A Biochemical-taxonomic Study of a Marine Micrococcus, *Gaffkya homari*, and a Terrestrial Counterpart

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SUMMARY: The morphology, minimal nutritional requirements, and biochemical reactions of the lobster pathogen, *Gaffkya homari*, and of a tetracoccus found contaminating meat products were compared. The marine and terrestrial cocci were identical by the *in vitro* methods used. The validity of the genus *Gaffkya* is discussed. The 4 strains of micrococci herein studied had an unusual nitrogen requirement: all required glutamic acid as nitrogen source; substrates were not utilized in the absence of glutamate.

Snieszko & Taylor (1947) described a Gram-positive, tetrad-forming micrococcus from septicæmic lobsters and called it *Gaffkya homari*. W. S. Sturges (1950, personal communication) isolated Gram-positive, tetrad-forming micrococci as occasional contaminants of meat products in a mid-western United States meat-packing plant. In the present study two strains each of the marine tetracoccus and of the terrestrial tetracoccus, were examined biochemically and morphologically. No clear differences were detected between them. This uniformity made it necessary to examine the criteria used to define the genus *Gaffkya*.

METHODS

Organisms. *Gaffkya homari* (ATCC 10400) was obtained from the American Type Culture Collection; *G. homari* RT-4 was obtained from Dr J. S. Getchell, Agricultural Experiment Station, Orono, Maine. The terrestrial tetrad-forming micrococci strain S-1 was isolated from the exposed cut surface of bologna sausage and strain S-5 was isolated from a discoloured area of meat, and were obtained through the courtesy of Dr W. S. Sturges, Cudahy Packing Co., Omaha, Nebraska. *G. tetragena* (ATCC 159) was obtained from Mr S. A. Rosenthal, Department of Bacteriology, University of Maine, Orono, Maine. *Micrococcus sodonensis* was obtained from Dr A. Bicknell, Michigan State College, East Lansing, Michigan; *Sarcina lutea* from Dr J. Webb, Biology Department, City College, New York, N.Y.; *Staphylococcus flavocyaneus* from the National Collection of Type Cultures (NCTC 7011).

The culture methods and the measurement of growth were essentially those used by Baker, Sobotka & Hutner (1953). Cultures were usually incubated at 35-37° for 5-7 days. Stock cultures were maintained on a medium described by Aaronson (1955). All chemicals were obtained from commercial sources.

Diagnostic tests for the identification of micrococci were those recommended in the Manual of Methods published by the Society of American Bacteriologists.

RESULTS

Morphology. These marine and terrestrial micrococci are Gram-positive, non-motile, *c.* 0.8–1.0 μ . diam., and form tetrads; their form is unchanged after several years on artificial media. On agar slopes and plates they grow as minute greyish white raised colonies (*c.* 1.0 mm. diam.) with rough surfaces and entire edges. They usually appear as beads rather than streaks or sheets on slopes; the rapid formation of acid may prevent confluent growth. In a chemically defined medium buffered with 0.5 % glutamic acid + 0.5 % *Tris*-(hydroxymethyl)-aminomethane and initially at pH 8.7, the pH value will be lowered below 5.0 by 5 days of growth.

Diagnostic biochemical reactions. All 4 strains gave the same diagnostic reactions by the tests used (Table 1). For comparison the reactions of other cocci are included (see also Table 2).

Response to antibiotics. As noted in Table 2, the differences between *Gaffkya homari* ATCC 10400 and S-1 were minor as compared with the difference between these organisms and the other cocci.

Minimal nutritional requirements. All 4 strains grew well in a chemically defined medium (Table 3). Final growth, however, was stimulated as much as 20 % after the addition of a mixture of amino acids (Table 4).

Sucrose, glycerol, glucose or gluconate served as both carbon and energy source, provided that a small amount of glutamic acid (*c.* 0.1 %, w/v) was present. Fumarate, succinate, lactate, citrate, acetate, glycine, L-glutamate, DL-aspartate, DL-asparagine, and DL-alanine did not support growth even with added glutamic acid.

Sulphate satisfied the sulphur requirement. In a defined medium the optimal pH was 8.7 (the highest pH value tested). The optimum temperature for growth was *c.* 35°.

Nitrogen requirement. All 4 strains had an absolute requirement for glutamic acid; it was not adequate as the sole source of energy, carbon and nitrogen but supported growth on the addition of suitable carbon and energy sources. Attempts to replace the glutamate with NH_4Cl , KNO_2 , KNO_3 , glycine, DL-alanine, DL-aspartate, DL-asparagine, and L-glutamine (added aseptically), each at several concentrations within the range 0.05–1.0 % (w/v) failed.

Osmotic and salt tolerances. All 4 strains were similar in their responses to increasing osmotic pressure and increasing salt concentration (Table 5). Pentaerythritol did not inhibit any of the 4 strains in the concentration range used (1.0–10.0 %).

DISCUSSION

Comparison of marine and terrigenous strains. The consistent similarities noted here argue that the strains should all be denoted as *Gaffkya homari* despite their diverse origins. The occurrence of related or identical species of bacteria in the sea and on the land has previously been noted (Wood, 1952; ZoBell, 1946). The marine and terrestrial environments of the cocci herein studied may be much alike in respect to osmotic pressure and high

Table 1. *Diagnostic biochemical reactions of Gaffkya homari strains and other cocci*

Strains	...	...	<i>G. homari</i>		Terrestrial strains	
			ATCC 10400	RT-4	S-1	S-5
Arabinose			Acid	Acid	Acid	Acid
Dulcitol			n.c.	n.c.	n.c.	n.c.
Glucose			Acid	Acid	Acid	Acid
Inositol			n.c.	n.c.	n.c.	n.c.
Lactose			Acid	Acid	Acid	Acid
Maltose			Acid	Acid	Acid	Acid
Mannitol			Acid	Acid	Acid	Acid
Sucrose			Acid	Acid	Acid	Acid
Xylose			n.c.	n.c.	n.c.	n.c.
Tryptone broth (indole)			n.c.	n.c.	n.c.	n.c.
Starch hydrolysis			n.c.	n.c.	n.c.	n.c.
Gelatin hydrolysis			n.c.	n.c.	n.c.	n.c.
Litmus milk			n.c.	n.c.	n.c.	n.c.
NO ₃ reduced to NO ₂			n.c.	n.c.	n.c.	n.c.
Urea broth			n.c.	n.c.	n.c.	n.c.
Citrate broth			n.c.	n.c.	n.c.	n.c.

Strains	...	...	<i>G. tetragena</i> ATCC 159	<i>Micrococcus</i> <i>sodonensis</i>	<i>Sarcina</i> <i>lutea</i>	<i>Staphylococcus</i> <i>flavocyaneus</i>
Arabinose			n.t.	n.t.	n.t.	n.t.
Dulcitol			n.t.	n.t.	n.t.	n.t.
Glucose			n.c.	n.c.	n.c.	n.c.
Inositol			n.t.	n.t.	n.t.	n.t.
Lactose			n.c.	n.c.	n.c.	n.c.
Maltose			n.c.	n.c.	n.c.	n.c.
Mannitol			n.c.	n.c.	n.c.	n.c.
Sucrose			n.c.	n.c.	n.c.	n.c.
Xylose			n.t.	n.t.	n.t.	n.t.
Indole			n.c.	n.c.	n.c.	n.c.
Starch			n.c.	n.c.	n.c.	n.t.
Gelatin			+	n.c.	+	+
Litmus milk			Basic	n.c.	Basic	n.t.
NO ₃ →NO ₂			+	n.c.	n.c.	n.c.
Urea broth			n.t.	n.t.	n.t.	n.t.
Citrate broth			+	+	n.c.	n.c.

n.c. = no change; n.t. = not tested; + = growth or change in medium.

Table 2. *Sensitivity of cocci to several antibiotics*

The organisms were grown on nutrient agar.

	<i>G. homari</i> ATCC 10400	<i>G. homari</i> S-1	<i>G. tetragena</i> ATCC 159	<i>Micrococcus</i> <i>sodonensis</i>	<i>Staphylococcus</i> <i>flavocyaneus</i>	<i>Sarcina</i> <i>lutea</i>
	Inhibition zone diameter (cm.) average of two experiments					
Streptomycin, <i>c.</i> 500 units/disk	2.9	2.7	4.1	3.3	4.3	4.5
Penicillin G, <i>c.</i> 500 units/disk	4.3	3.7	5.1	5.0	6.2	6.0
Aureomycin, <i>c.</i> 250 μ g./disk	3.5	3.6	3.2	2.2	4.4	4.1
Polymyxin, <i>c.</i> 500 units/disk	1.0	0.9	1.0	1.1	1.1	1.3

Table 3. *Defined medium for strains of Gaffkya homari*

Na ₂ glycerophosphate. 5H ₂ O	0.1 g.	Ca pantothenate*	0.2 mg.
MgSO ₄ . 7H ₂ O	0.05 g.	Thiamine HCl*	0.2 mg.
NH ₄ Cl	0.05 g.	Nicotinic acid*	0.2 mg.
L-Glutamic acid*	0.5 g.	Biotin*	1.0 µg.
Sucrose	1.0 g.	Mo (as Na ₂ Mo ₂ O ₄ . 2H ₂ O)	0.8 mg.
Glycerol	0.5 g.	Ca (as chloride)	1.0 mg.
Tris(hydroxymethyl)aminomethane	0.5 g.	Trace metals solution§	1.0 ml.
DL-Leucine†	5.0 mg.	Distilled water to 100 ml.;	
Hypoxanthine*‡	2.0 mg.	final pH value 8.5-8.8	

* Required.

† Stimulatory but not required.

‡ Purines may be replaced by *p*-aminobenzoic acid, folic acid or folinic acid

§ Formula in Aaronson (1955).

Table 4. *Amino acid supplement which stimulated growth when added to the defined medium of Table 3*

	g.		g.
DL-Alanine	0.04	DL-Methionine	0.006
L-Arginine (free base)	0.03	DL-Phenylalanine	0.004
DL-Aspartic acid	0.05	L-Proline	0.004
L-Glutamic acid	0.1	DL-Serine	0.01
Glycine	0.05	DL-Threonine	0.01
L-Histidine (free base)	0.02	DL-Tryptophan	0.005
DL-Isoleucine	0.005	L-Tyrosine	0.004
DL-Leucine	0.005	DL-Valine	0.005
DL-Lysine HCl	0.045	Distilled water to 100 ml.;	
		final pH value 6.5.	

Table 5. *Effect of osmotic pressure and salt concentration on strains of Gaffkya homari grown on a chemically defined medium. See Table 3*

		Strain			
		ATCC 10400	RT-4	S-1	S-5
Salt conc.		Growth expressed as optical density.			
% (w/v)					
0		1.08	1.04	0.83	0.75
NaCl	1.0	0.46	0.70	0.78	0.98
	3.0	0.72	0.77	0.67	0.86
	5.0	0.82	0.68	0.66	0.72
	7.0	0.64	0.73	0.74	0.67
	10.0	0.02	0.06	0	0.03
NaNO ₂	0.1	0.52	0.71	0.52	0.44
	0.3	0.32	0.64	0.40	0.38
	1.0	0.12	0.30	0.28	0.21
	3.0	0.02	0.18	0.12	0.02
	5.0	0	0	0	0
	7.0	0	0	0	0
NaNO ₃	1.0	1.30	0.94	1.00	0.90
	3.0	1.08	0.98	1.00	0.97
	5.0	0.83	0.84	0.66	0.89
	7.0	0.98	0.80	0.77	0.90
	10.0	0.76	0.61	0.64	1.00

concentrations of electrolytes and of organic compounds. These environmental similarities may account for the success in isolating similar strains. It would be of interest to know whether the strains isolated from meat products can cause septicaemia in lobsters.

Glutamic acid as an obligate nitrogen source. The strains studied have a requirement for glutamic acid as a nitrogen source which is neither spared or replaced by any of the nitrogen-containing compounds tested. Gilvarg & Davis (1954) reported an obligate glutamate requirement for an *Escherichia coli* mutant. Wiame & Storck (1953) reported that glutamate would serve as the sole nitrogen source for certain *Bacillus subtilis* mutants; several amino acids replaced the glutamate. As experiments with α -ketoglutarate were not tried with *Gaffkya homari* strains, it is not clear whether or not the specificity of this requirement resides in the carbon skeleton.

Critique of the genus Gaffkya. As defined by *Bergey's Manual* (1948) *Gaffkya* strains '...occur in animal body and in special media as tetrads, while in ordinary culture media they occur in pairs and irregular masses. Aerobic to anaerobic. Gram positive. Parasitic organisms.' The lobster pathogen fits this genus since it is a tetrad-forming Gram-positive coccus and a parasite. Parasitism seems to be an important—even essential—criterion for assigning a coccus to *Gaffkya*. If one followed *Bergey's Manual*, the saprophytic strains S-1 and S-5 would be excluded from *Gaffkya*. The strains S-1 and S-5 are nutritionally, biochemically and morphologically identical with the strains of *Gaffkya homari* by all the *in vitro* methods here used; these strains must be assigned to *G. homari* if the criterion of parasitism be abandoned.

The nomenclatural vicissitudes of the genus *Gaffkya* attest to the inadequacy of its definition. It was created by Trevisan (1885) for the tetrad coccus isolated by Gaffky (1888) and called by him *Micrococcus tetragenus*. The Winslows (1908) placed this species in the genus *Albococcus* with the white staphylococci. Buchanan (1917) placed it in *Staphylococcus*, Hucker (1924*a*, 1928) and Fleming (1929) in *Micrococcus*; and *Bergey's Manual* (1930) returned it to *Gaffkya*. Lehmann & Neumann (1931) placed it in *Sarcina*, but succeeding editions of *Bergey's Manual* retained *Gaffkya* as a genus. Wood (1950), Shaw, Stitt & Cowan (1951) and Bisset (1952) advocated the elimination of *Gaffkya* because of the close similarity of these bacteria to other micrococci with which they intergrade. It would appear from the present study that the criteria used to distinguish the genus in *Bergey's Manual*, especially parasitism, are dubious; the genus may have to be dismantled and its component species distributed elsewhere, e.g. *G. tetragera* may have to be placed in *Staphylococcus* (*Micrococcus*) occupying a position alongside *S. albus*, with which it shares colonial morphology, habitat, and antigens (Hucker, 1924*b*, Hucker & Robertson, 1926). *G. tetragera* and *G. homari* share tetrad-formation and parasitism, but they may not otherwise be closely related. They differ morphologically (tetragera cells are larger and colonial and slope growth is more abundant) and biochemically (tetragera does not ferment carbohydrates or milk and does reduce nitrate to ammonia; *Bergey's Manual*; Snieszko & Taylor, 1947) and they have no common agglutinogens (Rosenthal, 1950). Shaw *et al.* (1951)

described cocci ('group iii') which they placed among the staphylococci to which *G. homari* shows some resemblance; unfortunately their description does not permit detailed comparison.

The good agreement between the biochemical reactions and the morphological criteria currently used in bacterial taxonomy and the additional information on growth factors, carbon, nitrogen, and sulphur sources, and effects of chemical agents, including antibiotics, seen in these studies suggest that biochemical criteria may well complement the older taxonomic criteria.

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