

# Simple Method for Killing Halophilic Bacteria in Contaminated Solar Salt<sup>1</sup>

D. J. KUSHNER, G. MASSON, AND N. E. GIBBONS

*Division of Biosciences, National Research Council, Ottawa, Ontario, Canada*

Received for publication 23 October 1964

Adding acid to brines or mixing acid phosphate with the salt used in preserving fish was recommended by Gibbons (Progr. Rep. Atlantic Fish. Exp. Sta., no. 14, p. 13, 1935), because it was observed that under acid conditions the halophilic bacteria that spoil salted fish cannot grow. The red halophile, *Halobacterium cutirubrum*, was found to be extremely sensitive to pH changes (Kushner and Bayley, Can. J. Microbiol. 9: 53, 1963). At pH 4, for example, the normally rod-shaped bacteria form spheres which are no longer viable. This suggested that exposure to moderately low pH values might serve to prevent halophilic growth and also to sterilize solar salt.

Two samples of solar salt, contaminated with red and white halophiles, were tested. One of these had been used for preserving fish; after drying, it still contained a large number of halophiles. The other sample contained a small number of halophiles, but counting was difficult. Saturated solutions were prepared by adding 60 ml of sterile distilled water, a few milliliters at a time, to 20 g of salt; 1-ml samples of these saturated solutions were placed in sterile petri dishes and treated with hydrochloric or acetic acid. The solutions were then dried overnight at 37 C, and the salt was taken up in 5.0 ml of sterile 20% NaCl. The number of viable cells in this solution was counted by the drop-plate method on 2% agar in a growth medium prepared as previously described (Kushner and Bayley), except that the NaCl concentration was reduced to 20%. Reducing the pH of the saturated "used" salt solutions to 5 greatly lowered the number of living organisms; at pH 2.7 or below, no viable halophilic bacteria remained (Table 1). The unused salt was also sterilized by lowering the pH, but it had a much lower buffering capacity than the used salt, presumably because of the presence in the latter of material extracted from fish. To reduce the pH of a saturated solution to 3.0 required 0.005 M HCl for the used salt, but only 0.001 M HCl for the unused salt.

<sup>1</sup> Issued as N.R.C. No. 8353.

These results suggest that acidifying saturated salt solutions with HCl or acetic acid before the final crystallization begins might be a convenient and economical method of freeing solar salt from halophilic bacteria. The amount of technical concentrated HCl required to sterilize 3,025 liters of saturated brine containing 895 kg (1 ton) of unused salt is 378 ml, which at the present time costs roughly 9 cents. When brine treated in this way was dried in air and redissolved, the pH of the resulting solution was 6.2, as opposed to 6.6 in an unacidified control; titration showed that about 90% of the acid had escaped during drying. Thus, products salted with treated salt would not be exposed to strong acid. This is an advantage, since Gibbons showed that salted acid products are susceptible to "dunn" (*Sporendonema sebi*).

It is obvious that, in contaminated premises, the introduction of a halophile-free salt will not remedy the problem unless the premises are also freed of halophilic bacteria. For this purpose the use of large quantities of clean water is effective.

TABLE 1. Effect of acid on the number of viable halophilic bacteria in "used" solar salt

Expt	Final acid concn	pH	Colonies per g of salt*
1	0	6.5	75,000
	0.001 M HCl	5.4	27,500
	0.01 M HCl	1.5	0
	1% Acetic acid	2.7	0
2†	0	7.8	600,000
		6.0	210,000
		5.0	3,000
		2.4	0

\* All samples contained approximately equal numbers of red and white halophiles.

† HCl was added to the brine to produce the pH values shown, and samples were then dried. Note the differences in initial pH as well as in numbers of organisms between the two lots of "used" salt.