MINERAL DEFICIENCIES IN COMPLEX ORGANIC MEDIA AS LIMITING FACTORS IN THE SPORULATION OF AEROBIC BACILLI[1]

JACKSON W. FOSTER AND FRED HEILIGMAN

Department of Bacteriology, University of Texas, Austin, Texas

Received for publication March 7, 1949

When aerobic sporeforming bacteria are cultivated in a variety of the usual bacteriological organic media, good vegetative development is observed in all the cultures, but striking differences in degrees of sporulation may be observed. Table 1, dealing with *Bacillus cereus*, is representative of the behavior of six different species of *Bacillus*. Spore counts were made by the malachite green, mercurochrome method as described by Wynne (1948). A point of special interest is that in each medium the vegetative growth was good, differences in cell yields between the various media being due to a difference of not more than a few generations, and often less than that.

A detailed study of the negligible sporulation in "casamino acids" and "N-Z-case" media (asporogenic media) revealed the following points: (1) small proportions of yeast extract or Wilson liver "B" added to the asporogenic media induced good sporulation; (2) the effect was obtained by the ash of yeast extract or liver; and (3) the potassium ion was identified as the main factor critical for sporulation in the asporogenic media (table 2). Potassium, along with several other cations, has been shown by several authors to influence sporulation (Fabian and Bryant, 1933; Perdue, 1933), and spectrochemical analysis reveals its presence in aerobic spores (Curran *et al.*, 1943).

From table 2 one may compute that the addition of K^+ led to about a 30 per cent augmentation in total cell yield (Petroff-Hauser count), but to upwards of a 1,000 per cent increase in spore yield, suggesting a definite role for potassium in the sporulation process. Also it appears from these and numerous other experiments on this subject that the sporogenicity of a medium may be independent of the amount of vegetative growth it supports. In sporulation work it is likely that certain standard media may, as this study shows, be deficient in the minerals essential for maximum sporulation but adequate for abundant vegetative development. Some evidence was obtained that certain other minerals, such as magnesium and iron, may also be deficient. The exact status of the deficiency would depend on the particular lot of medium employed. One may conclude that a salts mixture should be used in organic media employed in sporulation studies.

[1] The research that this paper reports was undertaken in co-operation with the Committee on Food Research of the Quartermaster Food and Container Institute for the Armed Forces. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or having the endorsement of the War Department.

This finding appears to explain numerous reports of "stimulation" of sporulation by certain ions (Fabian and Bryant, 1933). It would appear that "stimulation" does not occur, but rather that the basal media were deficient in minerals for sporulation but sufficient for vegetative growth, and that presumably sporulation cannot be high without having the nutrient requirements for this process satisfied. Complex media favorable for sporulation without supplements contain

TABLE 1

Sporulation of Bacillus cereus in complex organic media that support good vegetative growth

INCUBATION	NUTRIENT BROTH*	YEAST EXTRACT*	WILSON LIVER B	CASAMINO ACIDS*	N-Z CASE†	PEPTONE*	BRAIN-HEART INFUSION*	VEAL INFUSION*	FRESH BEEF INFUSION
hours									
24	23	8	8	0	0	0	4	7	0
48	44	49	56	1	0	28	18	12	6
72	75	68	85	4	1	33	38	51	23
96	78	85	99	7	1	38	65	55	38

All media equivalent to standard nutrient broth, i.e., organic solids = 8 mg per ml in distilled water. Initial pH 7.2. Five ml medium per 50-ml Erlenmeyer flask. Incubation on a shaking machine at 30 C.

Figures represent percentage of total cells (vegetative plus spores) that are spores.

* Difco.

† Sheffield.

TABLE 2

Influence of the potassium ion on the sporulation of Bacillus cereus in asporogenic media (N-Z-case)

INCUBATION	BASAL MEDIUM		BASAL MEDIUM PLUS 0.36 mg K^+ AS KCl PER ml	
	Total population* $\times 10^{-9}$	% spores	Total population* $\times 10^{-9}$	% spores
hours				
48	6.2	1	8.0	34
72	7.4	4	9.6	43
96	Est.†	7†	Est.†	85†

Conditions similar to those in table 1. Medium: N-Z-case, 8 mg per ml in distilled water.

* Vegetative cells plus spores.

† Lysis difficulties made exact total counts impossible. The "% spores" are taken from stained slides.

adequate amounts of minerals; it seems that the well-known fact of differences in sporogenicity of complex media may be explained in part at least on their mineral content.

This applies, however, only to relatively dilute complex media. Attempts to obviate the potassium deficiency by using the media in higher concentration (2-, 3-, and 4-fold) were unsuccessful. In fact, in the case of the peptone in table 1, sporulation was even less in the more concentrated media, indicating

that other factors then come into play in the sporulation process, an observation similar to that of Roberts and Baldwin (1942), Williams (1931), and Tarr (1932). This is being studied further.

Attention is called to the value of the homogeneous conditions of shake cultures in sporulation studies: sporulation is more rapid and eventually higher than in stationary broth cultures. For example, stationary controls for the yeast extract medium in table 1 gave the following sporulation percentages on the four consecutive days: 1, 14, 33, and 45.

REFERENCES

CURRAN, H., BRUNSTETTER, B. C., AND MYERS, A. T. 1943 Spectrochemical analysis of vegetative cells and spores of bacteria. J. Bact., **45**, 485–494.

FABIAN, F. W., AND BRYANT, C. S. 1933 The influence of cations on aerobic sporogenesis in a liquid medium. J. Bact., **26**, 543–558.

PERDUE, L. 1933 The effects of inorganic salts in spore formation. Master's thesis, University of Texas.

ROBERTS, J. L., AND BALDWIN, I. L. 1942 Spore formation by *Bacillus subtilis* in peptone solutions altered by treatment with activated charcoal. J. Bact., **44**, 653–659.

TARR, H. C. A. 1932 The relation of the composition of the culture medium to the formation of endospores of aerobic bacilli. J. Hyg., **32**, 535–543.

WILLIAMS, O. B. 1931 Bacterial endospore formation in media of varying biologic value. Proc. Soc. Exptl. Biol. Med., **18**, 615–617.

WYNNE, E. S. 1948 Physiological studies on spore formation in *Clostridium botulinum*. J. Infectious Diseases, **83**, 243–249.