

INTERCHANGE OF BACTERIA BETWEEN THE FRESH WATER AND THE SEA

VICTOR BURKE

State College of Washington

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Burke and Baird (1931) have presented experimental evidence that many fresh water and land bacteria can exist and multiply in an environment having the salt concentration of the sea. If fresh water bacteria survive in the sea, it follows that at least some of the bacteria in the sea should survive in fresh water, and that there should occur, therefore, an interchange of bacteria between the sea and the fresh water and land environment. The experiments described in this paper were designed to produce further evidence as to the possible exchange of bacteria between the land and the sea.

This work was done in the summer of 1931, at the Hopkins Marine Station, Pacific Grove, California. At this time of the year the streams entering the Monterey Bay and nearby ocean have ceased flowing or are greatly reduced, so that the shore waters cannot be considered brackish.

EXPERIMENTAL

The medium used, adjusted to pH 8.0, consisted of 5 grams peptone, 3 grams beef extract, 30 grams gelatin, 100 cc. potato extract, 15 grams agar, and 900 cc. sea water or tap water. Material from various fresh water, soil, and marine sources was plated out on the two media and counts made on successive days.

The marine material consisted of sea water free of plankton, taken near shore, sea water taken a mile or more from shore rich in plankton, the same water incubated for two weeks, stomach contents of free-swimming and bottom fishes, sea water near the mouth of a small stream, decayed fish, and water from about a

decayed fish. In comparing growth on the two kinds of media, it was found that numbers varied from one to a thousand times as many and that colonies appeared sooner and were usually larger on the homologous medium. With the exception of the intestinal contents of fishes, the marine material never gave counts on the homologous medium of more than 15 times the count on the heterologous medium.

The fresh water and soil material consisted of water from two streams above high tide, decayed marine fish placed in streams for two to six weeks, the same fish left in the laboratory two weeks, water surrounding the same fish, and soil in front of laboratory. With some material the plate counts were the same on the two kinds of media, with others up to 40 times as many colonies appeared on the homologous medium. The colonies appeared earlier on the homologous medium and there was noted a difference in size, pigment production, and type of colony formation.

Anaerobic shake-agar cultures made from material heated to destroy vegetative cells gave comparable results. In general more colonies grew on the homologous medium and the anaerobic line was nearer the surface.

In all the sea and fresh-water material examined there were bacteria that grew in the heterologous medium. Whether the counts on the two kinds of media were identical or varied greatly depended on the source and nature of the material. In some specimens, the predominating organism grew equally well on the two kinds of media, and the counts were practically identical.

The experiments so far reported demonstrate that both fresh water and the sea contain many bacteria that will grow in the heterologous environment. They do not indicate whether certain species fail to grow. Cells of all the species present might grow and the counts differ. Also the counts might be identical and different species be present, as pointed out by Lipman (1926).

A side-by-side comparison of plates of fresh water and sea water on the two kinds of media suggested that different organisms were appearing on the heterologous agar as well as that some were not growing. If this were true, then the effect of the

changed environment would be greater than indicated by the comparative counts. We therefore decided to determine the effect of salt on pigment production and the effect of the heterologous environment on the viability of the cells of pure cultures. Burke and Baird showed that cells of a species vary in their tolerance for salt. It occurred to us that the reduced counts on the heterologous agar might be caused by the more sensitive cells of each species not growing, rather than by certain species failing to grow.

Staph. aureus, *Sarcina lutea*, *Pseudomonas pyocyanea*, *Pseudomonas fluorescens* and *Serratia marcescens* were grown in 1 to 6 per cent salt. Salt had no effect on the pigment production of *Staph. aureus* and *Sarcina lutea*. Pigment production by *Serratia marcescens* was retarded by 2 per cent salt and never reached its maximum in 3 per cent salt. Pigment production by both species of *Pseudomonas* was slight in 1 and 2 per cent salt and inhibited in 3 per cent salt. The results were the same on extract agar and potato extract agar. Smith (1933) has shown that salt affects the pigment production, morphology, and colony formation of *Bacillus megatherium*. In plating out soil on sea-water agar we noted that salt checked the "spreader" type of colony.

It is evident from these results that it is not possible by a comparison of colonies on the two kinds of media to determine whether some species have failed to grow on the heterologous agar.

To determine whether the reduced counts on the heterologous agar could be due in part at least to the failure of the most sensitive cells of each species to grow, twenty-four-hour cultures of seventeen species of bacteria isolated from sea water were plated out on the two kinds of agar. All the species grew on the salt-free agar but all the cells did not. With some species the counts on the heterologous medium were reduced slightly, with others more than 50 per cent. We found no species, either fresh water or marine, that failed to grow on the heterologous medium. The reduced counts on the heterologous medium of the material we examined were due, in part at least, to the failure of the more sensitive cells to grow. What percentage of bacteria will fail to grow on the heterologous agar remains to be determined.

Since salt affects spore formation, it occurred to us that the fate of spore-bearing organisms in a changed environment such as occurs between land and sea might depend upon the effect of salt on the formation and germination of the spores (Curran, 1931; Smith, 1933).

A series of experiments with *B. subtilis* demonstrated that a salt concentration equal to that of the sea causes an increase in the ratio of spores to vegetative cells. This occurred in agar, broth and water. Further experiments on spores freed of vegetative cells by heat demonstrated that spores of this organism will develop in a salt concentration greater than that of the sea. The fate of some spore-bearing organisms in the sea apparently does not depend upon the effect of the salt on the formation and germination of the spores.

The experiments described suggest that fresh water bacteria may be able to maintain themselves in the sea. There is considerable evidence that they can maintain themselves in brackish water. To determine whether fresh water forms could be isolated from the sea, bacteria were isolated from decayed fish and water in a small stream flowing into the ocean and from decayed fish and sea water 50 feet to one side of the mouth of the stream. One hundred and five cultures were isolated over a period of several weeks and studied as regards staining, morphology and growth characteristics. After duplicates were eliminated there were left fourteen species from the sea and eight from the stream. When these were compared, five species appeared to be common to the stream and to the ocean near the mouth of the stream. The dilution of the sea water by the stream was negligible.

DISCUSSION

Since many fresh-water bacteria can survive and carry on their activities in salt concentrations equivalent to or greater than that occurring in the sea, we should expect to find that the bacterial flora along the sea shore is enriched by additions from the land and fresh water entering the ocean. If such forms survive in the ocean, they will undoubtedly survive when carried back to the fresh water environment. Our experiments favor

the view that such an interchange of bacterial species between the sea and fresh water occurs. How extensive this interchange is, and whether it is greater than with higher forms of life, remains to be determined. Workers in marine bacteriology can, by keeping in mind the possible interchange of bacterial forms between land and sea, avoid some of the mistakes made by workers in other groups.

CONCLUSIONS

Bacterial counts of fresh water and sea water are usually but not always reduced when the material is plated out on a heterologous medium as regards salt. The reduction in counts is due in a large part at least to the fact that the more sensitive cells, of many if not all species, fail to grow. The more resistant cells of many species grow in the heterologous environment.

A salt concentration equal to that of the sea causes an increase in the rate of spore formation of *B. subtilis*. These spores will develop in the same salt concentration.

Changes in salt concentration affect the characteristics of some species. Suspected new species of fresh water and marine bacteria should be studied in both a fresh-water and marine environment. To compare the characteristics of marine bacteria on seawater agar with the characteristics of fresh water bacteria on fresh water agar is inadequate for the description of new species.

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