strains, but I have not found it possible definitely to separate them by biochemical tests. The mucoid strains will cause ropiness in milk and give sliminess in glucose, lactose, and sucrose broth and more or less slimy-

ness in galactose, raffinose, sorbitol, xylose and glucerol broth. There is no correlation between mucoid growth or nonmucoid growth and the liquefaction of gelatin.

In classifying Klebsiellae and Aërobacteres one is between Scylla and Charybdis, since on the one hand it is possible to simplify by restricting the number of tests, e.g. relying on IMViC and the fermentation of lactose and glucose, or on the other hand to complicate to the point of absurdity by differentiating on numerous fermentation and other tests. There is the further complication that the influence of medium, growth temperature and length of time of incubation has not always been fully recognized. Alford (1960) and Schultze et al. (1960) support our contention of the importance of incubation temperature when carrying out biochemical tests. Taylor's question (1959) "Why Christen a Salmonella?" is pertinent here, and the same question can be asked also regarding the Klebsiella-Aërobacter group. The cold-tolerant mesophilic strains of Aërobacter especially are to be regarded as an adaptable group capable of changing to meet changes in their environment (given time) whether it be biological or biochemical.

In discussing the legitimacy of the generic name Klebsiella to include both Klebsiella and Aërobacter, the report of the Coli-Aerogenes (1956) Subcommittee of the Society for Applied Bacteriology (1956) states "since the generic name Klebsiella has priority over Aërobacter, the aerogenes organism not only falls into the genus Klebsiella but, strictly, also loses its specific name. However, if the Rules of the Bacteriological Code (1948) are strictly applied, the correct names for many coli-aerogenes bacteria would be so unfamiliar as to cause confusion and error, and it will be necessary to ask for official opinions to conserve suitable names against earlier. The views expressed by Cowan (1954) concerning names for coliform organisms and his paper "Nonconformism in Nomenclature" (1959) are also relevant.

Hormaeche and Edwards (1960) have discussed the difficulty of classifying the bacteria of the Klebsiella-Aërobacter group. They proposed a new genus Enterobacter with the type species Enterobacter cloacae based on Bacillus cloacae Jordan (1890) and requested that the term Enterobacter Hormaeche and Edwards be placed in the list of conserved bacterial generic names and that Enterobacter cloacae Jor-

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dan comb. nov. (B. cloacae Jordan, 1890) be designated as its type species. This request of Hormaeche and Edwards deserves careful consideration, but it accepts in general only those strains of Aërobacter with an optimum temperature 37°C, and rejects the cold-tolerant mesophilic strains of Aërobacter of Grimes and Hennerty (1931). Ewing and Edwards (1960) state "that at present the Aërobacter group is divisible into three subgroups corresponding to the species A. cloacae, A. aërogenes and A. liquefaciens Grimes (1931)."

Hormaeche and Edwards (1958) make a valuable contribution in their observations on the genus Aërobacter with respect to the separation of Aërobacter from Klebsiella on the basis of motility and the urease and ornithine decarboxylase tests. They note that nonmotile variants of otherwise typical cultures are known to occur, likewise aberrant and intermediate strains. They state that there are at least two well-defined species—A. aërogenes and A. cloacae—and list their biochemical characteristics. I am of the opinion that sanitary and food bacteriologists would be in agreement in desiring that the generic name Aërobacter be retained even though Klebsiella has precedence. Classification is not intended to fit a concept of static genera and species. With the development of more and better taxonomic methods, particularly serological methods, the interrelationships of the strains that comprise the Klebsiella-Aërobacter group are now somewhat better realized. Can Klebsiella be differentiated from Aërobacter while acknowledging the adaptability of the latter group to adjust itself to its environment, resulting in a variety of intermediate strains, which may also be termed "variant" or "aberrant"?

The description of the genus Klebsiella, as given by the Enterobacteriaceae Subcommittee (1958) and in Bergey's Manual (1957), I would amend as follows: Nonmotile short rods, Gram-negative, encapsulated in the mucoid phase, conforming to the definition of the family Enterobacteriaceae, methyl-red negative, Voges-Proskauer positive, gelatin not liquefied, citrates utilized as sole source of carbon, nitrites produced from nitrates, sodium malonate positive, capable of fermenting glucose, lactose, sucrose, mannitol, salicin, and growing in KCN medium. The type species is Klebsiella pneumoniae. Encountered frequently in the respiratory, intestinal and urogenital tracts of man and recognized

as one of the causes of mastitis in cattle (Barnes, 1953; Buntain and Field, 1953; Esterbrooks and Plastridge, 1956; Guillot et al. 1961; Smith and Henderson, 1934; White, 1957; The type species represents the strains of the group that are adjusted to a human or animal habitat, and may be more or less pathogenic.

As to the value of biochemical tests in distinguishing A. cloacae from A. aërogenes, the Enterobacteriaceae Subcommittee (1958) give the biochemical characteristics of Cloaca (Aërobacter) and state "although these biochemical characteristics can be considered as typical, there are aberrant cultures not fermenting lactose, sucrose, rhamnose, sorbitol, raffinose, methyl-red positive, Voges-Proskauer, citrate or KCN negative, Two biochemical types can be differentiated. Type A does not generally ferment inositol nor glycerol, and when doing so does not produce gas in four days; it is arginine positive, lysine negative and liquefies gelatin. Type B promptly ferments with gas inositol and glycerol, is arginine negative, lysine positive and about half the strains do not liquefy gelatin."

Breed (1957) states "usually motile" gelatin colonies—"thin, circular, bluish, translucent" (no mention of liquefaction). Gelatin stab—"slow liquefaction, liquefying power sometimes lost."

I regard a nonmotile A. cloacae strain as a variant of A. aërogenes Beijerinck, 1900, i.e. a Klebsiella strain; a motile A. cloacae that does not liquefy gelatin as not properly identified and the motile A. cloacae strain that liquefies gelatin, more or less slowly, as a variant of A. lipolyticus nom. nov. described below.

Aërobacter is defined as follows: motile, Gram-negative rods, mucoid phase common in media containing lactose, conforming to the definition of the family Enterobacteriaceae, methyl-red negative, Voges-Proskauer positive, uric acid, citric acid and citrates utilized as sole source of carbon, nitrites formed from nitrates, fermenting with the production of acid and gas—glucose, levulose, galactose, lactose, sucrose, maltose, cellobiose, mannitol, raffinose, sorbitol, inositol, rhamnose, arabinose, xylose, glycerol, salicin, aesculin, α -methyl glucoside; positive for arginine dihydrolase, lysine and ornithine decarboxylases; sodium formate and sodium malonate positive. Growth in KCN medium. Phenylalanine deaminase negative. Dulcitol,

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inulin, potato starch, destrin may or may not be fermented. Indole test usually negative.

These biochemical reactions are typical for the strains that comprise the cold-tolerant mesophilic strains, optimum temperature, 20°C to 30°C; good growth at 15°C. growth at 4°C; poor or no growth at 37°C.

Soil and plant strains, isolated from soil, grasses, cereals, water, milk and milk products, and cold-stored meat. Cause of ropymilk. Aërobacter lipolyticus nom. nov. liquefies gelatin. Pectin not liquefied.

A strain with the same biochemical characteristics, but incapable of liquefying gelatin, has been named Aërobacter hibernicum.

The name Aërobacter liquefaciens Grimes and Hennerty 1931 is a later homonym of Aërobacter liquefaciens Beijerinck 1900, hence illegitimate. The name Aërobacter lipolyticus nom. nov. is proposed to replace A. liquefaciens Grimes and Hennerty. A detailed description is given here-with.

Aërobacter lipolyticus nom. nov.

Synonym: Aërobacter liquefaciens Grimes and Hennerty 1931, not A. liquefaciens Beijerinck 1900.

Morphology: Rods, 0.75-1.0 x 1.0-2μ, motile, possessing pertrichous flagella, Gram-negative, aerobic, facultative, nonspore-forming.

Nutrient gelatin: Two days at 21°C. Punctiform, entire dirty greyish colonies, gelatin liquefied.

Nutrient gelatin stab: Two days at 21°C. Filiform growth with liquefaction. Five to seven days—above 10% liquefaction, infundibuliform. Two to three weeks—around 40% liquefaction.

Tryptone dextrose agar: Two days—colonies, circular, smooth, moist, glistening, greyish-white, viscid, convex, opaque, raised, entire.

Tryptone dextrose agar slant: Two days—abundant greyish-white growth, smooth, moist, glistening, opaque, viscid.

Nutrient broth: Good growth in 24 hours, turbid.

Levine medium: One to two days—good, nonchromogenic growth. Colonies or streak-smooth, glistening, opaque, viscid.

Potato slope: Two to five days—good growth, smooth, moist, glistening, opaque, viscid, muddy grey to brownish grey colour.

Biochemical Tests at 20-30°C

Methyl-red: Negative.

Voges-Proskauer test: Positive.

Uric acid media: Two days—good growth. pH 4.4-5.0.

Koser's citrate medium: Two days—good growth.

Hydrogen sulfide: Lead acetate filter paper strip—Negative.

Bismuth liquor technique. 1938. J. Bact. 35:183. Definite blackening around the top of the medium and along the line of the stab.

Nitrates reduced to nitrites.

Indol: Usually negative (positive strains found).

Catalase: Positive.

Diastatic action: Negative.

Milk: Acid coagulation, ropiness, pH 5.3 in two to three days. Acidity 0.5 to 0.6% lactic acid.

The following are fermented with production of acid and gas, with pH dropping to 5.0 or lower in two to four days:

Lactose, sucrose, fructose, galactose, maltose, rhamnose, arabinose, cellobiose, aesculin.

pH dropping to less than 5.5 but not lower than 5.1:

Mannitol, inositol, adonitol, xylose, raffinose, α -methyl glucoside, salicin.

pH dropping to 6.6 but not lower than 5.6: Sorbitol, glycerol, amygdalin.

Glucose: Drops to pH 5.5 in two days and is around 7.0 in four days.

Dulcitol may or may not be fermented; when fermented, pH varied from 5.5 to 6.5.

Erythritol: Not fermented.

Mucoid phase common in media containing glucose and lactose.

Inulin: Usually fermented, pH 6.3 (average).

Dextrin: May or may not be fermented; when fermented, pH 6.4 (average).

Starch: May or may not be fermented; when fermented, pH 6.0 (average).

Sodium malonate: Positive, pH 8.4 (average). One negative strain found.

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Sodium hippurate: May be negative or positive, pH 6.2 (average).
 Sodium formate: Positive, gas production, pH 8.4 (average).
 KCN: Positive.
 Asparagine: Positive, pH 8.0 (average).
 Arginine dihydrolase: Usually positive.
 Lysine decarboxylase: Positive.
 Phenylalanine deaminase: Negative.
 Ornithine decarboxylase: Positive
 Growth in 7.5% sodium chloride solution.

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