MICROBIOLOGY

Nitrogen Fixation in Cell-free Extracts of *Azotobacter vinelandii* prepared by Lysis with Phage A_{22}

WITHIN the past few years nitrogen fixation has been achieved in cell-free extracts of micro-organisms including *Clostridium pasteurianum*, *Azotobacter vinelandii*, *Bacillus polymyxa*, and in a variety of blue-green algae[1-5]. We now report that cell-free lysates of *A. vinelandii*, prepared by adding A_{22} phage[6] to cultures of the bacteria during early exponential growth, incorporate nitrogen gas.

A. vinelandii OP was grown in 4-l. aerated cultures in Burk's nitrogen-free medium at 30° for 12 h. At this stage when cell counts were about 2×10^7 cells/ml., A_{22} phage was added (phage to cell input 3:1) and incubation continued for another 6 h. Phage counts of the incubated cultures, made on agar plates layered with *Azotobacter* cells in a nitrogen-free medium, showed more than a 100-fold increase in phage plaque-forming units per ml. The lysed material was collected at 44,000*g* in a continuous-flow Servall centrifuge at 4°, and the residue was washed three times with 0·05 M phosphate (*p*H 7·5). The residue suspended in the dilute phosphate buffer was recentrifuged at the various *g* values shown in Table 1. Less than 10^3 cells/ml. were found by plate counts of the suspended residue. Before exposing to the nitrogen-15 gas mixture the pellets collected at various *g* values were suspended in 0·05 ml. phosphate buffer *p*H 7·5. The effects of adding medium from 6-h cultures, from which the bacteria had been removed by centrifuging at 40,000*g* for 1 h (absence of whole cells checked by plating experiments) to supernatant fractions and to the suspended pellets were investigated. In some experiments the cell-free medium was boiled for 15 min, cooled and then added to the test fractions. Samples containing between 0·2 and 1·5 mg protein were exposed for 2 h in Warburg flasks to a gas mixture containing (atmospheres) 0·2 nitrogen, 0·2 oxygen and 0·8 helium. The nitrogen gas contained 33·4 atomic per cent nitrogen-15 excess. The extracts were then digested in sulphuric acid containing a copper-selenate catalyst. Total nitrogen was determined by the micro-Kjeldahl procedure and nitrogen-15 by the method of Simms and Cocking[7] using a mass spectrometer.

Table 1. NITROGEN FIXATION IN EXTRACTS OF *Azotobacter vinelandii* PREPARED BY LYSING CELLS WITH PHAGE A_{22}. ATOMIC PER CENT NITROGEN-15 EXCESS

	Sample	Exp. I	Exp. II
	Whole cells (12 h growth)	0·56	0·68
	Cell-lysates* centrifuged successively at the following *g* values:		
1	5,000*g* for 30 min (pellet)	0·007	0·025
2	(1) plus *M*†	0·009	0·030
3	5,000–14,000*g* for 30 min (pellet)	0·018	0·026
4	(3) plus *M*†	0·490	0·254
5	14,000–27,000*g* for 30 min (supernatant fluid)	0·028	0·032
6	(5) plus *M*†	0·380	0·540
7	27,000–44,000*g* for 60 min (residue)	0·029	0·034
8	(7) plus *M*†	0·240	0·340
9	44,000*g* for 60 min (supernatant fluid)	0·016	0·023
10	(9) plus *M*†	0·104	0·132

* Whole cells (12 h growth) treated with A_{22} phage and incubated for a further 6 h to effect lysis. Lysate collected at 44,000*g* suspended in 0·05 *M* phosphate (*p*H 7·5) and centrifuged successively at the various *g* values.

† *M*, medium from 6 h cultures of *Azotobacter* centrifuged at 40,000*g* for 1 h to remove whole cells. An equal volume of the medium added to the fractions.

The results in Table 1 show that there is little fixation in the cell-debris left after centrifuging the suspended residue at 5,000*g*; but the fractions collected at 14,000*g* or above have an appreciable enrichment of the isotope. The addition of the cell-free culture medium to the various fractions stimulated nitrogen fixation as reported earlier[3,5]. The main activity was found in the particulate fractions collected between 14,000*g* and 44,000*g* although there was an appreciable enrichment even in the resultant supernatant fraction. When the latter was centrifuged at 144,000*g* for 1 h the activity was found in the pellet. The stimulatory effect of the medium on nitrogen fixation was not destroyed by boiling.

It is of interest that electron paramagnetic resonance spectra of the active particulate fractions showed pronounced signals at gauss 1·94, presumably non-hæm iron, and at gauss 1·97, probably Mo^{5+}, as well as one for a flavin semiquinone. These results are similar to those reported for active nitrogen-fixing fractions prepared from *Azotobacter* by other methods[8].

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Significance of Deoxyribonuclease Production by *Staphylococci*

METHODS of differentiating pathogenic species of *Staphylococci* (*Staphylococcus aureus*) from commensal strains (*Staph. albus*) are of prime importance to the medical bacteriologist. The method generally used to effect this separation is one of the many modifications of the coagulase test, and it is usually considered that new tests adopted for this purpose should correlate highly with the coagulase index. Among the alternatives that have been proposed to the coagulase test are colonial pigmentation, phenolphthalein phosphatase activity and the possession of a fibrinolysin.

None of these has found favour. Chromogenesis has been considered unreliable, partly because of the subjective difficulties involved, but the introduction of glycerol monoacetate agar by Willis and Turner[1] has done much to eliminate this problem. Further, this new medium permits a subdivision of epidemiological importance within the pathogenic group. Barber and Kuper[2] suggested that phosphatase activity paralleled coagulase production, but there is some disagreement about the value of this test because of the large number of coagulase-negative strains that produce a phosphatase. It was noted by Christie and Wilson[3] that the fibrinolytic activity of human strains of *Staphylococci* generally correlated with the coagulase test and with pathogenicity, but in our experience there are many important exceptions to this generalization.

More recently, Weckman and Catlin[4], using a viscometric technique, showed that high deoxyribonuclease production was a characteristic of coagulase-positive