A BRIEF SURVEY OF THE GROUP OF ACID-PRODUCING AND ACID-TOLERATING BACTERIA KNOWN AS THE LACTOBACILLUS GENUS*

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The Lactobacillus genus, which is without question one of the most widely distributed groups of microorganisms in nature, and to which important economic rôles have been attributed, possesses the following generic properties. The individual cells are rod-shaped, their length and thickness varying in a large measure with the nutrient medium in which they are grown, and according to the species. In young cultures they are Gram-positive, a property which is often lost, at least in part, as the cultures grow older. They are non-motile and non-sporulating. They produce delicate growths, as a rule, on artificial media, and in many instances do not submit readily to artificial cultivation. They vary in their oxygen requirements from facultatively anaerobic to micro-aerophilic.

The above description alone does not serve to distinguish the lactobacilli from other known forms, as, for example, the genus Kurthia (zopfii and zenkeri). The members of the Lactobacillus group are primarily fermentative, and possess at best but little proteolytic property. They act upon various carbohydrates, and produce lactic acid, often in considerable quantities. One of the properties which has attracted particular attention is their peculiar tolerance for free organic acids; hence the term "aciduric" as applied to the members of this group.

The different species vary considerably in their ability to attack carbohydrates, and in the amounts and kinds of acid produced. For example, while many decompose lactose, others do not. Again, some readily attack the pentoses, xylose and arabinose, while others are without this property. Then, whereas a large number form lactic acid, with only very small amounts of by-products, others produce, in addition to lactic acid, appreciable amounts of other substances, particularly acetic acid and alcohol.

The first known organism of the aciduric group was described by Kern, in 1881, as *Dispora caucasica*, and later studied and renamed by Beijerinck *Lactobacillus caucasicus*. It was isolated from kefir. It closely resembles the *L. bulgaricus* of Grigoroff, the first account

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of which was published in 1905, but it differs from the latter in that it appears granular and is decidedly less acidogenic than *L. bulgaricus*.

Many other organisms of the Lactobacillus group have been reported from time to time as definite and distinct types as, for example, the Boas-Oppler bacillus[3], *L. acidophilus* of Moro[28], *L. bifidus* of Tissier[39], *L. acidophilus-odontolyticus* of McIntosh, James and Lazarus-Barlow[23, 24], *L. delbrücki* of Leichmann[22], *L. leichmanni* of Henneberg[16], *L. lycopersici* of Mickle[25], and *L. pentoaceticus* of Fred, Peterson and Davenport[8].

Members of this genus have been found to occur in the intestine of man, as well as in that of rats, guinea pigs, rabbits, fowl, sheep, cows, horses and other wild and domestic animals; in the human stomach and vagina; in milk, butter, cheese, ensilage, sour-mash, wine, beer, compressed yeast, sour dough, tomatoes and tomato catsup; and in soil and on cereals and various raw cereal products.

The various organisms may be grouped for convenience under (1) the milk and dairy products types, (2) the intestinal types, (3) the oral and dental types, and (4) the raw cereal and vegetable products types. The first three of these groups are more or less well defined; the fourth is a mixed group and admits of further subdivision, largely on the basis of ability to decompose various carbohydrates and mannitol, and the amounts and kinds of acids formed.

Lactobacilli Found in Raw Milk and Dairy Products

While the natural souring of cow's milk is due mainly to an organism which is not a member of the Lactobacillus genus, namely *Streptococcus lacticus*, aciduric bacteria of the bulgaricus type are, as a rule, present in the milk, cream, cheese and butter when they leave the dairy plant, particularly in countries and sections having a warm climate. *L. bulgaricus* and the closely related types or varieties require a relatively high incubation temperature for rapid development (35-45° C.). *Streptococcus lacticus*, on the other hand, thrives at the lower temperatures, and continues to multiply even in the ordinary refrigerator (8-12° C.). In the colder or more temperate climates, therefore, the usual holding or storage conditions for milk and the various milk products are such as to discourage the free development of the bulgaricus type, and to encourage the milk-souring activities of *Streptococcus lacticus* and other milk organisms, particularly of the coli-aerogenes group.

L. bulgaricus came into prominence through the extensive studies of Metchnikoff and his followers on the relationship of high sour-milk diet to health and longevity of oriental people subsisting on their so-called national sour-milk diet. Conspicuous among these sour milks are kefir and yoghurt of the Balkans and Southern Russia, mazun of Armenia, huslanka of Bukowina, gioddu of Sardinia, and leben raib of Egypt. While these various products differ from each other more or less in certain respects, they all contain high acid-producing organisms which are apparently essential to, and characteristic of, the products.

Continued search for the supposedly characteristic organism resulted, in 1905, in the isolation of what is now generally known as *L. bulgaricus* by Grigoroff in Massol's laboratory, and frequently referred to as the bacillus of Massol. This was further established as a definite type or species by Metchnikoff in his use of the organism in the preparation of bulgaricus milk and other *L. bulgaricus* products for therapeutic purposes.

L. bulgaricus, or a very closely related organism, has been regarded for some time as playing an important rôle in the natural curing of corn silage. Heinemann and Hefferan[15] were apparently the first to isolate it from ensilage. Hunter and Bushnell[19] demonstrated its constant occurrence and probable importance in this fermentation product. Sherman[37] found it to be present in corn silage in very large numbers; also on corn fodder in its natural state. While the ensilage organism (or organisms) may not be identical with Grigoroff's *L. bulgaricus,* it resembles the latter more closely, especially in its high acid-producing property, than any other known forms, particularly the intestinal types.

Organisms of the Lactobacillus genus which are identical with, or more or less closely related to, *L. bulgaricus* have been observed also in various other fermentation products, for example, sauerkraut, pickles and sour-mash.

A large Gram-positive bacillus was reported by Boas and Oppler[3] which in more recent years has been regarded by observers as being in certain respects quite similar to *L. bulgaricus.* It was found originally in the gastric juice of patients suffering from carcinoma of the stomach. In the same year Schlesinger and Kaufmann[35] found what appeared to be the same organism in the stomach in 19 of 20 cases of gastric carcinoma. Others reported similar findings, as, for example, those of Galt and Iles[9]. Some even went so far as to state that malignant cases of gastric carcinoma could be distinguished from the benign by the presence of the Boas-Oppler bacillus in the gastric content.

Strauss[38] reported the presence of apparently the same aciduric organism in small numbers in normal gastric juice. Heinemann and Ecker[14] concluded from their observations that this bacillus may occur in normal gastric juice in moderate numbers, but that in stomach fluids which contain decidedly less than the normal amount of hydrochloric acid it is present in large numbers. Its presence in the gastric juice in large numbers is, according to them, an indication of lowered hydrochloric acid content, or complete absence of this acid, this condition being due to one of several causes as, for example, gastric ulcer, gastric carcinoma, gastritis and pernicious anemia. They held to the view that this organism is a true lactic acid bacillus, as Kuntz[21] and others had suggested, but that it does not belong to the group of aciduric intestinal bacilli so commonly observed in infants, because, among other things, it did not attack maltose.

Intestinal Types of Lactobacilli

In spite of the fact that the intestine is a veritable culture tube, and that the contents of the colon consist largely of bacterial cells, only relatively few species of bacteria find the conditions favorable for multiplication and continued existence. Of the known organisms which are characteristically of intestinal origin, four assume a numerically prominent rôle; namely, *Bacterium coli*, the enterococcus (*Streptococcus fecalis*) of Thiercelin, *L. acidophilus* and *Cl. welchii*. Other forms often play a more or less temporary rôle, as, for example, *Proteus vulgaris*, staphylococci and long-chain streptococci, and, of course, the various pathogenic bacteria which invade the intestine as a seat of infection or a route of elimination.

Soon after birth adventitious organisms make their entrance into the digestive tract, largely through the mouth. The temporary, heterogeneous intestinal flora is soon followed by a more definite and characteristic mixture, the exact composition of which depends on the diet. In infants subsisting entirely on mother's milk a characteristic Lactobacillus flora develops rapidly, in which 90 per cent or more of the bacterial population of the colon or feces is often made up of *L. acidophilus* and the closely related *L. bifidus*.

Marked changes can be brought about in the intestinal flora of man and of lower animals at all ages by the oral administration of dextrin or lactose or a lactose-containing food like milk. For example, by feeding from 2 to 3 grams of lactose daily to adult white rats subsisting on normal mixed diet, the intestinal flora becomes changed in the course of 2 or 3 days to one in which *L. acidophilus* predominates, at times to the extent of 90 or 95 per cent of the intestinal population. When the amount of lactose is increased to 3 or 4 grams daily, the *L. acidophilus* type is rapidly superseded by a flora which is largely or almost wholly *L. bifidus*. No other carbohydrates are known to exert the same influence. These observations have been fully substantiated in various laboratories.

The favorable influence of lactose and dextrin on the aciduric flora apparently rests on the fact that these carbohydrates are not completely decomposed in, or absorbed by, the upper portion of the intestine, and are therefore carried to the colon where they offer favorable pabulum for the development of the aciduric organisms. It may rest in part on increased H-ion concentration in the colon caused by the bacterial decomposition of the lactose or dextrin, and a subsequent selective environment for the aciduric types, according to the suggestions of Cannon[5].

The administration of large numbers of *L. acidophilus* to man results, as a rule, in the appearance of this organism in large numbers in the feces. This is particularly true when the material is taken in the form of acidophilus milk, or bouillon culture accompanied by liberal amounts of lactose. The ingestion of *L. bulgaricus* cultures in the same amounts and manner is not followed by the appearance of the living cells in the feces, as various investigators have shown.

The extent to which *L. acidophilus* is implanted in the intestine through the ingestion of cultures or suspensions of this organism is perhaps a debatable question. The problem is complicated by the possibility that the administration of *L. acidophilus* in milk or along with lactose stimulates the development of strains of this organism which are native in the intestine, and their elimination in the feces. This may be done by the carbohydrates alone, or in combination with the administered organism, the latter acting in a way more or less analogous to crystals which, when added to a concentrated solution of the same substance, cause the solution to crystallize.

Successful implantation of *L. acidophilus* must depend largely on the strains of the organism which are selected for the purpose, and on its acclimatization to the varied intestinal environment which is to be encountered in different subjects or hosts. Experiments are now being conducted in our laboratory with what appears to be a particularly favorable strain, and one that may be recovered readily from the subjects and identified as the original ingested type. While promising results have already been obtained, considerable time will be required to establish this important principle.

L. bifidus and *L. acidophilus* are characteristically intestinal organisms. They require carbohydrates for their growth, and even under the most favorable conditions are isolated and grown on artificial media with some difficulty, and at best produce only comparatively delicate growths. Development at temperatures below 30° C. is slow and limited, if it takes place at all.

While the two organisms are very closely related, they possess marks of difference in morphology and cultural reactions, which have again been emphasized recently by Cruickshank[6]. *L. bifidus* is decidedly more pleomorphic and, according to Tissier and various other observers, shows distinct branching. Cruickshank, however, claims that real branching is rare, and that what others have regarded as branched cells are not true branching, but pseudobranching forms. *L. bifidus* has more exacting oxygen-carbon dioxide requirements than those of the Moro bacillus, being unable to grow on the open plate, in primary culture. It also has a greater tendency than has *L. acidophilus* to lose its viability in old cultures. One of the most striking differences, however, lies in its high energy requirements.

Another aciduric organism which is very closely related to, or identical with, *L. acidophilus* is the bacillus of Döderlein, which was found originally in the human vagina, often in large numbers, by this investigator[7]. These claims have been substantiated by various other observers. Thomas[40] regards the organism as indistinguishable from Moro's bacillus. He found it to be present in the normal vagina in less than 10 per cent of young children. He concluded that when it invades the vagina it does so by exterior passage from the intestinal tract. He failed to demonstrate the presence of the Döderlein bacillus in the vagina in cases of gonococcal vaginitis, and, since this organism was found by him to exert an inhibitive action on the growth of the gonococcus *in vitro*, he assumed that it has a distinct inhibiting effect on the latter in the vagina.

In spite of certain isolated claims to the contrary, *L. acidophilus* and *L. bulgaricus* should be regarded as distinct types or species. As first pointed out by Rahe[30], *L. acidophilus* ferments maltose, whereas *L. bulgaricus* does not possess this property. Their deportment toward levulose is also different. In some border-line strains these differences disappear, of course. According to Albus and Holm[1], and Kopeloff and Berman[20] the Moro bacillus is able to develop at a much lower surface tension than the bulgaricus organism. Furthermore, *L. acidophilus* is a slow and low acid-producer, as compared with the other. Finally, the former is distinctively an intestinal type of organism, constituting at times the bulk of the fecal flora. *L. bulgaricus*, on the other hand, is destroyed when introduced into the gastro-intestinal tract, even in enormous quantities and under the most favorable dietary system, as has been shown repeatedly by various independent workers.

The Oral and Dental Types of Lactobacilli

Stimulated by the theory long held in some quarters that dental decay owes its origin to the activities of acid-producing bacteria of the mouth, numerous attempts have been made to incriminate some particular organism or organisms, and for some time chief attention was directed to certain types of streptococci as, for example, in the work of Goadby[10], Baumgartner[2], Hartzell and Henrici[13] and Seitz[36]. Howe and Hatch[17] isolated organisms of the Lactobacillus group from carious teeth and referred to them as *L. acidophilus* and *L. bifidus*. McIntosh, James and Lazarus-Barlow[23] called attention to a member of this genus which they found in caries mouths and which they believed to bear a definite relationship to dental caries. While it resembled *L. acidophilus* in certain aspects, they regarded it as being distinctly different from the Moro bacillus. They named their organism *Bacillus acidophilus-odontolyticus*. In an investigation conducted independently, Rodriguez[31] found what appeared to be a similar bacterium, to which he attached considerable significance.

Bunting and Parmelee[4], in reporting their earlier findings, briefly described a lactobacillus which they found to be a constant inhabitant of decayed teeth, and with which they claimed to have demonstrated by *in vitro* experiments a direct relationship between the organism and tooth destruction. They applied the name *B. acidophilus* to this bacillus, presumably because this was the easiest expedient in finding a name for the christening, and because the name would require no effort on their part to familiarize the reading public with it.

The very incomplete description given the organism by Bunting and by Hadley did not warrant their naming it *B. acidophilus*. In subsequent publications they have made rather weak attempts to justify their action. Hadley, Bunting and Delves[12] announced that they had established the identity of their dental organism by dissociating it (a smooth type) and deriving a rough dissociant which resembles the ordinary rough type of *L. acidophilus*. They did not show, however, that with the change from smooth to rough the rough type assumed the other characteristics which the intestinal type possesses, and particularly the property of viability and continued multiplication in the intestine when introduced into the digestive tract. Rosebury, Linton and Buchbinder[33] and Howitt and van-Meter[18] have published results of comparative studies of so-called intestinal and oral strains of Lactobacillus, and arrived at the conclusion that the two are not essentially different. In carefully reviewing their work one is struck with the lack of authenticity of some of their so-called intestinal strains, and particularly those upon which their conclusions as to identity of types are largely based.

Quite recently Rodriguez[32], in discussing certain phases of dental caries, constantly referred to the organism associated with dental caries, and which by his quantitative method he demonstrated to be present in enormous numbers in the saliva of caries mouths, as *L. acidophilus*, being largely influenced apparently by the claims of the Michigan group. He offers no reasons or observations of his own to support his action. However, the paper of Rodriguez can carry little force regarding the relationship of the so-called dental *L. acidophilus* to caries, for he admits that the relationship is still to be proved, in the following words; "irrespective of whether *L. acidophilus* is the specific bacterial agent of tooth decay, a proposition which I willingly admit remains within the confines of debatable grounds . . ." Indeed, there appears to be a rapidly accumulating wealth of evidence that the basic and underlying cause of dental decay is essentially faulty nutrition.

Following several years of intensive research on dental caries, and on the relationship of intestinal to oral strains of lactobacilli, Morishita[26, 27] concluded that the intestinal and dental organisms are not one and the same type. He also made clear his belief that "in spite of their [high acid-producing lactobacilli] occurrence in active pathological parts of the teeth and in saliva, it is impossible as yet to offer final proof that these organisms are directly responsible for the production of tooth decay"

The death of Dr. Morishita in 1929 interrupted his investigation at a most critical point, but certain phases of the work are being continued in this laboratory. These studies, including a comparison of different types of lactobacilli, and experiments on dissociation, thus far fully support Morishita's claims that *L. acidophilus* of the intestine is a type distinct from the common oral type or types of McIntosh, Rodriguez, Bunting and others.

While the commonly recognized intestinal type may be found at times in dental cavities and in the saliva, it is in all probability only an adventitious organism here and is numerically insignificant. The dental type which is found in such large numbers in tooth cavities and in the saliva of caries mouths has certain characteristics which should distinguish it from the generally recognized intestinal *L. acidophilus*. It produces, as a rule, smooth, solid, comparatively large and dense colonies on appropriate agar plates, when grown at incubator temperature. It grows readily at room temperature. Its cultural requirements are not extremely exacting. It is a rapid and high acid-producing organism and acts in a wider carbohydrate range than does *L. acidophilus*. Finally, there is apparently no evidence to show that an aciduric organism of the odontolyticus (common dental) type occurs in the intestine ordinarily or following the use of high lactose or dextrin diet.

If we accept the views of the extreme pleomorphists we perhaps should conclude that all aciduric organisms constitute one type or species. Likewise, we would also have to assume that all of the known paratyphoid organisms have no right to existence as definite species, and perhaps even that the tubercle bacillus is but a variant or special phase of the common spore-forming species, *B. mesentericus*. However, the paths of the extreme pleomorphists are still far from leading us to the point where the entire present system of taxonomy and nomenclature is to be discarded. After all, there is a remarkable fixedness of bacterial species or types, in spite of the multitudinous environmental influences to which bacterial cells are exposed when growing under either artificial or natural conditions.

Types of Lactobacilli Commonly Found on Grain, and on Cereals and Vegetable Products

The organisms to be discussed here constitute a so-called mixed group, and are treated as such largely as a matter of convenience, as has already been stated. There are included in this more or less artificial group certain species, the outstanding characteristics of which are ability to decompose one or both of the pentoses, xylose and arabinose, and to produce, beside lactic acid, relatively large amounts of acetic acid. *L. pentoaceticus* of Fred, Peterson and Davenport[8] is perhaps the most important and best-known member of this subgroup.

There are included, also, organisms which possess, as a distinguishing mark, the property of high lactic acid production from the hexaldoses. In this division are included *L. delbrücki* of Leichmann and *L. leichmanni* of Henneberg. However, not all high acid-formers belong in this category. For example, *L. bulgaricus,* which is primarily of dairy and dairy products origin, and certain of the oral and dental aciduric types also produce relatively large amounts of lactic acid under appropriate conditions.

Included here, also, is a group of aciduric bacteria which at times plays an important rôle in the spoilage of tomatoes and tomato products; this group comprises the following organisms, among others: *L. lycopersici* of Mickle, *L. mannitopoea* and *L. gayoni* of Müller-Thurgau and Osterwalder, and *L. plantarum* of Orla-Jensen. *L. pentoaceticus* is also found at times in spoiled tomato products. A description of these organisms and of their biochemical properties has been published recently by Pederson[29].

L. plantarum forms very little, if any, product other than lactic acid, while the other three species produce both lactic and acetic acids in appreciable amounts from glucose, together with some ethyl alcohol and carbon dioxide. Mannitol is produced from fructose by these same three organisms. The lactic acid formed by all of the above species or types is inactive.

Another interesting and economically important organism of this mixed group of lactobacilli is Henneberg's *L. panis,* which was isolated originally from sour dough; this and closely related lactobacilli appear to play an important part in the baking of "salt-rising" bread. The acid produced by these bacteria from the sugar in the dough acts on the baking-powder, liberating the carbon dioxide upon which the lightening process in the dough depends.

Space does not permit of a detailed description of the various organisms referred to in this paper. However, some special attention will be given to the well-known pentose-fermenter, *L. pentoaceticus*, and to the high lactic acid producing type or types, of which *L. delbrücki* and *L. leichmanni* are the best-known representatives.

Lactobacillus pentoaceticus and Pentose Fermentation

Aside from the work of Gayon and Dubourg, Maze and Perrier, and Müller-Thurgau and Osterwalder, little concentrated effort was devoted to the study of pentose fermentation by microorganisms until 1919, when Fred, Peterson and Davenport published their first work on *L. pentoaceticus*, which was isolated by them from various grains, soils, manures and silage.

They found that this organism readily decomposes xylose, with the formation of large amounts of lactic and acetic acids, and traces of ethyl alcohol and carbon dioxide. The maximum quantities of acid were obtained in cultures containing from two to three per cent xylose, and the proportion of the volatile to the non-volatile acid was found to be approximately the same throughout the period of fermentation, namely, 40 per cent of acetic to 60 per cent of lactic acid.

More recently our laboratory made a rather intensive study of *L. pentoaceticus*, in which the work of the Wisconsin investigators was corroborated, and our knowledge concerning the distribution and properties of this aciduric type somewhat broadened. The following conclusions, among others, were arrived at: (1) pentose-destroying bacteria of the *L. pentoaceticus* type are widely distributed in nature; (2) no definite, close relationship can be established between *L. pentoaceticus* and aciduric organisms of dental or intestinal origin; (3) xylose is fermented by this bacterium with the formation of acetic and inactive lactic acids in the ratio of 42: 58; (4) an excess of calcium carbonate is necessary for complete fermentation of the pentose; (5) the amounts of acid formed in the liquid medium do not seem to be influenced by oxygen tension; (6) from 85 to 90 per cent of the xylose destroyed is represented by the lactic and acetic acids formed; and (7) the optimum temperature for the production of the acids from xylose by *L. pentoaceticus* is 33° C.

All of the strains of *L. pentoaceticus* studied fermented glucose, levulose, galactose, maltose, sucrose, mannose, mannitol, xylose and arabinose, but failed to attack lactose and the various other carbohydrates and alcohols usually employed in fermentation tests.

High Lactic Acid Production by Members of the Lactobacillus Genus

Quite recently our laboratory made a series of comparative quantitative studies of lactic acid production of various members or types of the Lactobacillus genus, including *L. bulgaricus, L. acidophilus, L. acidophil-aerogenes, L. odontolyticus* (dental strain), *L. pentoaceticus, L. fermentatae, L. plantari, L. delbrücki* and *L. leichmanni.* Of these various types, *L. delbrücki* stood out conspicuously as a high lactic acid producer. Certain strains of *L. leichmanni* and *L. odontolyticus* (mouth and dental types) also possessed this property in a large measure. As the strains of *L. delbrücki* were consistently high acid producers, and this organism has been used in recent years in commercial lactic acid production by the biological process, special emphasis is placed on this type or species in the following discussion.

L. delbrücki was first described by Leichmann in 1896, having been isolated by him from sour potato-mash in a distillery. It is ordinarily a medium-sized Gram-positive rod, occurring singly, or in short or long chains. It grows well in casein-digest and in yeast-water broth to which a fermentable carbohydrate has been added, and in tomato-juice broth. It rapidly acidifies the medium. It readily ferments glucose, levulose, galactose, and maltose, but does not attack lactose and the other carbohydrates and alcohols which are commonly employed for differential purposes. Its action on sucrose, mannitol, mannose and dextrin was somewhat variable, according to our observations. Its action on carbohydrates is limited within a rather narrow range, but it possesses unusual ability to produce large amounts of lactic acid from the hexoses, particularly glucose.

The production of commercial lactic acid by the biological process is a highly specialized field. A mash containing from 10 to 12 per cent sugar is heated for at least an hour, with frequent stirring or agitation. After cooling, it is inoculated liberally with a freshly prepared bulk starter (pure culture of *L. delbrücki*). Fermentation sets in in the course of four or five hours, the acidity of the mash increasing rapidly. Excessive acidity is prevented by the use of milk of lime. In from a week to ten days the sugar is completely destroyed, being converted almost quantitatively into lactic acid, which is finally harvested as commercial lactic acid, after acidification with sulfuric acid and removal of the precipitated calcium sulfate. The resultant solution contains as much as from 15 to 20 per cent lactic acid. Special precautions are necessary to avoid contamination of the mash with foreign organisms before the fermentation process is completed. The acid produced is levorotatory lactic acid.

Some strains of *L. leichmanni* also lend themselves to the production of lactic acid in volume, and in some instances this organism has been used alone or along with *L. bulgaricus*. Our own observations on the high acid-producing property of certain dental strains (*L. odontolyticus*) of Lactobacillus should warrant further experimentation with this species or type, with the view of employing it in the biological production of lactic acid in volume.

A discussion of the very large and complex group of lactic acid producing organisms which constitute the Lactobacillus genus must at best be very inadequate in a paper of this length. Furthermore, in the present limited state of our knowledge regarding the exact relationships of the different members or types to each other and to the various processes in which they may and do play a rôle, it would be futile to attempt anything like a thorough systematic classification. What is of particular interest thus far is that, in spite of the various properties which all of the members of this genus have in common, the differences are often as pronounced as those which are observed between widely different genera, especially in so far as the biochemical properties are concerned.

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