THE DIFFERENTIATION OF AEROBACTER AEROGENES AND AEROBACTER CLOACAE[1]

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Many attempts, based on biochemical properties, have been made to differentiate strains of the tribe Eschericheae—a biochemically heterogeneous collection of the *Enterobacteriaceae*—into genera. In the sixth edition of *Bergey's Manual* (Breed *et al.*, 1948), these strains, if of animal origin, are classified into three genera: *Escherichia*, *Aerobacter*, and *Klebsiella*. The genus *Aerobacter* is subdivided into two species, *Aerobacter aerogenes* and *Aerobacter cloacae*. More recently Kauffmann (1951) has claimed that the Friedländer (Klebsiella), Aerobacter, and Aerogenes "types" belong to one common group, which should be termed *Klebsiella*, as according to the rules of nomenclature this name has priority over the other two terms. In this article, using biochemical and morphological criteria, the relationship between *A. aerogenes* and *A. cloacae* will be discussed.

It is generally accepted that according to their action on gelatin, Aerobacter strains may be divided into nonliquefying *A. aerogenes* and gelatin liquefying *A. cloacae*. The latter are claimed to be the only Escherichieae strains which liquefy gelatin. Working with a few strains, Kligler (1914) found that *A. aerogenes* attacked glycerol whereas *A. cloacae* did not. Johnson and Levine (1917) stated that *A. aerogenes* differs from *A. cloacae* in that it forms gas from glycerol, is nonmotile, and rarely liquefies gelatin. Inositol was first used for the differentiation of Escherichieae strains by MacConkey (1905). Mackie (1921) observed absolute positive correlation between capsule formation, fermentation of inositol, and absence of motility, and Hay (1932) noted that the feature most characteristic of the mucosus capsulatus group (Klebsiella), apart from capsulation, was its ability to ferment inositol, but that this was shared also by *A. aerogenes*. Malcolm (1938), on the basis of inositol, indole, Voges-Proskauer and citrate tests, recognized eight groups of Escherichieae; all his *A. aerogenes* strains were inositol positive; all his *E. coli* strains, negative.

MATERIALS AND METHODS

Strains. The material studied consisted of 411 strains, most of which were isolated in the State Serum Institute, Copenhagen. All the strains were from human material, most of them being isolated from urines, but some from uterine secretions, expectorates, cerebrospinal fluids, etc. The strains selected were gram negative rods with the classical IMViC (indole −, methyl red −, Voges-Proskauer +, citrate utilization +) reactions of *A. aerogenes* although a few strains gave aberrant results in one or two of the IMViC tests. The ability to ferment lactose was not considered basic, and many of the strains selected lacked this property.

Media. The following media were used in the biochemical examination of the strains: arabinose, xylose, rhamnose, glucose, sucrose, maltose, lactose, glycerol, adonitol, mannitol, dulcitol, sorbitol, salicin, and inositol: 0.5 per cent of each of these "sugars" was added to a basal medium consisting of 0.5 per cent Bullox's meat extract, 1.0 per cent of Riedel's peptone, and 0.5 per cent NaCl. Casein digest broth (for indole production), nitrate medium (for nitrate reduction), fluid urea medium,[2] fluid and solid ammonium-glucose and ammonium-sodium-citrate medium, glucose-phosphate broth (for the determination of the methyl red reaction and acetyl-methyl-carbinol formation), and ferro-chloride gelatin (for gelatin liquefaction and H_2S production).

Indole production was determined after one day's incubation at 37 C; the production of

[1] Most of the experimental work reported here was done in Statens Seruminstitut, Copenhagen, Denmark (Director, J. Ørskov), while the author was holding a British Council Scholarship.

[2] The composition of the urea medium was: peptone (Difco), 1 g; NaCl, 5 g; KH_2PO_4, 2 g; glucose, 1 g; urea, 20 g; phenol red (1:500), 6 ml; distilled water, 1,000 ml.

acetyl-methyl-carbinol using the Barritt method (1936) after two days' incubation at 37 C; the methyl red reaction and the reduction of nitrates after four days' incubation at 37 C. The urea, ammonium-glucose, and ammonium-citrate media (both fluid and solid) were observed for four days; the ferro-chloride gelatin for at least 60 days; the "sugars" for 30 days.

Determination of motility. The strains were tested for motility by examining microscopically, with an oil immersion lens, wet preparations of 3 to 4 hours 37 C casein digest broth cultures.

Capsule determination. This was done by Ørskov's direct agar microscopy method (1922) and also using Klieneberger-Nobel's method A2 (1948) for capsule staining.

RESULTS

The morphological and biochemical properties of the strains studied are recorded in table 1.

Nearly all strains promptly fermented arabinose, xylose, rhamnose, glucose, sucrose, maltose, mannitol, and sorbitol although with a few strains the fermentation of one or more of the "sugars" was late, and with some strains negative. Only two strains failed to form gas in glucose and mannitol. (A Durham tube was used only in these two substrates and in glycerol.) Nearly all strains gave the classical IMViC reactions of *Aerobacter* (*aerogenes* and *cloacae*). However, based on all biochemical properties tested the strains fall readily into two large groups—*A. cloacae* hereinafter designated as group I and *A. aerogenes* hereinafter designated as group II. All group I strains examined were nonencapsulated; all but 13 group II strains were encapsulated. Some of the group I strains were motile, whereas all group II strains were nonmotile. The similarity of biochemical properties of encapsulated and nonencapsulated group II strains suggests some common origin. This view is strengthened by the fact that some nonencapsulated and encapsulated group II strains isolated from the same specimen had identical biochemical properties.

The substrates important in the biochemical differentiation of strains of the two groups are: salicin, inositol, glycerol, and gelatin, and of less importance, dulcitol and urea. Only one group I strain fermented inositol and only one fermented dulcitol; none fermented glycerol. The fermentation of salicin was usually late, occurring between the third and seventh day of incubation although a few strains promptly fermented this glucoside and a few strains failed to attack it. All strains liquefied gelatin. The utilization of urea varied; some strains promptly utilized urea, others did not decompose it until between the second and fourth days' incubation, while still others did not attack this substrate. The important properties of some typical group I strains are given in table 2.

All but 7 group II strains promptly fermented inositol. With 6 strains the fermentation occurred between the second and fifth days, but the seventh strain failed to ferment this substrate. Inositol fermenting mutants were obtained later from this strain. (Attempts to obtain an inositol fermenting mutant from a group I strain have proven unsuccessful until now.) Some strains fermented dulcitol. All group II strains tested promptly fermented glycerol with acid and gas production, and all but one promptly fermented salicin. Seventeen group II strains liquefied gelatin. All but 3 group II strains promptly utilized urea.

The fermentation of glycerol by *A. cloacae* strains has been reported by Griffin and Stuart (1940) and by Brooke (1951). These workers agree that no gas is formed in this fermentation. The main point of differentiation between *A. aerogenes* and *A. cloacae* in *Bergey's Manual* is that the latter organisms ferment glycerol without gas production. Brooke claims that glycerol fermentation by *A. cloacae* does not occur until between the second and fifth days of incubation. This late fermentation may be due to aerobic adaptation with different end products being formed than those formed when glycerol is fermented by group II strains. This is being investigated. It is suggested that glycerol tests be read after 24 hours' incubation.

Recent experiments with one typical group I and one typical group II strain have shown that when strict fermentation tests are done, virtually all oxygen being removed, glycerol is not fermented by the group I strain although it is still fermented by the group II strain. The addition of different naturally occurring hydrogen acceptors —sodium fumarate, sodium pyruvate, peptone (Difco)—to the medium still did not result in the group I strain fermenting glycerol (Magasanik *et al.*, 1953). These workers also have found that glycerol can be used for respiration by both groups of organisms, the enzyme being adaptive

TABLE 1

The properties of group I (Aerobacter) and group II (Klebsiella) strains

	(*A. cloacae*)	(*A. aerogenes*)	
	Aerobacter	Klebsiella	
		Nonencapsulated	Encapsulated
Arabinose	108 +	13 +	290 +
Xylose	104 +, 4 −	13 +	290 +
Rhamnose	102 +, 6 ×	13 +	290 +
Glucose	108 +	13 +	290 +
Sucrose	108 +	13 +	288 +, 2 ×
Lactose	67 +, 20 ×, 21 −	12 +, 1 ×	290 +
Maltose	108 +	13 +	290 +
Glycerol	103 ×, 5 −	13 +	117 +*
Adonitol	31 +, 77 −	13 +	287 +, 3 −
Mannitol	108 +	13 +	290 +
Dulcitol	1 +, 107 −	2 +, 11 −	89 +, 201 −
Sorbitol	105 +, 3 ×	13 +	290 +
Salicin	9 +, 95 +, 4 −	12 +, 1 ×	290 +
Inositol	1 +, 107 −	11 +, 2 ×	285 +, 4 ×, 1 −
Gas (Glucose)	106 +, 2 −	13 +	288 +, 2 ×
Gas (Mannitol)	106 +, 2 −	13 +	288 +, 2 ×
Gas (Glycerol)	108 −	13 +	117 +*
Indole	108 −	13 −	278 −, 12 +
Gelatin	108 +	10 −, 3 +	276 −, 14 +
H_2S	108 −	13 −	290 −
KNO_3	108 +	13 +	290 +
V. P.	106 +, 2 −	13 +	289 +, 1 −
M. R.	96 −, 12 +	12 −, 1 +	278 −, 12 +
Urea	20 +, 44 ×, 44 −	11 +, 2 ×	289 +, 1 ×
Ammonium-glucose agar	108 +	13 +	290 +
Ammonium-glucose fluid	108 +	13 +	290 +
Na citrate agar	108 +	13 +	285 +, 4 ×, 1 −
Na citrate fluid	108 +	11 +, 2 ×	283 +, 5 ×, 2 −
Capsule	108 −	13 −	290 +
Motility	71 +, 37 −	13 −	290 −

Key: "Sugar"

+ = fermentation during the first day of incubation.

× = fermentation occurring between the second and thirtieth day of incubation (both inclusive).

− = no fermentation even after 30 days' incubation.

Gelatin

+ = liquefaction

− = no liquefaction (observed for sixty days).

Utilization tests

+ = utilization during the first day of incubation.

× = utilization between the second and fourth day of incubation (both inclusive).

− = no utilization even after 4 days' incubation.

* Only 117 strains tested.

in all strains tested. Magasanik (1953) working with the same strains showed that, whereas the group II strain could use inositol for respiration (the enzyme being adaptive), the group I strain could not attack inositol.

The large number of nonlactose fermenters or of late-lactose fermenters in group I does not of necessity invalidate the inclusion of these organisms in the tribe Escherichieae. The fact that many of these organisms are nonlactose or late-

lactose fermenters suggests that they be designated as paracolons. However, it is usually accepted that the term paracolon has no definitive meaning and merely serves as a useful repository in which to place gram negative Escherichieae which cannot be otherwise classified. Stuart *et al.* (1943) speak of *Paracolon aerobacter*, and the properties of many of these strains are very similar to the properties of the organisms designated in this paper as group I. We do not consider lactose fermentation as important a criterion as do Stuart *et al.* The readiness with which mutative lactose fermentation occurs among *Escherichia coli* and *Klebsiella* is well known. Edwards (1929) speaking of Friedländer's bacillus states: "if lactose fermentation is used as a criterion for the differentiation of species it will result in the separation of organisms serologically identical into different species. Such a procedure hardly seems justified when the majority of organisms labeled as Friedländer's bacillus are lactose fermenters."

The ability or the failure to ferment promptly inositol and glycerol clearly differentiates the encapsulated group II and the nonencapsulated group I strains. In their ability to liquefy gelatin and by their late fermentation of salicin, the latter group may also be differentiated from the former, strains of which generally do not liquefy gelatin and which readily ferment salicin. The nonencapsulated group II strains occupy an intermediate position and are distinguishable from group I strains by their ability to ferment inositol and glycerol, to utilize urea promptly and to ferment salicin promptly. Further, in contrast to group I strains most nonencapsulated group II strains do not liquefy gelatin. Group I strains do not attack dulcitol, many of them do not utilize urea, and many of them are motile. On the other hand, many group II strains ferment dulcitol, nearly all utilize urea, and all studied were nonmotile.

TABLE 2

The important biochemical properties of some typical group I (Aerobacter) strains

	STRAIN NO.							
	9089	7815	1099	6574	5002	5325	5471	6498
Lactose	+	−	+	+	×	−	×	+
Glycerol	×	×	×	×	×	×	×	×
Gas (glycerol)	−	−	−	−	−	−	−	−
Adonitol	−	−	+	+	−	−	+	+
Dulcitol	−	−	−	−	−	−	−	−
Salicin	×	×	+	×	×	×	+	−
Inositol	−	−	−	−	−	−	−	−
Indole	−	−	−	−	−	−	−	−
Gelatin	+	+	+	+	+	+	+	+
V. P.	+	+	+	+	+	+	+	+
M. R.	−	−	+	−	−	−	−	−
Urea	−	×	×	−	+	×	+	×
Ammonium-citrate agar	+	+	+	+	+	+	+	+

Symbols as in table 1.

DISCUSSION

Taxonomical criteria at present applied to the classification of bacteria are purely arbitrary, and with newer knowledge such criteria will be displaced by others of a more fundamental nature and more amenable to bacterial classification. The main criteria in use at present are (1) source, (2) pathogenicity, (3) biochemical properties, (4) antigenic structure, and to a lesser extent (5) morphology. Although biochemical classification, as generally used by the bacteriologist, is not fundamental, at least it does allow the formation of arbitrary divisions, which is expedient and useful from the epidemiological standpoint. Little is known yet of bacterial morphology, but since early in the history of bacteriology the presence on the surface of bacteria of capsules or other ectoplasmic layers (envelopes) has been known. In this work, combining biochemical tests and

"capsular and envelope" morphology, a division of the indole negative, methyl-red negative, Voges-Proskauer positive, citrate positive strains of the tribe Eschericheae into two groups is possible. Other Eschericheae with the same IMViC reactions also probably exist.

Kauffmann (1951) in his latest book *Enterobacteriaceae* defines two groups of Eschericheae—the Escherichia and the Klebsiella groups. The latter is defined as "a large group of serologically related, Gram-negative, non-sporing and non-motile rods, which usually possess capsules and form mucus. Usually, they do not form indole. They split adonitol and inositol. Often they decompose urea, give a positive Voges-Proskauer reaction, but a negative methyl-red reaction. They usually grow on ammonium citrate agar and ferment lactose." The Escherichia group is defined as "a large group of serologically related Gram-negative, non-sporing rods, showing, with certain exceptions, a motile peritrichous phase in which they normally occur. They usually form indole and give a negative Voges-Proskauer test, but a positive methyl-red reaction. They do not decompose urea and usually they do not grow on ammonium citrate agar. Gelatin is not liquefied." Certainly, the group of organisms designated in this paper as group I does not fill the requirements of either of these two groups of Kauffmann. On the basis of the IMViC tests group I strains are identical with Kauffmann's *Klebsiella*, but thereafter they differ widely from the Klebsiella organisms. They frequently are motile, they do not ferment inositol, they frequently do not attack urea, and do not form a capsule or "mucus". In contrast to the Escherichia group they have the opposite IMViC reactions and liquefy gelatin.

It is not the author's intention to enter into the polemics of bacterial nomenclature. Strains described in this paper under the heading of group II have the same properties as strains designated by Kauffmann (1951) as *Klebsiella*. Such strains are generally encapsulated. The position of rhinoscleroma and ozenae organisms in relation to the Klebsiella group will not be discussed. The organisms classified in this paper as group I differ from the Klebsiella organisms in that they are nonencapsulated, do not ferment inositol or glycerol, and liquefy gelatin. They are also late salicin fermenters, do not ferment dulcitol, and frequently do not utilize urea. They may or may not be motile. Serologically, it is possible that the O and R antigens of the groups I and II are the same. The final taxonomic position and naming of these two groups, I and II, are a matter for the International Enterobacteriaceae Subcommittee.

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SUMMARY

A description is given of 411 Eschericheae strains, most of which have the IMViC reactions $--++$. On the basis of morphology and biochemical tests it is suggested that those strains at present called *Aerobacter cloacae* and designated in this paper as group I constitute a homogeneous group of organisms with properties which distinguish them from the Klebsiella group of organisms. The characteristic features of this new group are: inability to ferment inositol or glycerol and the liquefaction of gelatin. The strains are nonencapsulated and are frequently motile. In addition they are late salicin fermenters, do not ferment dulcitol, and frequently do not utilize urea. The Klebsiella group includes those strains generally called *Aerobacter aerogenes* and designated in this paper as group II. The differential characteristics of the two groups are discussed.

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