THE PRINCIPAL DIVISIONS
AND GROUPS OF ENTEROBACTERIACEAE
AND THEIR DIFFERENTIATION

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It is not the writers' purpose to attempt a review of the literature on taxonomy and nomenclature within the family Enterobacteriaceae¹. It is wished to present only in outline a taxonomic system which we have found to be practical and which may be useful to others. The system outlined in the following pages is the result of many years of discussion and consideration on the part of the authors and it has been presented at a number of seminars, workshops, and informal discussions during the past few years. Also, persons primarily interested in taxonomy or nomenclature have expressed interest in the system because it is logical and can be directly transposed into a formal system of taxonomy and nomenclature consisting of tribes and genera by anyone interesting in so doing.

The system is presented in the nine tables that follow. The tables are self-explanatory and contain the essential information required in the differentiation of the various groups of Enterobacteriaceae. The reactions listed are those given by the majority of cultures but notes are included where necessary to explain certain important exceptions. The methods used in the determination of the biochemical reactivities of the microorganisms were those

¹Those interested in reading further on these subjects are referred to the following publications, which contain discussions and extensive bibliographies: Borman, et al. (1944), Cowan (1956), Edwards and Ewing (1955), Kauffmann and Edwards (1952), and Kauffmann (1949, 1953, 1954, 1956, 1959). Also, Bergey's Manual, the Report of the International Enterobacteriaceae Subcommittee (1958), and a file of the International Bulletin of Bacteriological Nomenclature and Taxonomy should be consulted.

which may be termed "recommended" or "standard" methods. A compilation of these methods is available upon request.

The family Enterobacteriaceae¹ is composed of a number of delineated groups of bacteria, each of which consists of types that give similar biochemical reactions. Some of these groups, or genera, are related to each other through the similarity of their biochemical reactions and, in many cases at least, by means of close antigenic relationships. For example, the Salmonella, Arizona and Citrobacter groups are related biochemically and may be said to belong to the same principal division. Similarly, the Shigella and Escherichia groups are more closely related to each other, both biochemically and serologically, than either is related to any other group, hence, are placed in another principal division. In the outline classification given herein, the authors have placed groups in the same principal division if the members of those groups possessed a number of common biochemical characteristics and have not relied upon any single characteristic, such as failure to ferment lactose, for this purpose. Therefore, the resulting taxonomic schema consists of only four principal divisions, each of which is composed of two or more groups. It is the writers' firm belief that this arrangement is more logical than certain classifications, which place quite different bacteria such as those of the Salmonella and Shigella groups in the same tribe simply because members of these groups usually fail to ferment lactose. It will be noted that the Serratia group has been incorporated into the Klebsiella-Aerobacter-Serratia principal division. This was done because the Serratiae have many properties in common with members of the Klebsiella and Aerobacter groups and because studies have indicated that there is no justification for a separate tribe Serratiae erected primarily upon pigment production.

It is well known that Enterobacteriaceae exist that may not be readily classified in one of the delineated groups, although the principal division to which such a culture belongs may be perfectly apparent. Such strains should be collected and studied carefully, since a collection of re-

¹Definitions of the family may be found in the Reports of the International Enterobacteriaceae Subcommittee (1954, 1958).

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lated biotypes may constitute a new group or subgroup. Or, a culture may be sui generis and another like it may not be seen again. To the authors the fact that occasional unclassifiable strains occur seems much less remarkable than the fact that the majority of cultures isolated daily in diagnostic work are perfectly typical and easily classified.

The principal divisions and groups are erected upon a biochemical basis and subgrouping within the various biochemical groups may be accomplished by means of further biochemical tests, on the basis of serology, or by a combination of both methods. Generally, the groups are subdivided on the basis of their O antigens as in the case of the Salmonella, Arizona, Citrobacter, Escherichia, Providencia, and Serratia groups. The O antigen groups are further subdivided into serotypes, which are characterized by the possession of particular O antigen fractions, K (sheath or capsular) antigens, and H (flagellar) antigens. Further, some serotypes may be composed of two or more biotypes or bacteriophage types. Some biochemical groups are divided into subgroups by means of the biochemical and serological characteristics of the serotypes contained in each of the subgroups. For example, the Shigella group is composed of four subgroups, which are differentiated by means of the biochemical and serological characteristics of the serotypes contained in each. In the Klebsiella group, subdivision generally is made directly into capsular types by means of K antigen determination, although at least three principal biochemical subgroups also are recognized by many within the Klebsiella group. These correspond to the species Klebsiella pneumoniae, K. rhinoscleromatis, and K. ozaenae. If biochemically defined species are to be erected within this group, it is possible that the indol positive, gelatin positive Klebsiella oxytoca (Bacterium oxytoccum Flüge) Lautrop 1956, should be included.

At present the Aerobacter group is divisible into three subgroups by means of biochemical characteristics. These subgroups correspond to the species Aerobacter cloacae, A. aerogenes, and A. liquefaciens Grimes (1931). No biochemical subgroups have been established within the Hafnia group since cultures studied to date are quite similar. It is the opinion of the authors that the biochemical interrelationships of Hafnia cultures and members of Aerobacter subgroups should be studied and further elucidated

before a final decision is made regarding the status of the Hafnia group and before generic status is assigned to it as suggested by Cowan (1956). Data presently available indicate that the Hafnia probably should be regarded as a subgroup of the Aerobacter group or perhaps simply as a biotype within one of the existing Aerobacter subgroups. It may be recalled that Stuart et al. (1943) considered cultures that now are called Hafnia as a subdivision of Aerobacter.

The authors have elected to retain the Rustigian and Stuart (1943, 1945) classification of the Proteus group and have placed the Proteus and Providencia groups in the same principal division. However, reorganization within the Proteus-Providencia division according to the suggestions made by Kauffmann (1953), by Ewing (1958), or according to other proposals may be desirable in future.

It may be noted that in the foregoing discussion of principal divisions nothing has been said about "the paracolon group." This omission was intentional, since in reality, there is no such thing as "a paracolon group." The term "paracolon" has been applied to a wide variety of bacteria, including certain salmonellae, and has lost any meaning it once may have had. Into a so-called "paracolon group" have been placed a large number of diverse microorganisms that have only one common differential characteristic: the inability to ferment lactose promptly. Each group of bacteria that ferments lactose promptly also includes counterparts that do not attack this substrate or do so only after prolonged incubation. Failure to ferment lactose should not exclude a culture from a group of which it is otherwise a typical member. An Escherichia strain and an Aerobacter culture should not be placed together in a "paracolon group" because each produces acid from lactose after some delay. Instead each should be classified in its appropriate group regardless of its reluctance to utilize lactose, particularly since the rate of lactose fermentation is not a stable characteristic. For example, a "Paracolobactrum coliforme" may be changed to Escherichia coli simply by selection of components that ferment lactose rapidly. Therefore, the writers do not believe that genera should be erected upon the single characteristic of failure to ferment lactose. Hence, we cannot accept the genus Paracolobactrum (Bergey's Manual, 7th ed.) in which totally different microorganisms are placed together simply because of their re-

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luctance to utilize lactose. Parenthetically, it may be added that the International Enterobacteriaceae Subcommittee does not recognize the genus Paracolobactrum nor the paracolobactrum group (Minutes of Subcommittee Meetings, Stockholm, 1958). At the risk of being repetitious we shall state again that the group to which a microorganism belongs must be determined by a combination of biochemical reactions, not by a single property. Only in this manner can an orderly arrangement of biochemical groups within the family be achieved.

Table 1. The Principal Divisions and Groups of Enterobacteriaceae.

Principal Divisions	Groups
Shigella - Escherichia	Shigella Escherichia (<u>E. coli</u> , including <u>Alkalescens-Dispar</u>)
Salmonella - Arizona - Citrobacter*	Salmonella Arizona Citrobacter* (including Bethesda-Ballerup)
Klebsiella - Aerobacter - Serratia	Klebsiella Aerobacter Hafnia Serratia
Proteus - Providence	Proteus Providence

*Formerly Escherichia freundii.

Table 2. Differentiation of the Principal Divisions Within the Enterobacteriaceae by Biochemical Methods
(Reactions of Typical Cultures)

Substrate or Test	Shigella - Escherichia Division	Salmonella - Arizona - Citrobacter Division	Klebsiella - Aerobacter - Serratia Division	Proteus - Provi- dence Division
Indol	d	-	-	d
Methyl-red	+	+	-	+
Voges- Proskauer	-	-	+	-
Simmons' citrate	-	+	+	d
Hydrogen sulfide (TSI)	-	+	- or (+)	d
Urease	-	-	- or (+)	d
KCN	-	d	+	+
Phenylal- anine	-	-	-	+

N.B. S. typhi, S. paratyphi A and some of the rare types are citrate negative. S. paratyphi and other rare types are H₂S negative.

+ = positive in one or two days.

- = negative.

(+) = delayed positive.

d = different biochemical types.

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Table 3. The Shigella-Escherichia Division.

Substrate or Test	Shigella Group	Escherichia Group
Gas from glucose	-*	+ (-)
Lactose	-*	+ or x
Salicin	-	d (approx. 80% +)
Motility	-	+ (-)
Lysine decarboxylase	-	+
Mucate	-	+ (-)
Christensen's citrate	-	+ (-)

*Certain biotypes of S. flexneri 6 form gas and S. sonnei cultures ferment lactose slowly.

N.B. There is no difficulty in the differentiation of typical E. coli cultures and shigellae. However the anaerogenic nonmotile, varieties of E. coli, some of which are often referred to as Alkaescens-Dispar types, may require closer examination before they can be definitely classified as E. coli. In attempting to classify a particular strain as E. coli or as a member of the Shigella group, the biochemical reactivities of the culture should be considered as a whole. Shigellae are much less reactive than E. coli strains and a culture that produces acid promptly (i. e., within 24 hrs.) from all, or most of a wide variety of carbohydrates, such as maltose, rhamnose, xylose, sorbitol, and dulcitol, undoubtedly is not a member of the Shigella group.

- + positive in one or two days.
- negative.
- + (-) majority of strains positive, negative varieties occur.
- x late and irregularly positive.
- d different biochemical types.

Table 4. The Salmonella - Arizona - Citrobacter Division

Substrate or Test	Salmonella Group	Arizona Group	Citrobacter Group
Lactose	-	+ or x	+ or x
Dulcitol	+	-	d
Gelatin	-	(+)	-
KCN	-	-	+
Lysine decarboxylase	+	+	-
Malonate	-	+	-

N.B. The majority of salmonellae ferment dulcitol promptly, but S. typhi, S. pullorum, S. paratyphi A, S. cholerae-suis, and a few others do not do so. Members of the Arizona group are uniformly negative on this substrate. S. paratyphi A is lysine negative.

+ positive in one or two days.

- negative.

(+) delayed positive.

x late and irregularly positive.

d different biochemical types.

Table 5. The Salmonella - Arizona - Citrobacter Division
Reactions in Organic Acid Media¹

Substrate or Test	Salmonella	Arizona	Citrobacter
Mucate	+	- (+)	+
D-tartrate	+	-	-
Citrate	+	-	-
Malonate	-	+	-

¹Organic acids as used by Kauffmann and Petersen (1956) and by Ellis, Edwards and Fife (1957).

N.B. The reactions given in Table 5 are the "pattern reactions" given by the majority of strains belonging to the three groups. Occasional strains may give a slightly different pattern and the publications quoted should be consulted when exceptional results are obtained.

+ positive in one or two days. - negative.
- (+) majority of strains negative, occasional strains positive.

Table 6. The Klebsiella - Aerobacter - Serratia Division

Substrate or Test	Klebsiella and Aerobacter Groups	Hafnia Group		Serratia Group
		37°C	22°C	
Gas from glucose	+	+	+	d
Gas from cellobiose	+	+	+	-
Gas from glycerol	d	+	+	-
Sorbitol	+	-	-	+
Methyl red	-	+	-	-
Voges-				
Proskauer	+	d	+	+
Gelatin	d		-	+

N.B. When gas is formed from glucose by Serratia, the volumes are small (10% or less).

+ = positive in one or two days. - = negative.
d = different biochemical types.

Table 7. The Klebsiella - Aerobacter - Serratia Division

Substrate or Test	Klebsiella		Aerobacter Group		Hafnia Group		Aerobacter Group		Serratia Group
	Group		Subgp. A	Subgp. B	37°C	22°C	37°C	22°C	
Gas from glucose	+		+	+	+	+	+	+	d
Gas from inositol	+		-	+	-	-	+	+	-
Gas from glycerol	+		-	+	+	+	d	(+)	-
Gas from cellobiose	+		+	+	+	+	d	(+)	-
Sorbitol	+		+	+	-	-	+	+	+
Raffinose	+		+	+	-	-	+	+	-
Lysine decar- boxylase	+		-	+	+	+	d	+	+
Arginine dihydro- lase	-		+	-	-	-	-	-	-
Ornithine decar- boxylase	-		+	+	+	+	+	+	+
Gelatin	-		(+)	x	-	-	+	+	+
Motility	-		+	+	d	+	+	+	+

N.B. When gas is formed from glucose by Serratia, the volumes are small (10% or less).

- Aerobacter Subgroup A corresponds to Aerobacter cloacae.

Subgroup B corresponds to Aerobacter aerogenes.

Subgroup C corresponds to Aerobacter liquefaciens (a psychrophilic bacterium).

+ positive in one or two days. - negative. (+) delayed positive.

x late and irregular positive. d different biochemical types.

Table 8. Differentiation of *Aerobacter* subgroup C and the *Hafnia* and *Serratia* Groups.

Substrate or Test	Aerobacter Group Subgroup C		Hafnia Group		Serratia Group
	37°C	22°C	37°C	22°C	
Gas from glucose	+	+	+	+	d
Gas from glycerol	d	(+)	+	+	-
Gas from inositol	+	+	-	-	-
Gas from cello-biose	d	(+)	+	+	-
Sorbitol	+	+	-	-	+
Arabinose	+	+	+	+	-
Raffinose	+	+	-	-	-
Rhamnose	-	-	+	+	-
Gelatin		+		-	+

N.B. When gas is formed from glucose by *Serratia*, the volumes are small (10% or less).

+ positive in one or two days. - negative.

(+) delayed positive. d different biochemical types

Table 9. The *Proteus* - *Providencia* Division

Substrate or Test	Proteus Group				Providencia Group
	vulgaris	mirabilis	morganii	rettgeri	
Mannitol	-	-	-	+	d
Indol	+	-	+	+	+
Simmons' citrate	d	d	-	+	+
Hydrogen sulfide (TSI)	+	+	-	-	-
Gelatin	+	+	-	-	-
Urease	+	+	+	+	-

+ positive in one or two days. - negative.

d different biochemical types.

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