BASE EXCHANGE AND HEAT RESISTANCE IN BACTERIAL SPORES

Gordon Alderton and Neva Snell

Western Regional Research Laboratory*, Albany, California

Received December 3, 1962

Dormant bacterial spores can be added to the many naturally occurring structures, including vegetative cells, that show base exchange behavior. This function is pronounced, and compositional manipulation by this route can be used to reduce, restore, and enhance heat resistance of fully formed spores. Some properties of spores may be correlated with the behavior of ion exchange gels.

METHODS AND MATERIALS

B. megaterium Northern Regional Research Laboratory B-938 was grown from spore inoculum in JL medium (Sacks and Alderton, 1961) overnight at 29-31° in shaken Fernbach flasks. The culture was allowed to autolyze overnight at 5°, and then the spores were cleaned by a polymer two-phase system (Sacks and Alderton, 1961) and dried in vacuum.

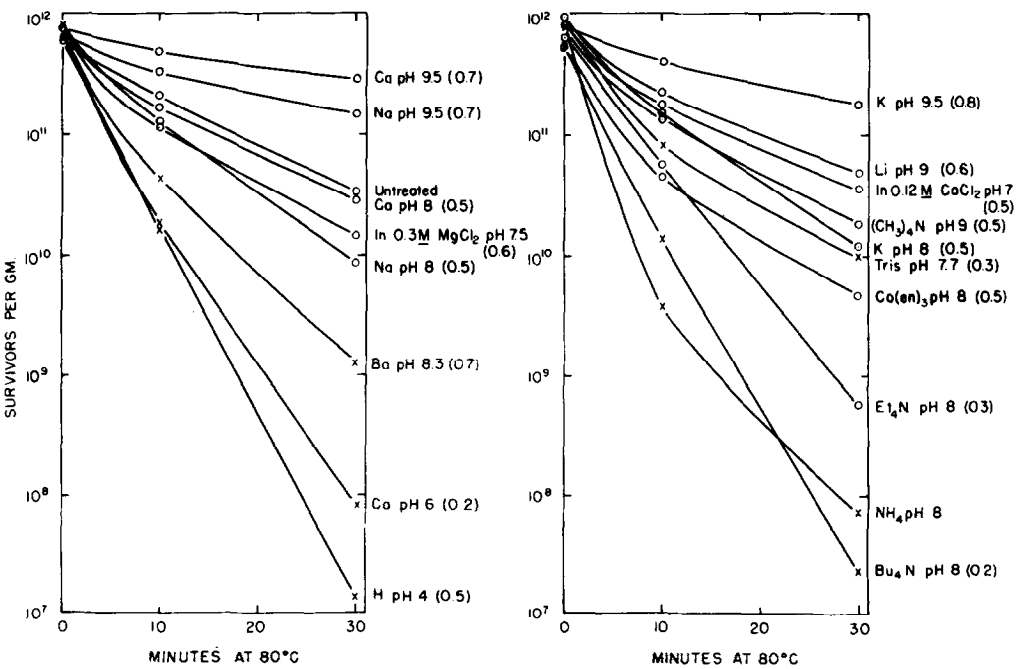
Cation exchange was measured and followed kinetically by pH methods in an International Instruments Co. pH stat-titrator** at 25° under flowing nitrogen in 4 ml. volumes containing 40 mg. of spores. When untreated spores were immersed in salt solutions, sharp pH drops occurred. Except in the two cases in which external salt solutions were involved, cation loads were applied by titrating lyophilized pH 4 stripped spores with the appropriate hydroxide. A pH of 4 was chosen arbitrarily for approaching the stripped

---

* A laboratory of the Western Utilization and Research Division, Agricultural Research Service, U. S. Department of Agriculture.

** Reference to a company or product name does not imply approval or recommendation of the product by the U. S. Department of Agriculture to the exclusion of others that may be suitable.

(hydrogen) form of the spore exchanger system, because no appreciable dipicolinic acid leakage was noted at this pH. At pH 3, a very slow release of dipicolinic acid was noted with an aged B. megaterium preparation. The later stages of approach to equilibrium with spores resemble those of the methacrylic acid polymers in that they are slow. For the preparations in the figure, equilibration times were about 2-3 hours, at which times neither hydrogen ion stripping nor cation loading at the noted pH values was complete. For the heat resistance tests shown in the figure the titrated suspensions were readjusted to compensate any pH drop during refrigerated storage and then heated at a spore concentration of about 10 mg./ml. Survivors were measured by colony outgrowth at 35° overnight on beet molasses agar (Lewis and Ijichi, 1957). The approximate capacity values in milliequivalents per gram for titrating the pH 4 stripped spores to the indicated slurry pH under these conditions are given in parentheses in the figure. The hydroxides $(CH_3)_4NOH$, $(C_2H_5)_4NOH$, $(Butyl)_4NOH$, and $Co(en)_3(OH)_3$ were prepared by Dowex 1 column treatment of the halide salts.



Heat Resistance of Cation Loaded B. megaterium Spores.

RESULTS AND DISCUSSION

The relatively large cation exchange capacity of B. megaterium spores is a chemical function which allows compositional manipulation of fully formed spores without killing them. Large changes in cation composition are possible by this 'in vitro' method without the necessity of manipulating conditions in the parent bacterial culture. The binding capacity vs. pH pattern for the spore exchanger system resembles more that of the resinous weak cation exchangers (Howe and Kitchener, 1955; Kunin, 1958), such as methacrylic acid polymers, rather than the strong sulfonic acid type. In the former type of exchanger, hydrogen ion has by far the highest exchange potential.

Major changes in cation composition might be expected to influence various properties of the spore. The data in the figure indicate large effects of cation load on the property of heat resistance. The presence in spores of a base exchange function with attendant influence on heat sensitivity suggests a new route for a systematic attack on the practical food and industrial problem of killing spores. Several well known spore phenomena are consistent with the assumption that heat resistance is promoted by particular types of cation load. To mention a few: I. Heat resistance magnitudes are notoriously intractable, apparently varying with, among other things, heating medium, pH, culture history and composition, storage medium, isolation procedure, and osmotic concentration. One would expect the cation load on the spore exchanger system to vary to a greater or less extent with all of these variables, in fact, to vary with almost any treatment that involved exposure to an aqueous or other ionizing environment. II. Spores are more easily heat killed at low pH. For the weak cation exchangers, hydrogen ion with the highest exchange potential would displace all other cations. III. Phosphate buffer concentration and additions of inorganic salts (Curran, 1952) have been reported to affect heat resistance. Such treatments would alter the load on a base exchanger. IV. It has been reported (Levinson et al., 1961; Lechowich and Ordal, 1962) that heat resistance increases with increasing ratio of calcium to dipicolinic acid, the calcium being in excess of the

dipicolinic acid chelational equivalent. The base exchange mechanism described above furnishes a site for this excess calcium.

The titrimetric base binding beyond only pH 4 indicates that the phenomenon is not confined to the visible surface, since an exchange site for about every square Å would be required. Even an exchange system of high specific capacity would occupy an appreciable fraction of the spore mass and, thus, require that the spore be porous. Gerhardt and Black (Gerhardt and Black, 1961) have reported that the spore has considerable free space accessible to a wide variety of solutes, with some charged solutes giving anomalous space results consistent with cation exchanger behavior. The nature of the functional groups for base binding remains to be determined. Cation-exchanging carboxyl, phosphorous-containing, and chelating groups would be pH controlled and, thus, are among the possibilities.

The Lewis, Snell, and Burr mechano-chemical theory (Lewis et al., 1960) of spore dehydration and dormancy requires an elastic organ whose dimensional changes would exert pressure on the vital core. Non-mineral ion exchangers are elastic gels. When the degree of crosslinking is low, many-fold volume changes can occur during exchange, especially hydrogen exchange in the case of the weak cation exchangers. Typical swelling pressures (Kitchener, 1957) are well above the requirements of the Lewis, Snell, and Burr model. The usual natural environment of the spore and the pH conditions of heat stability indicate that the spore's cation exchange system would normally be at least partially loaded with cations other than hydrogen. This should correspond to a more or less swollen condition, and thus the Lewis, Snell, and Burr model would need a geometric arrangement in which a swollen elastic organ would exert pressure on the core. One of the simplest arrangements would consist of an outer, relatively inextensible shell against which an inner shell of elastic ion exchanging gel would press and thus exert more or less pressure on the core depending on the degree of swelling brought about by the cation load, temperature, external osmotic concentration and other factors influencing exchanger gel swelling. More complex but more likely structures

might involve construction from anistropically swelling members similar to Kuhn's (Kuhn et al., 1960) cross striated 'muscle' system (suitably oriented) or to cotton and wool fibers which also exhibit lateral swelling.

It might be expected from such models that any conditions of cation load and environment capable of swelling the exchanger gel to a sufficient extent should confer heat resistance. Conversely, the mechano-chemical model would require that the dehydrating pressure be released at germination. On the basis of such a model the very recent reports of Rode and Foster (Rode and Foster, 1962a,b) on "ionic germination" might be interpreted as involving a volume change of the elastic gel, through changed cation load, to a point of core hydration at which metabolic activity could begin.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the excellent assistance of Mrs. P. A. Thompson, and thank Dr. J. C. Lewis for helpful discussion.

REFERENCES

Curran, H. R., Bact. Rev. 16, 111 (1952).
Gerhardt, Phillip and Black, S. H., J. Bact. 82, 750 (1961).
Howe, P. G. and Kitchener, J. A., J. Chem. Soc. (London), 2143 (1955).
Kitchener, J. A. (1957). In: Ion Exchangers in Organic and Biochemistry (edited by Calvin Calmon and T. R. E. Kressman), Interscience Publishers, Inc., New York.
Kuhn, W., Ramel, A., and Walters, D. H. (1960). In: Size and Shape Changes of Contractile Polymers (edited by A. Wasserman), Pergamon Press, New York.
Kunin, Robert (1958). Ion Exchange Resins, 2nd ed. John Wiley & Sons, New York, 466 p.
Lechowich, R. V. and Ordal, Z. John, Can. J. Microbiol. 8, 287 (1962).
Levinson, Hillel S., Hyatt, Mildred T., and Moore, Florence E., Biochem. Biophys. Res. Comm. 5, 417 (1961).
Lewis, J. C., Snell, N. S., and Burr, H. K., Science 132, 544 (1960).
Lewis, J. C. and Ijichi, K., Appl. Microbiol. 5, 124 (1957).
Rode, L. J. and Foster, J. W., Arch. Mikrobiol. 43, 183, 201 (1962).
Rode, L. J. and Foster, J. W., Nature 194, 1300 (1962).
Sacks, L. E. and Alderton, Gordon, J. Bact. 82, 331 (1961).