COMPARATIVE STUDY OF *GAFFKYA HOMARI*, *AEROCOCCUS VIRIDANS*, TETRAD-FORMING COCCI FROM MEAT CURING BRINES, AND THE GENUS *PEDIOCOCCUS*¹

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Received for publication May 20, 1959

Snieszko and Taylor (1947), and Hitchner and Snieszko (1947) described gram-positive, tetrad-forming micrococci associated with a septicemic disease of lobsters. These organisms grew poorly on laboratory media unless a fermentable sugar was added. They were further characterized by their ability to ferment mannitol and their inability to reduce nitrate or to hydrolyze gelatin. Because of their morphology and pathogenicity for the lobster, the authors suggested a new species, *Gaffkya homari*.

In 1951, Dr. W. S. Sturges, Jr., of The Cudahy Packing Company Laboratory, Omaha, Nebraska, submitted to this laboratory two strains of cocci which had been isolated from cured meat products. These microorganisms were characterized by small colony formation on ordinary laboratory agar media, tendency to occur as tetrads, and ability to produce a greenish discoloration of cured meat products. Aaronson (1956) also obtained two cultures of these tetrad-forming cocci from Dr. Sturges and noted that they appeared to be identical to *G. homari* with respect to morphology, minimal nutritional requirements, and biochemical characteristics.

Williams *et al.* (1953) attempted to enumerate the oral streptococci from the air of occupied rooms by the use of a selective culture medium containing potassium tellurite and crystal violet. They noted the frequent occurrence of a unique coccus that had biochemical characteristics similar to the enterococci. But, no tendency toward chain formation could be demonstrated. These microorganisms were catalase-negative, grew in the presence of 40 per cent bile, and produced strong greening on blood agar. In view of these unique features, the authors proposed a new genus and species, *Aerococcus viridans*.

The present report describes a group of tetrad-

forming, gram-positive cocci that have been found to comprise a minor proportion of the normal flora of meat curing brines. These microorganisms appear to be identical, or very similar, to *Aerococcus viridans*, *Gaffkya homari*, and the cultures of Dr. Sturges. Since all of these microorganisms have several characteristics common to the genus *Pediococcus*, it is proposed that they be included in this genus.

MATERIALS AND METHODS

Used ham curing pickle samples were obtained from a number of meat packing companies and plated quantitatively on APT agar (Evans and Niven, 1951). After incubation for 3 days at 30 C, individual colonies were picked, streaked on APT agar, and reisolated. One tetrad-forming coccus from each packing plant was saved for further study. Although these organisms did not represent the predominant morphological type in these brines, some were found in most of the brine samples examined. Similar organisms also were isolated from the skin of pig carcasses that had been chilled overnight after slaughter, and from outbreaks of bacterial greening of bacon. Six strains from curing brines, 1 from a hog carcass, and the 2 from bacon were subjected to a detailed taxonomic study.

For comparative purposes, 6 cultures of *A. viridans* were obtained from Dr. R. E. O. Williams. The 2 strains from Dr. Sturges and 2 *G. homari* strains from Dr. Aaronson also were included in the study. The *G. homari* strains (ATCC 10400 and RT-4) were the same as those employed in the comparative study by Aaronson (1956).

The laboratory methods employed were similar to those described in the previous publications (Deibel and Niven, 1958, 1959). Nitrate reduction was tested in the semi-solid indole-nitrite medium (BBL) supplemented with 0.5 per cent

¹ Journal paper no. 178, American Meat Institute Foundation.

TABLE 1

Physiological characteristics of tetrad-forming cocci from meat brines and cured meat products, Aerococcus viridans, and Gaffkya homari

	Tetracocci (Meat Brines, etc.) (9 strains)	Tetracocci (Sturges) (2 strains)	<i>Aerococcus viridans</i> (6 strains)	<i>Gaffkya homari</i> (2 strains)
Relation to oxygen.....	Facultative	Facultative	Facultative	Facultative
Catalase.....	—	+	4+; 2—	1+; 1—
Greening on blood agar.....	+	+	+	+
Nitrate reduced.....	—	—	—	—
Final pH, glucose broth.....	4.7–5.0	5.0	4.8–5.1	4.7–4.9
CO ₂ from glucose.....	—	—	—	—
Litmus milk.....	2 acid; 7 no change	1 acid; 1 no change	4 acid; 2 no change	1 acid; 1 no change
Polysaccharide from sucrose.....	—	—	—	—
Growth:				
10% NaCl broth.....	+	+	+	+
10 C.....	+	+	+	+
40 C.....	+	+	+	+
45 C.....	—	—	2+; 4—	—
40% bile agar.....	+	+	+	+
Hydrolysis:				
Sodium hippurate.....	7+; 2—	+	+	—
Esculin.....	6+; 3—	1+; 1—	4+; 2—	1+; 1—
Arginine.....	2+; 7—	—	1+; 5—	—
Starch.....	—	—	—	—
Gelatin.....	—	—	—	—
Fermentation:				
Xylose.....	—	—	4+; 2—	—
Arabinose.....	—	—	4+; 2—	—
Galactose.....	+	1+; 1—	+	1+; 1—
Lactose.....	4+; 5—	1+; 1—	+	1+; 1—
Trehalose.....	7+; 2—	—	5+; 1—	+
Cellobiose.....	+	1+; 1—	4+; 2—	1+; 1—
Melibiose.....	4+; 5—	—	2+; 4—	1+; 1—
Raffinose.....	6+; 3—	—	4+; 2—	1+; 1—
Inulin.....	2+; 7—	—	1+; 5—	1+; 1—
Glycerol.....	3+; 6—	+	5+; 1—	1+; 1—
Mannitol.....	+	—	5+; 1—	1+; 1—
Sorbitol.....	—	—	4+; 2—	—
Inositol.....	1+; 8—	+	—	—
Salicin.....	7+; 2—	1+; 1—	4+; 2—	1+; 1—

All cultures fermented glucose, mannose, fructose, maltose, and sucrose. None fermented rhamnose, melezitose, or dulcitol.

yeast extract. Nitrite was detected using the sulfanilic acid- α -naphthylamine reagent. Catalase production was determined in APT broth and on an agar medium containing only 0.05 per cent glucose (Felton *et al.*, 1953). Growth in relation to oxygen was determined in agar shake cultures using trypticase soy broth (BBL) plus 1.5 per cent agar.

RESULTS

Table 1 summarizes the physiological characteristics of the 9 tetracocci from meat curing pickles, bacon, and hog carcass; the 2 tetracocci from Dr. Sturges; 6 strains of *A. viridans*; and 2 strains of *G. homari*. Although some variations were noted in the characteristics among the individual strains, no single test served to differ-

Figure 1. A comparison of the morphology and cell size of *Pediococcus cerevisiae* (left) and *Pediococcus homari* (right). Stained with crystal violet and magnified 730 $\times$.

entiate one group from another. The characteristics of the 2 *G. homari* strains as reported by Aaronson were largely confirmed, although some deviations were noted. In contrast to Aaronson's results, strain RT-4 failed to ferment lactose or mannitol.

Morphology. Regardless of source, stained preparations of all cultures grown in APT broth showed numerous tetrad formations, some pairs, and many larger clusters. All strains were gram-positive and were similar in appearance. The tendency to form tetrads was diminished when these organisms were grown in or on other media which yielded reduced growth. Therefore, smears were routinely prepared from APT broth cultures after 20 to 24 hr at 30 C. As demonstrated in figure 1, *Pediococcus cerevisiae* produces similar groupings of cells, but the individual cells generally are smaller when cultured in APT broth or similar media.

Catalase production. Regardless of source, all cultures either failed to produce detectable catalase or showed only weak catalase activity. Indeed, all the tetrads isolated in the present study failed to produce catalase even when cultured on the low-glucose agar of Felton *et al.* (1953). However, of the 6 strains of *A. viridans*, 4 cultures (strains 7764, 7765, 7766, and 7794)

showed weak catalase activity on this agar medium. Both strains from Dr. Sturges were weakly catalase-positive, even in broth culture. One of the *G. homari* strains (ATCC 10400) was weakly positive on the low-glucose agar. The inability to demonstrate catalase or the finding of weak catalase activity parallels closely the distribution and activity of this enzyme in the genus *Pediococcus*.

Cultural characteristics. When cultured in a broth medium, most of the strains produced uniform turbidity, the cells tended to settle rather quickly to form a packed sediment. The sediment can be resuspended uniformly with ease. Some of the cultures of *A. viridans* produced a more granular type of growth that was difficult to resuspend uniformly. None of the cultures produced any evidence of pellicle formation. In general, broth cultures appeared very similar to lactic acid bacteria.

After 48 hr at 30 C, surface colonies on agar media were 2 mm or less in diameter. The colonies were greyish white and compact, and had characteristics similar to *P. cerevisiae* or other lactic acid bacteria.

Relation to oxygen. In suitable agar media such as the trypticase soy medium, all cultures were facultative with respect to oxygen. In other agar media most cultures exhibited superior

growth just beneath the surface of the agar. However, in no instance did any of the cultures grow extensively on the surface of the agar shake culture as would be expected for a micrococcus or a staphylococcus.

Glucose fermentation. All strains from all sources proved to be homofermentative and achieved a final pH value of 4.7 to 5.1. The lactic acid produced by 5 strains isolated in this study, and the type culture *A. viridans* (NCTC 8251), was the L(+)-isomer. In contrast to these results, *P. cerevisiae* cultures tested under the same conditions produced a final pH value between 3.7 and 4.0 and accumulated inactive lactic acid as the predominant fermentation product.

Miscellaneous tests. All cultures were relatively tolerant to salt and had a rather low temperature range for growth. The temperature limits of growth of some of the tetracocci isolated in this study have been shown to depend on the amount of salt in the medium (Deibel, Goldman and Niven, unpublished data).

In contrast with *P. cerevisiae* (Felton and Niven, 1953), none of the tetracocci required the citrovorum factor (Leucovorin) when tested in a folic acid-free basal medium. The growth of each of the tetracoccus strains was inhibited in an agar medium containing 0.02 per cent sodium azide, except in those areas having a heavy inoculum. *P. cerevisiae* did not evidence a similar inhibition of growth in this medium.

Characteristically, all strains produced a strong greening reaction when individual colonies were examined in sheep blood agar pour plates. In contrast, the strains of *P. cerevisiae* tested produced either a very weak greening reaction or no evident change on blood agar. As opposed to *P. cerevisiae*, all of the tetracocci, regardless of source, were capable of producing a green discoloration when streaked upon the cut surface of cured meats and incubated for 24 hr at 30 C.

Other minor differences were noted between the tetracoccus collection and *P. cerevisiae*. For example, the majority of the tetracocci hydrolyzed sodium hippurate, whereas *P. cerevisiae* cultures tested in this laboratory, as well as those reported by Jensen and Seeley (1954), failed to attack this substrate. Also, the majority of the tetracocci fermented mannitol whereas *P. cerevisiae* does not (Pederson, 1949; Williams *et al.*, 1953).

DISCUSSION

From the results, it seems reasonable to group the tetracocci from meat sources, *Aerococcus viridans* and *Gaffkya homari*, into one species. Although variations existed among strains, no single characteristic served to separate one group of microorganisms from another. Furthermore, these microorganisms have characteristics very similar to the genus *Pediococcus*. This genus, of which only two species are established, is characterized as being catalase-negative or weakly catalase-positive, facultative or microaerophilic, homofermentative, nonproteolytic, incapable of reducing nitrate, producing poor growth on the surface of agar media, and having a tendency to form tetrads. All the cultures here studied have these characters. Therefore, it is logical to conclude that they belong to the genus *Pediococcus*.

Williams *et al.* (1953) recognized the similarity of *A. viridans* and the genus *Pediococcus* but differentiated the two groups of microorganisms on the basis of morphology, relation to oxygen, final pH value, mannitol fermentation, and tolerance to crystal violet and potassium tellurite. Our study has demonstrated that the morphology, and relation to oxygen of these groups, are not markedly different, and the other characteristics would not appear to justify differentiation on a generic basis.

In selecting a species name for the tetracocci here studied, it would be desirable to keep in mind the apparent ubiquity of these organisms and to recognize the inherent weakness of employing parasitism as a criterion of species delineation. Nevertheless, on the basis of priority as prescribed by the *International Code of Nomenclature of Bacteria and Viruses* (1958), it becomes necessary to classify the tetracocci as *Pediococcus homari* nov. comb. if it is accepted that all of them belong to one species within the genus *Pediococcus*. Rule 23 of the Bacteriological Code states that "a legitimate name or epithet must not be rejected merely because it is inappropriate, or disagreeable, or because another is preferable or better known, or because it has lost its original meaning." Indeed, it would be interesting to test the pathogenicity of representative cultures of *A. viridans* and the meat tetracocci for the lobster. The proposed classification places these microorganisms appropriately among the lactic acid bacteria (family *Lactobacillaceae*, tribe *Streptococceae*).

There are other tetrad-forming cocci that have been described and which may be related to *P. homari*. Hornsey and Malloes (1955) observed the occurrence of a catalase-negative *Micrococcus* species in beef curing brines. Although the published description does not permit detailed comparison, there is suggestive evidence that these organisms are similar or identical to *P. homari*.

Orla-Jensen (1919) described a catalase-negative tetracoccus from anchovy pickle that possessed a weak fermentative capacity and produced *dextro*-rotatory lactic acid from glucose fermentation. Mees (1934) confirmed these findings and proposed the name *Tetracoccus halophilus*, since the growth of this species was stimulated by sodium chloride.

Since completion of this study, attention has been called to the description of a coccus from soy sauce mash and soy-koji (Sakaguchi, 1958) which appears to be identical or very similar to the microorganisms described here. They existed as tetrads, were microaerophilic, salt tolerant, catalase-negative cultures which had a rather low optimal temperature for growth. They were weakly fermenting, homofermentative microorganisms which produced either L(+) or inactive lactic acid from glucose. They failed to reduce nitrate, and the majority of the cultures fermented mannitol. The author proposed the name *Pediococcus soyae* for this group of microorganisms. It would be interesting to determine whether they are identical to the microorganisms included in the present study.

Although *P. homari* has characteristics much in common with the other *Pediococcus* species, it may be differentiated in that *P. homari* is relatively tolerant to salt, produces *dextro*-rotatory lactic acid from glucose, ferments sugars with a higher limiting pH value, produces strong greening on blood agar, generally ferments mannitol, and evidences a marked inhibition when grown on an agar medium containing 0.02 per cent sodium azide. In contrast with *P. cerevisiae*, *P. homari* does not require the citrovorum factor for growth, and the individual cells of *P. homari* tend to be larger than those of *P. cerevisiae*.

Aaronson (1956) reviewed the genus *Gaffkya*, and suggested that it may have to be dismantled. In view of the fact that the two species of this genus could well be distributed into other genera, it would be desirable to give serious consideration to abandoning the genus *Gaffkya*.

The partial overlapping of some physiological characteristics of *P. homari* with the enterococci merits comment. When grown under somewhat unfavorable conditions the cellular morphology of *P. homari* may be confused with that of the enterococci. Furthermore, catalase may be absent or undetected in *P. homari*, the degree of salt tolerance is similar, and the strong greening reaction in blood agar is very similar to that produced by *Streptococcus faecium*. Consequently, these tests fail to differentiate these two groups of organisms.

ACKNOWLEDGMENTS

The authors wish to express their gratitude to Dr. W. S. Sturges, Jr., Dr. R. E. O. Williams, and Dr. S. Aaronson for kindly providing the cultures included in this study.

SUMMARY

A comparative study was made on a collection of gram-positive, tetrad-forming cocci from cured meat and meat curing brines, *Gaffkya homari*, and *Aerococcus viridans*. These cultures were sufficiently similar in their morphological and physiological characteristics to justify placing them into a single species. Because of their close relationship to the genus *Pediococcus*, it is proposed that they be placed into the species *Pediococcus homari* nov. comb.

In addition to characteristic morphological features, *P. homari* is a facultative, salt tolerant, homofermentative bacterium that produces little or no catalase, forms *dextro*-rotatory lactic acid from sugar fermentation, and fails to reduce nitrate, or liquefy gelatin. It differs from *Pediococcus cerevisiae* in that it has a rather high limiting pH, produces strong greening on blood agar, generally ferments mannitol, is less tolerant to sodium azide, and does not require the citrovorum factor for growth.

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