A PRELIMINARY REPORT UPON SOME HALOPHILIC BACTERIA

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While studying the microörganisms concerned in the fermentation of cucumber pickles a group of bacteria were isolated and studied which appear sufficiently interesting to deserve special comment. These organisms were isolated from the scum which grows on the surface of the brine after active fermentation (respiration) has ceased. The interesting fact about these organisms is that certain members of the group demand an appreciable concentration of sodium chloride for development.

HISTORICAL

Many investigators, among others Forster (1889), de Freytag (1890), Petri (1890) and Peuch (1887) have studied the salt tolerance of some of the well-known species of pathogenic bacteria such as *B. tuberculosis*, *B. typhosus*, *B. diphtheriae*, *Vibrio cholerae*, *B. anthracis*, and the bacillus of swine plague (Schweinrotlauf). Many of these types were found surprisingly resistant, being still living and virulent after months of exposure to saturated salt solutions. Sperlich (1912) studied the reaction of bacteria found in air, water and soil to varying percentages of salt. He found that many organisms grew better in the presence of a certain percentage of salt, the optimum varying from 0.5 per cent to 5 to 6 per cent of salt in the case of *Bact. constrictium* (Zimmermann).

Farlow (1867, 1886), Poulsen (1879–1880), Edington (1887), Leyet (1887), Ewart (1887), Le Dantec (1891), Hoye (1901, 1904, 1906, 1908), Beckwith (1911), Kellermann (1914–1915)

and Bitting (1911), have studied the bacteria concerned in the reddening of salted fish. The bacteriology of this phenomenon seems to have been fairly well worked out. Kellermann speaks of other non-pigment-forming bacteria associated with the causative agent but none of these investigators have called attention to a group of bacteria that demand for growth a certain percentage of sodium chloride. In this respect we believe the cucumber brine organisms to be unique.

Rapin and Grosseron (1914) have studied the possibilities of bacterial contamination of salt during the process of refining and Wolff (1914) made a study of highly refined salt used for butter making and of a less highly refined grade used for cheese making. Both investigators found that the more highly refined the salt the smaller the number of microörganisms present. Wolff showed that many of the bacteria found had a very marked effect upon butter when applied in pure culture to sterile material.

EXPERIMENTAL

From the pickle scum mentioned above 21 bacilli were isolated. The brine from which the scum was taken contained approximately 10 per cent of salt. The medium used for isolation was cucumber juice agar to which 10 per cent of sodium chloride had been added. On this medium at room temperature there appeared first a large number of rugose, cretaceous, raised colonies of mycoderma, in appearance quite characteristic of the scum as it appears on the surface of the pickle vats. After a few days smaller, creamy, glistening, colonies appeared, 1 to 2 mm. in diameter. These colonies were raised, opaque, with entire edge and of a butyrous consistency. Many of the colonies when viewed from the bottom of the plate had a very bright red center with a dark green periphery. When transferred to cucumber agar slants with ten per cent salt no further chromogenic action was noted except, in some cases, a browning of the medium underneath the stroke. It was found that these organisms would grow apparently just as well on meat extract medium with 5 per cent salt as on the cucumber medium and so for

further transfer of stock cultures and for differential purposes meat extract medium with 5 and 10 per cent salt was used. A study of the cultural characteristics of the organisms divided them into five groups as follows:

Group 1. Non-motile, non-spore-bearing bacilli, average size 0.5 by 2μ. On salt agar smooth, opaque, white, glistening colonies after forty-eight hours at 30°C.

Group 2. Non-motile, non-spore-bearing, Gram-negative bacilli 0.5 by 4 to 6μ. Abundant opaque, glistening colonies on salt agar. Motility doubtful.

Group 3. Gram-positive bacilli 0.5 by 4μ showing elliptical terminal spores. On salt agar plates there were formed at first large myceloid colonies which did not reappear in subcultures.

Group 4. Short, non-spore-forming, Gram-positive, average size 0.4 by 3μ with a tumbling form of motility. Colonies on salt agar convex, round, glistening, butyrous. No spores.

Group 5. Small, motile, non-spore-forming, Gram-positive bacilli 0.3 by 2μ. Colony formation not uniform.

Other cultural characteristics are shown in the following table.

The most conspicuous feature about these organisms is their ability to grow in highly concentrated salt solutions, the majority growing readily in a 25 per cent solution of sodium chloride.[1] In only one of the groups (no. 1), could growths be obtained without the aid of added salt. The optimum temperature was 30°C. But under the most favorable conditions growth was slow. The organisms were very sensitive to acids. Inoculated into pickle juice with varying degrees of acidity, all failed to grow above + 1. All except 3 cultures grew in salt broth with a P_H value of 6. In broth with a P_H value of 5, growth occurred in but one culture.[2]

All the cultures were facultative, although growth was more abundant in the presence of air.

No indol was formed and nitrates were reduced by but one of the groups (group 5).

[1] Medium containing 25 grams of sodium chloride in 100 cc. This method was followed in making all the media containing salt.

[2] The Colorimetric Determinations of Hydrogen Ion Concentration and Its Application in Bacteriology by Clark and Lubs, Journ. Bact., **2**, nos. 1, 2 and 3.

Table showing cultural characteristics of five groups of Halophilic organisms

GROUP NUMBER	GRAM STAIN	LIQUEFACTION OF GELATIN	ACTION IN MILK	GROWTH ON POTATO	INDOL PRODUCTION	NITRATE PRODUCTION	ACID PRODUCTION* IN			SPECIAL CHARACTERISTICS	RELATION TO SALT	
							Glucose	Lactose	Sucrose.		Minimum requirement	Maximum requirement
											per cent	*per cent*
1	0	0	No change in plain milk after 20 days but milk with salt added coagulated in 18 days	Moderate raised, glistening	0	0	0	0	0	Decided ropiness in sugar broths	0.0†	15
2	0	0	Coagulated in 5 to 10 days	Raised, spreading, glistening	0	0	0	0	0	Odor of butyric acid in sugar media	5	25
3	+	+	Coagulated in 5 days	Moderate, raised, spreading	0	0	+	0	+	Spore formation	5	25
4	+	0	Not coagulated	Membranous, flat, glistening	0	0	+	0	0	Characteristic growth on potato resembling a coating of shellac	5	25
5	0	0	Not coagulated	Moderate, smooth and glistening	0	+	+	0	0	Cultural characteristics not uniform as in other groups	5	25

* If medium titrated less than 1 per cent normal acid to phenolphthalein, it was recorded as a negative fermentation.

† Ordinary nutrient broth containing 0.5 per cent sodium chloride.

These organisms show great resistance to drying. Thoroughly dried out cultures kept in the laboratory for one year were easily revived by adding salt broth to the tubes.

The form of growth shown in salt broth by most of these cultures was quite characteristic. In this medium there was slowly formed a moderate, uniform cloudiness with a light flaky pellicle, which later became thick and creamy. After several days' incubation, the pellicle broke up, forming viscid, stringy material suspended horizontally in the medium. This settled later and formed a viscid mass at the bottom. In group 1 marked ropiness of the liquid media occurred.

While the tolerance of certain organisms for high concentrations of sodium chloride and the favorable influence of certain concentrations upon growth has been determined for many organisms, no one, so far as the writers are aware, has called attention to a group that absolutely demand sodium chloride for their development.

Investigations have indicated that the chlorides are not essential to the growth and development of bacteria and experience has shown that the presence of salt in more than small percentages is inhibitory to the growth of many types (Sperlich, 1912). All these organisms showed a marked preference for salt and all except group 1 apparently demanded a certain concentration, 2 per cent, for their growth.

No explanation of this phenomenon is offered but the inquiry suggests itself as to whether they are essentially salt organisms which by long environmental influences have come to require salt in high concentration as essential to their vital processes, such as we might expect to find in sea water, or whether they are only common saprophytic bacteria which by growth for only a short time in salt solution have developed mutants which demand salt for their growth.

The question of the influence of osmotic pressure upon these organisms has not been studied. There is the possibility that the essential factor is not sodium chloride but a certain osmotic pressure which is necessary to create a suitable environment for growth. If it is only an increase in osmotic pressure that is

demanded, other salts in proper concentration would act in the same manner as sodium chloride. Further work on this point is contemplated.

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