THE GENUS PEDIOCOCCUS[1]

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A lactic acid producing sarcinalike organism has been associated with a type of spoilage in beer since the condition was first described by Pasteur (1876). He found that these organisms, which occur in pairs and fours and were sometimes associated with long rod bacteria, are the cause of the socalled "beer sickness." Their relationship to the true lactic acid bacteria of the genera *Lactobacillus* and *Streptococcus* was not realized until years later. The organism in spoiled beer was first referred to as a sarcina by Hansen (1879). The spoilage was studied by Balcke (1884*a*); and in Balcke's (1884*b*) second paper he recognized the assistance of Dr. Kurth in determining that the organism was not a true sarcina. Balcke applied the binomial *Pediococcus cerevisiae* to this species. In a series of papers, Lindner (1887, 1888, 1889) described the organisms involved in "beer sickness" more completely and proposed new specific names for types producing somewhat different reactions from that of the strain which he believed was *Pediococcus cerevisiae*.

The pediococci were not associated with other fermentations until years afterward, and it is doubtful whether anyone except Henneberg (1926) associated them with desirable fermentations such as those brought about by some species in the closely related genera *Streptococcus* and *Lactobacillus*. In this respect they might be compared with the genus *Leuconostoc*. The ability of species of *Leuconostoc* to bring about desirable changes was not recognized until Hammer (1923), Orla-Jensen (1919), and Pederson (1929, 1930) found that they were important in certain fermentations. The pediococci may be found to be very important in some vegetative fermentations when more is learned about their true role in nature.

The name "*Sarcina*" may continue to be attached to these organisms by brewing technologists but they should be classified properly by bacteriologists in relation to other organisms.

Since the name *Pediococcus* was first used by Balcke, this has been accepted and used as a generic name in connection with other specific names by Lindner (1887, 1927, 1928), De Toni and Trevisan (1889), Reichard (1894), Fischer (1897), Claussen (1903), Sollied (1903), Henneberg (1926), Pribram (1933), Mees (1934), and others. Buchanan (1925) states: "If the cocci which are arranged in tetrads are to receive generic recognition, the name *Pediococcus* would appear to be valid. The type species is *Pediococcus cerevisiae* Balcke."

In the 6th edition of Bergey's Manual (Breed *et al.*, 1948) the genus is included in an appendix to the family *Micrococcaceae*, with the type species *Pediococcus*

[1] Journal Paper No. 780 New York State Agricultural Experiment Station, Cornell University, Geneva, New York.

cerevisiae Balcke. Twelve other species are listed as having been described. In regard to the genus, it is stated: "The following genus is recognized by workers in the brewing industry. It includes species that present characters intermediate between *Micrococcus*, *Sarcina*, and *Streptococcus*. Many students prefer to regard these as species of *Sarcina* (Macé, 1901)."

Still others (Lehmann and Neumann, 1896, Migula, 1900, Hucker, 1924) have felt that *Pediococcus* may be regarded as a synonym of *Micrococcus*. Buchanan (1925) stated that if *Pediococcus* does not receive generic recognition it perhaps may become a synonym of *Micrococcus*.

Beijerinck (1908) applied the name *Lactosarcina* to this genus in line with his names *Lactococcus* and *Lactobacillus*. Shimwell (1947) considers that they should be included among the plant types of the genus *Streptococcus*. Mees (1934) would include in the genus two strains described as *Tetracoccus* by Orla-Jensen (1919). Macé (1901) included these under *Sarcina*. Pribram (1933) placed the genus in a new family, *Pediococcaceae* and attributed the generic name to Balcke, but gave as the type *Pediococcus tetragenus* Koch and Gaffky. This as well as the other species named, *P. tetrus*, *P. flavus* and *P. gadidarum*, apparently do not belong in the genus.

Lindner credited Hayduck (1885) with a study of two sarcinalike organisms. Lindner studied two types of organisms, the first a low acid producer which he considered to be a strain of *Pediococcus cerevisiae*. The second produced more acid and was thus given the name *Pediococcus acidi lactici*. The acid was identified as lactic acid. The optimum temperature of growth was found to be 41 C. In a second paper Lindner (1888) described another species, *Pediococcus albus*. In this second study, reference is made to the fact that he studied the strain of *Pediococcus cerevisiae* that von Huth had studied. However, according to Mees (1934) von Huth studied an organism from horse urine which was believed to be the source of the organism in beer. There seems to have been no definite study of the original culture of Balcke and thus Lindner was possibly wrong in calling von Huth's culture *Pediococcus cerevisiae*. Lindner (1889) applied a third name *Pediococcus viscosus* to a slimy variety.

De Toni and Trevisan (1889) accepted the generic name and listed the first three specific names mentioned above and three others, *Pediococcus kochii*, *Pediococcus maggiorae*, and *Pediococcus aurantiacus*. However, they credited the name to Lindner and list *Pediococcus acidi-lactici* first. These three other species are different and should not be included in the genus. A number of other names have been applied to sarcina like organisms in beer, including *Pediococcus sarcinaeformis* by Reichard (1894), *Pediococcus damnosus* and *Pediococcus perniciosus* by Claussen (1903), *Pediococcus kiliensis* by Bettges (1906), *Pediococcus odoris mellisimalis* and *Pediococcus acidulefaciens* by Schönfeld (1904), *Pediococcus lindneri* by Henneberg (1926), and *Pediococcus halophilus*, *Pediococcus pentosaceus*, and *Pediococcus urinae equi* by Mees (1934). Many of these early studies centered around the technique of isolating the organisms in pure culture and of course also involved the particular type of beer. Sollied (1903) isolated a strain from a potato mash and applied the name *Pediococcus hennebergi*. This

apparently was the first type isolated from a product other than beer or beer mash and it differed from all of the others except possibly *Pediococcus acidi-lactici* in that it had a higher optimum temperature for growth, 40 C, and was an active arabinose fermenter. It produced inactive lactic acid. The pediococci have been studied by Hansen (1890), Brown and Morris (1895), and Reichard and Riehl (1895) some of whom have referred to them as sarcina.

The names *Pediococcus damnosus* and *Pediococcus perniciosus* were included in Lehmann and Neumann (1927), but no reference was made to the names proposed by Balcke or by Lindner.

Henneberg (1926) studied cultures isolated from grain mash and potato mash and observed them in sauerkraut, molasses, salt beans, and in pickle fermentations. In addition, he referred to the tetracocci of Orla-Jensen (1919). He included three species, *Pediococcus acidi-lactici* of Lindner, *Pediococcus hennebergi* of Sollied, the wild lactic acid bacteria of potato mash, and *Pediococcus lindneri*, a new type which he stated he originally confused with Lindner's *Pediococcus acidi-lactici*. He also discussed briefly a slime producing variety from kraut which produced gas and less acid than the other types and which he stated might be used as a starter for sauerkraut since it produced a good aroma. He noted that this type sometimes occurred in short chains. It seems obvious, however, that he had confused the pediococci with leuconostocs, a type whose importance in kraut fermentation he had not recognized. Henneberg's separation of species is based upon colony and morphological differences, utilization of different sugars, and the amounts of acid produced. He pointed out that they all produce inactive lactic acid with a trace of volatile acid.

Mees (1934) presented a historical review of the studies on the spoilage of beer by these organisms. Mees (1934) studied eight strains that he had isolated by various methods as well as strains of the genus *Tetracoccus* Orla-Jensen (1919), a culture he had received from van Niel, a culture from horse urine, and several others which eventually he decided were not of this genus. He noted that the cultures considered to be pediococci were all nonmotile, gram positive, nonspore-forming, catalase negative, spherical bacteria. On acid media a strong tendency toward tetrad formation was observed, but in neutral media they were mostly diplococci. Complex protein degradation products were required for growth. With the exception of one of the tetracoccus cultures, all cultures produced inactive lactic acid, grew best at 23 to 24 C, and readily fermented glucose, fructose, maltose, and galactose. Lactose, sucrose, xylose, and arabinose were utilized only to a limited degree, if at all. Mees accepted the generic name *Pediococcus*, but used *Pediococcus damnosus* Claussen as his type with *Pediococcus cerevisiae* Balcke as a questionable synonym. He characterized *Pediococcus perniciosus* Claussen as a variety of *P. damnosus* and named a new variety *P. salicinaceus*. Species names, *Pediococcus pentosaceus* and *Pediococcus halophilus*, were applied to organisms included by Orla Jensen (1919) in *Tetracoccus*. Still another name *Pediococcus urinae equi* was given for the type isolated from horse urine.

In a series of papers, Shimwell and Kirkpatrick (1939) and Shimwell (1940,

1947, 1948) have reviewed the subject of beer disease organisms and conclude that the beer sarcina or pediococci should be accepted in the plant division of the genus *Streptococcus* because they are not regarded as true sarcinae or as micrococci. Shimwell (1940) credits Mees with the first scientific study of the problem. Shimwell and Kirkpatrick pointed out that the production of lactic acid with a small amount of volatile acid and carbon dioxide, and the production under certain conditions of diacetyl (the aromatic substance of butter) are all characters of the streptococci. The fact that the organisms produce inactive lactic acid and occur in tetrad formation is not considered sufficient cause for a generic separation of these organisms from streptococci. Accordingly they consider the beer cocci to be intermediate, in the classification of Davis (1936), between the *dextro*-lactic acid producing *Streptococcus lactis* group and the heterofermentative *levo*-lactic acid, diacetyl producing vegetative type of the aroma group. Shimwell proposed a first species differentiation based on temperature optimum and a second one on sugar fermentation.

Recent observations. The pediococci were observed in this laboratory in microscopic preparations from fermenting sauerkraut a number of years ago. However, they apparently do not play an important part in a normal kraut fermentation and actually were not isolated until 1944 when five strains were obtained. Later, 49 cultures were isolated from fermenting beans in salt brine and 645 from fermenting salt stock cucumbers. They played an important part in the fermentation of the beans and in some of the series of cucumber fermentations, particularly in the 1946 series in those brines containing 2½ to 5 per cent of salt. All cultures were isolated from small, round or lens shaped colonies and grew well in a stab of glucose yeast extract tryptone agar. After preliminary studies, 121 representative cultures were selected for a more complete study. These were remarkably similar. They were gram positive, nonmotile, catalase negative coccus forms which tended to occur in packets of four cells. None of the cultures liquefied gelatin or produced indole. Using the same 0.5 per cent tryptone, 0.3 per cent yeast extract base with added buffer salts, no significant variation was noted in regard to the fermentation of sugars and the related carbon compounds.

The cultures varied somewhat in regard to the amount of acid produced in sugar media. All cultures produced from 0.5 to 0.9 per cent acid in glucose, fructose, mannose, galactose, and maltose with an approximate average or frequency distribution peak between 0.6 and 0.7 per cent. Final hydrogen ion concentration was high, pH 3.25 to 3.4 compared with 3.05 to 3.25 for cultures of homofermentative lactobacilli and 3.7 to 3.9 for high acid producing streptococci. The majority of the cultures fermented sucrose, lactose, raffinose, salicin, and amygdalin, the frequency distribution curve having a peak at 0.5 to 0.6 per cent. The majority of the cultures also fermented arabinose but the frequency peak was lower, 0.2 per cent, and the maximum acidity 0.6 per cent. Xylose in the same way supported a slight growth but only 20 per cent of the cultures produced more than 0.1 per cent acid, although 0.6 per cent was the maximum. Rhamnose was utilized to a slight degree by 35 per cent of the cultures, but the maximum acidity was 0.3 per cent. Nearly all cultures, 96 to 100 per cent failed to utilize mannitol,

alpha-methylglucoside, inulin, dextrin, or starch. One culture produced 0.54 per cent acid in inulin. In all other cases, 0.2 per cent acid was the maximum produced.

All cultures selected for the study produced inactive lactic acid with a trace of volatile acid and a small amount of carbon dioxide, approximately 2 per cent of the sugar utilized. All cultures produced their maximum acidity between 22 and 32 C, but they also produced acid at 7 C and at 45 C. Some growth occurred in broth containing 10 per cent salt. However, the amount of acid produced increased as the salt concentration was decreased. In other words, salt does have some inhibitory action upon acid production at even 2 per cent concentration. The cultures varied in regard to growth in litmus milk. None grew rapidly; some curdled milk after a week at 32 C, but others failed to produce more than a slight acidity in two weeks.

On the basis of these results it would seem that there may be some differences between the various cultures which may be used in differentiating species. However, although one could select many strains which showed differences in regard to carbon compounds fermented, in no case could any significant trend be demonstrated. Therefore, one must conclude that all strains should be considered as belonging to one species.

In the isolation and study of these organisms from fermenting vegetables, 1438 strains of *Lactobacillus plantarum*, 282 strains of *Lactobacillus brevis*, 602 strains of *Leuconostoc mesenteroides*, and 783 strains of *Streptococcus faecalis* and closely related streptococci were also isolated and identified. Even though the pediococci are coccus forms, they were not confused morphologically or physiologically with the streptococci or leuconostocs at any time. The fact that the pediococci produced approximately twice as much or more lactic acid than either of the other coccus types was sufficient in itself to distinguish between them. But, in addition, morphologically they are more rounded like the micrococci, and tetrad grouping was very common. With respect to the acidity produced in sugar media, they were more often confused with the gas forming lactobacilli and with the low acid producing strains of the non-gas forming lactobacilli. They could, however, be readily distinguished by microscopic examination. Thus one might say that in respect to acid production, they are intermediate between the homofermentative lactobacilli and the streptococci. Little difference was found between the many isolates from widely differing sources such as fermenting beans which are fairly high in protein, fermenting sauerkraut, and cucumber pickles in $2\frac{1}{2}$ to 5 per cent salt. It therefore seems very doubtful that the different strains from beer vary sufficiently to warrant the recognition of a large number of species.

Concluding comments. Lindner, on the basis of a study of von Huth's culture from horse urine assumed that the organism studied by Kurth and named by Balcke was a low acid producing strain. There is no evidence that Lindner had Balcke's culture or one similar to it. The fact that it was assumed at that time that the pediococci may have been introduced from the stable is not sufficient reason to consider that the organism isolated from horse urine was the same as

that in the spoiled beer. Until such time that a sufficient difference can be noted between the various strains showing an optimum temperature range for growth between 20 and 30 C, and which include the strains described by Lindner, Claussen, Mees, and others, it seems most logical to consider that they are all similar to the original strain of *Pediococcus cerevisiae* Balcke or the slimy variety, *Pediococcus viscosus* Lindner.

Lindner's *Pediococcus acidi-lactici* and Sollied's *Pediococcus hennebergi* which apparently have an optimum temperature growth range at about 40 C and the two strains of tetracocci of Orla-Jensen may differ sufficiently to be considered as other species. Henneberg mentions pediococci of sauerkraut which he considered different from the other species.

The lactic acid producing cocci responsible for the socalled "sarcina sickness of beer" were given the name *Pediococcus cerevisiae* by Balcke (1884*b*). They are present in other fermenting materials in some of which they may have an important part in the fermentation. They are readily distinguished from the species of the genus *Streptococcus* by their tetrad grouping and their comparatively high acid production. These morphological and physiological characters and the fact that they produce inactive lactic acid seem sufficient to exclude them from the genera *Streptococcus*, *Micrococcus*, or *Sarcina*. The group should be considered as a separate genus, *Pediococcus* Balcke with the species *Pediococcus cerevisiae* Balcke as the type. There is no justification for the selection by Mees (1934) of *Pediococcus damnosus* Claussen, or by De Toni and Trevisan (1889) of *Pediococcus acidi lactici* Lindner as the type. Although it is granted that Balcke did not give a complete description of either his genus or species, they were well enough described so that other workers have recognized the organisms. The studies of Lindner (1887, 1888, 1889) further identified the organisms, and they were well recognized long before the work of Claussen (1903). The genus should be included in the tribe *Streptococceae* of the family *Lactobacteriaceae* with the genera *Diplococcus*, *Streptococcus*, and *Leuconostoc*, rather than in the family *Micrococcaceae* as in Bergey's Manual (1948).

The genus should include those gram-positive nonmotile, nonsporeforming cocci that occur in tetrads and sometimes singly or in pairs, show poor surface growth because they are microaerophilic, are high acid homofermentative lactic acid producers and do not reduce nitrates, liquefy gelatin or produce catalase.

The type species is *Pediococcus cerevisiae* Balcke. The species produces inactive lactic acid from sugars, always fermenting glucose, fructose, mannose, galactose, maltose and usually arabinose, sucrose, lactose, raffinose, salicin and amygdalin and, sometimes to a lesser degree, xylose and rhamnose. Nearly all strains fail to ferment mannitol, *alpha*-methylglucoside, inulin, dextrin, and starch. Milk may be fermented slowly by some strains. The optimum temperature range for fermentations is between 25 and 32 C. The organism was first observed in "sarcina-sick" beer but may occur in the fermenting materials such as sauerkraut, or pickles.

If a slimy variety is definitely shown in the future to be distinct enough to be given species or varietal recognition, the name proposed by Lindner, *Pediococcus*

viscosus has priority over other names. Similarly if a type is shown in future studies to have the higher optimum temperature for growth and warrants species or varietal recognition *Pediococcus acidi-lactici* Lindner or *Pediococcus hennebergi* Sollied may be recognized.

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