INFLUENCE OF OSMOTIC PRESSURE ON SPORULATION BY
BACILLUS SUBTILIS

JAMES L. ROBERTS, WELDON C. WHITE AND
ELIZABETH OJERHOLM

(WITH TWO FIGURES)

Studies on the physiology of bacterial endospore formation have in many cases involved the addition of one or more compounds to a basal medium, and a determination of the effect of these upon the percentage of cells appearing as spores. The consequences, so far as the spore crop is concerned, of the addition of electrolytes (FITZ-GERALD, 5; FABIAN and BRYAN, 3), fermentable carbohydrates (ESTY, 2; DE SMIDT, 7), vapors of fat solvents (MICHAILOWSKY, 6), antiseptics, and many other compounds have been studied by this method.

Among the bacteria, forms can be found which are more resistant to great changes in osmotic pressure than are any other forms of life (FALK, 4). Many species of bacteria, however, in common with other plants and animals, are readily affected by changes in the osmotic pressure of their menstruum (CURRAN, 1). It is well known that certain physical properties of a medium may influence the degree of endospore formation occurring within that medium. According to our best knowledge, little or no work has been done to determine the influence of osmotic pressure on sporulation. Studies involving the addition of materials of small molecular size could be more easily interpreted if there were available more definite knowledge of the effect of osmotic pressure on spore formation. The present work was undertaken to determine whether osmotic pressure is a factor sufficiently important to warrant consideration in future studies of bacterial sporulation.

Methods

A 0.5 per cent. Bacto-peptone water was used as a basal medium to which the various materials used in raising the osmotic pressure were added. The basal solution was prepared in one large vessel and distributed in 25-cc. volumes into 6-oz. prescription bottles with screw caps. The following materials were then weighed or measured into the bottles of basal solution:

1. Ten concentrations of KCl (0.05 M to 0.5 M)
2. Ten concentrations of NaCl (0.05 M to 0.5 M)
3. Ten concentrations of MgSO₄
4. Ten concentrations of MgCl₂ (0.033 M to 0.333 M)
5. Ten concentrations of CaCl₂ (0.033 M to 0.333 M)
6. Ten concentrations of sodium acetate (0.05 M to 0.5 M)
7. Ten concentrations of glycerol (0.1 M to 1 M)
8. Six concentrations of lactose (0.1 M to 0.6 M)
9. Ten concentrations of agar (0.01 to 0.1 per cent.)
10. Ten concentrations or NaCl (0.05 M to 0.5 M)

in basal medium with 0.1 per cent. agar

The media were sterilized in 15 pounds of steam pressure for 30 minutes and the hydrogen-ion concentrations of the various solutions were then ascertained to be between pH 6.4 and 6.6. Inoculation was accomplished by two drops of a 24-hour peptone water culture of the "K strain" of Bacillus subtilis obtained from the culture collection of the University of Texas. The cultures were incubated in a horizontal position with the bottle caps loosened. The incubation temperature used was 37° C. Under these conditions there was little or no pellicle formation.

Smears were prepared from each of the cultures after two and four days of incubation. It was intended that the final pH and osmotic pressure values should be determined at the completion of four days of incubation, but time did not permit a determination of these until the eighth day.

The smears were steamed in 5 per cent. aqueous malachite green, destained with distilled water, and counterstained with 5 per cent. mercurochrome. Approximately 500 cells were counted on each smear, and from these the percentage of spores was calculated.

In general, growth was not visibly inhibited by the osmotic pressures of the solutions we have used in these studies. Osmotic pressures greater than 23 atm. have not been investigated since only rarely do media contain sufficient osmotically active materials to produce greater pressures.

Results

As would be expected, the pH of most of the media became increasingly alkaline to 7.5 to 8.0 during eight days of growth. Those media containing glycerol and lactose were exceptions in that they became slightly more acid than the uninoculated control media. Because the results of spore counts in media containing glycerol and lactose may not be comparable with the results obtained in the remainder of the test solutions, they are omitted in future considerations. The percentage of sporulation in the presence of each of these two materials was lower than with the other chemicals tested.

Growth of B. subtilis seemed to cause a slight increase in the osmotic pressure of the menstruum after eight days of incubation. Since this change was always less than one atmosphere, it is considered negligible.

The curves representing sporulation in any series of media after two and four days of incubation (fig. 1) are sufficiently similar to indicate no excessively great experimental error in the mechanics of the determinations. The gravest danger is in drawing conclusions when so few compounds have been studied, and when it has been impossible to completely isolate the effects
of the osmotic pressure from the specific effects of the added chemicals. Specific influences of the chemicals are evident in the results presented in figure 1. It will be noted, however, that the various curves have some characteristics in common. In view of this, it seemed both fair and helpful to compound the results from all the media plotted in figure 1. The resulting histogram is shown in figure 2.

The percentages of sporulating cells in media containing agar are much higher than in other media. Therefore, the results with agar are of little value for comparison and are omitted from figures 1 and 2. The osmotic pressure of media containing agar has little or no effect upon sporulation.

The influence of osmotic pressure, at least in the case of B. subtilis, seems not to be sufficiently great to warrant serious attention in future spore studies. As shown in figure 2, the percentages of sporulation in media with osmotic pressures ranging from 2 to 18 atm. are not significantly different.
There does seem to be a slight inhibition of sporulation by pressures above 18 atmospheres and below 2 atmospheres.
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