asian region constitute an excellent series exemplifying this. *O. nigricans* appears to possess the strongest marine affinities, as it has a free-swimming veliger, while species such as *O. flavescens* and *O. campbelli* have taken one step towards the invasion of a terrestrial habitat, in that they possess direct development. *Onchidium damelli* forms the next link in the invasion of land, this species being restricted to estuarine waters and mangrove swamps. Finally, *Onchidina australis* can be found up to 30 ft. above high-tide mark.

B. L. STRINGER

Department of Zoology,
University of Canterbury,
Christchurch,
New Zealand.

[1] Joyeux-Laffuie, J., *Arch. Zool. exp. gen.*, **10**, 225 (1882).
[2] Fretter, V., *J. Mar. Biol. Assoc.*, **25**, 685 (1943).
[3] Needham, J., *Biol. Rev.*, **13**, 249 (1938).

## MICROBIOLOGY

### Sensitization of Bacterial Spores to Lysozyme and Hydrogen Peroxide with Reagents which Rupture Disuphide Bonds

VINTER[1-4] showed that the coat fractions of bacterial spores contain a high concentration of cystine disulphide bonds. Spore coats also contain a protein component[5,6] with which cystine could be associated. We have therefore subjected spores to reagents known to rupture protein disulphide bonds, and have investigated the results of these treatments.

Spores of *Bacillus cereus* NCTC 945 were incubated at 37° C with performic acid (2·5–25 per cent v/v); thioglycollic acid (1–25 per cent v/v) in the presence or absence of 8 M urea; 2-mercapto-ethanol (10 per cent v/v) at *p*H 3 in 8 M urea; sodium sulphite (12·5 per cent w/v) in the presence of 0·05 M cuprammonium ions and 8 M urea. Formic acid (2·5–25 per cent v/v) and hydrochloric acid (*p*H 3) were used as controls. None of these treatments altered the brightness of spores viewed by phase-contrast microscopy. Treated spores were washed with water and incubated with lysozyme at 37° C. Lysozyme caused phase darkening of the chemically treated spores resembling that which occurs during normal spore germination. The rate of phase darkening in lysozyme was quicker when urea was included with the disulphide bond-breaking reagents. Lysozyme did not alter the phase-contrast appearance of control or untreated spores.

Sensitization of spores to lysozyme with thioglycollic acid could be reversed by slow oxidation of the spores in aerated water. When attempts were made to reverse the effect of the thioglycollic acid treatment with hydrogen peroxide (0·04–4·0 M) it was found that the peroxide also caused phase darkening of the treated spores. Hydrogen peroxide had the same effect on spores treated with performic acid and 2-mercaptoethanol whereas up to 6 M hydrogen peroxide did not affect control spores. Phase darkening was followed by almost complete solubilization of the spores leaving only cell ghosts, presumably spore coat material, and therefore differed from the phase darkening which occurs during normal germination.

The *p*H activity curves of the lysozyme and hydrogen peroxide reactions showed important differences. The lysozyme reaction only occurred between *p*H 6·0 and 10·2 whereas the hydrogen peroxide reaction was most rapid above *p*H 10 and in the presence of metal ions and was inhibited by sequestrants. This suggested that the lysozyme reaction was physiological but the hydrogen peroxide reaction was non-physiological and depended on catalytic decomposition of the peroxide.

The reactions appeared to be general in their effects on bacterial spores. Spores of *Bacillus cereus* (three strains), *B. megaterium*, *B. polymyxa*, *B. brevis*, *B. fusiformis*, *B. mesentericus*, *B. licheniformis*, *B. subtilis*, *B. subtilis* v. *aterrimus* and *B. globigii* which were treated with performic or thioglycollic acids all became susceptible to lysozyme and to hydrogen peroxide, as also did spores of *Clostridium butyricum* (two strains), *C. saccharobutyricum*, *C. tertium*, *C. septicum* and *C. bifermentans*.

These results are compatible with: (1) Vinter's observation[1] of the high content of disulphide bonds in the coat fraction of spores; (2) chemical rupture of these bonds; (3) consequent disclosure of new substrates aided by the hydrogen bond breaking activity of urea; (4) action of lysozyme on previously protected lysozyme substrate which is present in spore mucopeptide[7]; (5) action of hydrogen peroxide on previously protected but unknown substrates. These results are also compatible with a spore structure in which the cystine disulphide-rich[1] and proteinaceous[5,6] coat surrounds the lysozyme-sensitive mucopeptide[5] and the calcium dipicolinate[8,9] of the cortex.

Normal germination of bacterial spores may involve similar reactions to those described here. Vinter[3] has suggested that spore disulphide bonds may not be solely concerned with resistance to irradiation but may also be involved in the sudden activity during germination. He demonstrated a qualitative reduction of spore disulphide bonds during germination[2]. Enzymatic rupture of spore disulphide bonds, followed by activation of a lytic system, could invoke changes in permeability[10] and swelling[11] of the spore typical of germination. For example, enzymatic rupture of disulphide bonds in yeast pseudokeratin has been described[3,12], and a spore enzyme capable of attacking spore mucopeptide was discovered by Strange and Dark[13]. Changes induced by such enzymes could parallel changes induced by disulphide bond-breakers and lysozyme. It is clearly possible that during normal spore germination endogenous enzyme systems could bring about changes similar to those induced by the exogenous systems we have described. The relevance of these reactions to normal germination is therefore now being examined.

G. W. GOULD
A. D. HITCHINS

Unilever Research Laboratory,
Colworth House,
Sharnbrook,
Bedford.

[1] Vinter, V., *Folia Microbiol.*, **5**, 217 (1960).
[2] Vinter, V., *Spores II*, edit. by Halvorson, H. O., 127 (Burgess Pub. Co., Minneapolis, 1961).
[3] Vinter, V., *Nature*, **189**, 589 (1961).
[4] Vinter, V., *Folia Microbiol.*, **7**, 115 (1962).
[5] Ohye, D. F., and Murrell, W. G., *J. Cell. Biol.*, **14**, 111 (1962).
[6] Salton, M. R. J., and Marshall, B. J., *J. Gen. Microbiol.*, **21**, 415 (1959).
[7] Strange, R. E., and Dark, F. A., *Biochem. J.*, **62**, 459 (1956).
[8] Knaysi, G., *J. Bact.*, **82**, 556 (1961).
[9] Rode, L. J., Lewis, jun., C. W., and Foster, J. W., *J. Cell Biol.*, **13**, 423 (1962).
[10] Black, S. H., and Gerhardt, P., *J. Bact.*, **83**, 301 (1962).
[11] Hitchins, A. D., and Gould, G. W., *J. Gen. Microbiol.*, **28**, iii (1962).
[12] Nickerson, W. J., and Falcone, G., *Science*, **124**, 318 (1956).
[13] Strange, R. E., and Dark, F. A., *J. Gen. Microbiol.*, **16**, 236 (1957).

## CYTOLOGY

### Chromosomal Dynamics of *Gastrimargus*

CHROMOSOME number and morphology have been regarded as most constant properties of a species both within and between different individuals. This is also[1-4] true of short-horned grasshoppers The concept of absolute karyotypic stability entails an evolutionary cul-de-sac, and therefore a relative stability alone can afford scope for further genotypic change. Though it is known that spontaneous chromosomal mutations in stable genetic systems is low[5-10], yet chromosomal variations within an

©1963 Nature Publishing Group