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Spore formation and spore germination of anaerobic food spoilage organisms, especially Clostridium botulinum.

SUMMARY

Data obtained in this study, together with information in the literature lead to the conclusion that the classical "dormancy" observed in anaerobic as well as aerobic spores is not a biological character of the spore itself, but is rather a function of the medium. "Dormancy" may be considered as a manifestation of the presence of anti-germination substances in normal media. "Dormancy" can be eliminated with varying degrees of success, depending on the medium and the nature of the inhibitive substances by the incorporation of various supplements, in particular soluble starch (0.1 percent), which absorbs some, but not all, the inhibitors.

The term "dormancy" would appear to be of dubious value in connection with bacterial spores.

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The Problem of "Dormancy" in Bacterial Spores[1]

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Our experience with germination of spores of various species of Clostridia, especially C. botulinum, has led to conclusions which we believe warrant the consideration of investigators of this group of organisms and of the aerobic spore formers as well. Pertinent observations have been made by previous authors, but it appears that all the recent available evidence and various aspects of the dormancy problem have not hitherto been crystallized into one comprehensive concept.

The essential points already established (Morrison and Rettger, 1930a and 1930b for review of previous literature; Curran and Evans, 1937; Olsen and Scott, 1946; Wynne and Foster, 1948a; Foster and Wynne, 1948) are these:

(1) Colony counts from a spore suspension may be submaximum in a few days incubation, and increase with time over prolonged periods. This indicates delayed germination and is a well-known phenomenon for both aerobic and anaerobic spores. This is the characteristic feature of the classical dormancy.

(2) This effect depends on the medium used, it being marked in some, and non-existent in others.

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(3) The final spore counts of a given suspension depend greatly upon the medium employed, though in each medium counts are at the maximum obtainable in it.

(4) Certain substances, notably soluble starch (0.1 per cent), eliminate the delayed germination in some media. In others the starch increases the final maximum count, whereas in still others it has no apparent effect. In general, all media containing complex nitrogenous materials preferred for counting spores, show the starch effect.

Data exemplifying all these points may be found in Table 1. From the information in the literature, as well as in Table 1, the following points may be made:

(a) The so-called "dormancy" in these organisms is not an inherent function of the spores, but rather of the medium in which the spores are placed to germinate.

(b) Even where "dormancy" occurred, or in media with low final counts, the large size of colonies speaks against a nutritional inadequacy.

(c) Accordingly, a plausible conclusion is that "dormancy" i.e., delayed germination, and also submaximal counts, are due to substances in normal media inhibitory to spore germination. "Dormancy" would then be only a manifestation of anti-germination substances in the medium and seemingly is, therefore, merely an artifact.

(d) In the case of the characteristic delayed rate of spore germination during "dormancy" one may consider this to be a function of sub-inhibitory concentrations of certain substances in the normal medium.

(e) In the case of submaximal counts, the inhibitory substances are of such nature and concentration that the germination rate of a portion of the spore population is zero, i.e., they never germinate, for all practical purposes.

(f) The inhibitors evidently are of different types, but one is the C_{18} unsaturated fatty acids (oleic, linoleic, lenolenic) which are present in almost

(g) The appreciable increase in germination rate, occasioned by the soluble starch in some media, is due to absorbtion of certain inhibitory substances, including the C_{18} fatty acids. In some media starch has little or no effect in enhancing counts known to be low.

(h) The different degrees of inactivation of germination inhibitors by starch indicate that different media contain varying amounts of different inhibitors. Solvent extraction of some media indicates some of these naturally occurring inhibitors are non-lipid.

(i) The significance of these substances inhibitory to germination is evidenced by the fact that the fresh infusions of beef, pork, and liver, ordinarily considered ideal nutrients, contain significant amounts of germination inhibitors, as demonstrated by the considerable enhancement of germination caused by addition of starch. Incidentally, fresh beef or liver infusion with starch is just as suitable as the widely used (for anaerobes) pork infusion medium of Vesair, with added starch.

(j) Effects similar to that of starch have been observed in the past when supplements containing native proteins and polysaccharides are added to the usual bacteriological media. Though this action has been arbitrarily ascribed to unspecified nutritional factors, an equally plausible idea is that they function physically in the manner of starch, through colloid absorbtion of inhibitory substances, and in some cases this has been proved definitely.

(k) Unless shown that no effect is obtained by starch, the above data and ideas make it imperative that this substance be an invariable component of all media in work on anaerobic as well as aerobic spores where maximum counts in the shortest time are desired. Even though starch apparently does not counteract all known cases of retarded germination, it is the simplest overall treatment available without altering the nutritional status of the basal medium.

(1) The term "dormancy" in the above connection no longer has the degree of validity it formerly enjoyed, and there now appears to be some question as to the justification of its continued use, unless in cases where the effect is shown to be independent of the germination medium.

Table 1

C. botulinum 62A Spore Recoveries in Various Agar Media[1]

Basal Medium	1 Per Cent Soluble Starch	Incubation, Days 3	6	17	33
Yesair's plus 0.1 percent starch	-	45	41	36[2]	-
Difco nutrient	-	28	30	22	-
Difco nutrient	+	35	42	35	-
Difco brain heart infusion	-	4	6	6	-
Difco brain heart infusion	+	12	23	30	30
BBL anaerobic	-	0	0	2	-
BBL anaerobic	+	0	1	6	8
Difco yeast extract, 1.0 percent	-	19	17	15	-
Difco yeast extract, 1.0 per cent	+	15	12	11	-
Corn steep liquor solids, 1.0 per cent	-	8	10	8	-
Corn steep liquor solids, 1.0 percent	+	7	9	11	14
Fresh beef infusion[3]	-	43	50	47	-
Fresh beef infusion	+	60	60	53	-
Fresh liver infusion[3]	-	24	24	23	-
Fresh liver infusion	+	46	52	49	-

[1] All media contained 0.2 per cent glucose and 0.2 per cent thioglycolate.

[2] The somewhat lower counts in this column as compared to the 6-day values is due to the obscuring of small colonies by development of gas bubbles and cloudiness in the media.

[3] Prepared exactly like Yesair's medium (cf. Wynne and Foster, 1948.)

 Continued

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