SHORT COMMUNICATION

Sensitivity of Synchronous Cultures of *Bacillus subtilis* to Lysozyme

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Rates of lysis by lysozyme (expressed as the decrease in $A_{550} \text{ min}^{-1}$) of synchronously dividing cells of *Bacillus subtilis* rose in concert with the stepwise increase in cell numbers. Asynchronously growing cells showed no periodicities in sensitivity; rates of lysis reflected the exponential increase in culture density.

INTRODUCTION

It is generally accepted that synchronous cultures of micro-organisms prepared by selection methods are less perturbed metabolically than those prepared by induction methods (Helmstetter, 1969; Mitchison, 1971). Such cultures facilitate the investigation of cellular events at defined stages of the cell-division cycle.

In this paper, the sensitivity to lysozyme of exponentially growing cultures of *Bacillus subtilis* is compared with that of a selected population of synchronously dividing cells. The results indicate an increase in sensitivity to lysozyme at cell division, suggesting possible periodic variations in lytic sites during the cell cycle.

METHODS

Organism and growth conditions. *Bacillus subtilis* (NC113) was grown in a minimal salts medium which contained (g l^{-1}): K_2HPO_4 , 14; KH_2PO_4 , 6; $(\text{NH}_4)_2\text{SO}_4$, 2; sodium citrate, 1; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2; FeCl_3 , 0.002; MnCl_2 , 0.002. The pH of the medium was adjusted to 7.0 before autoclaving. Filter-sterilized glucose (40%, w/v) was added to a concentration of 50 mM as carbon source. All cultures were incubated at 30 °C with shaking.

Preparation of synchronous cultures. Synchronous cultures were prepared by a modification of the method described by Sargent (1973). An exponentially growing culture (100 ml) at a density which gave an A_{550} of 0.6 to 0.7 was drawn once by suction through a single GF/C filter (Whatman, 47 mm diam.), using a Perspex Millipore filtration apparatus. The filtrate contained approximately 10% of the original culture and was immediately returned to the incubator and assessed for synchronous growth. The time taken for the procedure was approximately 5 min.

Assessment of synchrony. Cell numbers were determined in a standard counting chamber slide (Hawksley, Lancing, Sussex) after fixing the organisms in formaldehyde (4%, v/v). The degree of synchrony was assessed by the synchrony index (F) of Blumenthal & Zahler (1962), calculated from the equation:

$$F = (N/N_0) - 2^{t/g}$$

in which F has a maximum value of 1.0 in a culture exhibiting ideal synchrony, N is the number of organisms at time t , N_0 is the number of organisms at zero time, and g is the mean generation time.

Determination of cell lysis. Samples (2.9 ml) were removed into cuvettes during growth of cultures and their A_{550} was measured in a CE272 spectrophotometer (Cecil Instruments, Cambridge) linked to a chart recorder. Lysozyme (Sigma; 200 mg in 0.1 ml) was then added and the rate of lysis was calculated from the initial rate of decrease in A_{550} in a total volume of 3 ml and expressed as $\Delta A_{550} \text{ min}^{-1}$.

Fig. 1. Effect of lysozyme on cells from (a) an asynchronous culture and (b) a synchronous culture: $\circ$, cell numbers; $\blacktriangledown$, A_{550} ; ∇ , rates of lysis by lysozyme (200 mg) expressed as $A_{550} \text{ min}^{-1}$. In (b), the cell cycle is defined as the time from the mid-point of the first division to the mid-point of the second division; F_1 and F_2 denote synchrony indices for the first and second divisions, respectively.

RESULTS

Lysozyme sensitivity in asynchronous control cultures

An asynchronous culture was filtered as described in Methods and the cells retained on the filter were resuspended in the filtrate, thus restoring the original population. This suspension was incubated and the degree of lysozyme sensitivity was assessed during the subsequent growth period. Cell numbers, absorbance (A_{550}) and rates of lysis ($\Delta A_{550} \text{ min}^{-1}$) all increased in a smooth exponential fashion with a doubling time of 80 min (Fig. 1a). These results indicated that the filtration procedure caused little perturbation to the cells since the mean generation time of untreated exponentially growing cells was also 80 min. The similar increases in rates of lysis and in cell numbers suggested that the lysozyme sensitivity of the bacteria remained constant during asynchronous growth. Control experiments at different stages of asynchronous growth indicated that the sensitivity to lysozyme was similar for cells in culture medium and for cells removed by filtration and resuspended in uninoculated growth medium. These results suggest that sensitivity to lysozyme is not modified by changes in the culture medium during growth (e.g. pH).

Lysozyme sensitivity in synchronous cultures

Growth of a typical filtrate obtained by the procedure outlined in Methods is shown in Fig. 1(b). Cell numbers doubled rapidly after a lag of approximately 40 min indicating a synchronous division ($F_1 = 0.76$). A second synchronous division occurred at 120 min ($F_2 = 0.60$). The cell cycle, defined as the time between the mid-point of the first division and

the mid-point of the second, lasted approximately 80 min, which was similar to the mean generation time of the organism in unselected populations. The absorbance (A_{550}) of the culture doubled every 80 min. In marked contrast to the pattern of lysis exhibited by asynchronous cells, the rates of lysis measured during synchronous growth remained relatively constant during the period preceding cell division and increased concomitantly with the rise in cell numbers (Fig. 1*b*).

DISCUSSION

The method employed here to prepare synchronous cultures of *B. subtilis* gave a high degree of synchrony and is simple and rapid to execute. Its application to other bacterial systems has not been investigated.

The rise in sensitivity to lysozyme at cell division suggests a possible reorganization of the cell surface. Previous work has shown that, in *Escherichia coli*, sensitivity of synchronously dividing cells to penicillin and chloramphenicol also rises to a maximum at cell division (Mathison, 1968). The periodicities in rates of lysis described here may reflect periodic synthesis of lytic sites, wall-synthesizing enzymes or 'remodelling' autolytic enzymes. Evidence for the latter has been presented for *Streptococcus faecium* where autolytic activity in synchronized populations increases at cell division (Hinks *et al.*, 1978). Lysozyme has also been shown to enhance autolysin activity in *B. cereus* (Westmacott & Perkins, 1979). The differential response of *B. subtilis* to lysis by lysozyme has important implications. Cells taken from different stages of synchronous growth may not be equally sensitive to disruption methods. This will influence the amount of estimable intracellular components released at different stages in the cell cycle.

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