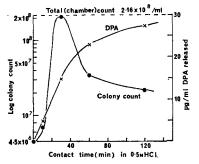
Activation of Bacillus stearothermophilus spores and release of dipicolinic acid after hydrochloric acid treatment

SIR,—Without prior heat activation less than 10% of spores of Bacillus stearothermophilus germinate and produce colonies on a nutrient agar medium (Cook & Brown, 1964, 1965). Optimum heat activation results in about 50% of the spores germinating and forming colonies.

We have found that in the presence of 0.5 N hydrochloric acid at 25° the colony count increases to the value of the total (chamber) count and that this increase is accompanied by progressive release of dipicolinic acid (DPA), a characteristic component of bacterial spores (Powell, 1953). Spore suspensions of B. stearothermophilus (NCIB 8919) were prepared according to Cook & Brown (1964). The DPA released was measured polarographically using method 2 of Porter, Brown & Brown (1967). Initially about 2% of the spores germinated and formed colonies after plating. After 30 min this rose to about 97%, by which time about half the DPA had been released (Fig. 1).



Effect of 0.5 N hydrochloric Fig. 1. acid on increase in colony count and release of DPA from B. stearothermophilus spores.

temperatures which are needed for heat activation and which may kill a proportion of heat-activated spores.

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References

Cook, A. M. & Brown, M. R. W. (1964). J. Pharm. Pharmac., 16, 725-732.
Cook, A. M. & Brown, M. R. W.(1965). J. appl. Bact., 28, 361-364.
Keynan, A., Issahary-Brand, G. & Evenchik, Z. (1965). Spores III, editors Campbell, L. L. & Halvorson, H. O., pp. 180-187. American Society for Microbiology, Ann Arbor, Michigan, U.S.A.

Lewis, J. C., Snell, N. S. & Alderton, G. (1965). *Ibid.*, pp. 47-54. Porter, G. S., Brown, M. W. & Brown, M. R. W. (1967). *Biochem. J.*, 102, 19c. Powell, J. F. (1953). Biochem. J., 54, 210-211.

Keynan, Issahary-Brand & Evenchik (1965) showed that lowering the pH imitated the effects of heat activation as measured by germination rates, but they did not correlate the pH effect with release of DPA. Lewis, Snell & Alderton (1965) reported that treatment of B. stearothermophilus spores at pH 1.5 for 80 min at 25° increased the colony count but did not remove DPA.

Although a causal relation between pH and DPA release has not been established by the present work, it might well be that low pH imitates the effect of heat activation by releasing DPA but without the noxious effects of the relatively high

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