chromatids. The low frequency of true chromatid breaks and the absence of isochromatid aberrations showing no reunion (*Nupd* types[5]) are more in accord with the exchange mechanism for aberration production, as proposed by Revell[7].

Further details and discussion of this work will be published elsewhere.

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Nitrogen Fixation by Gram-Negative Bacteria

Although claims for nitrogen fixation by Gram-negative bacteria other than the *Azotobacter* have been made in the past[1], confirmation of such claims is comparatively recent[2–5]. Nitrogen fixation by particular strains of *Pseudomonas* has been reported by Anderson[2] and by Voets and Debacker[3]. Recently, Jensen[5] isolated from a Danish water-course a nitrogen-fixing organism that appears to be a species of *Achromobacter*. In most of these studies, the investigators were attempting to obtain species such as *Azotobacter* that are known to fix nitrogen; hence the techniques employed would select for this characteristic. Because of the significance of the discovery of these new agents for both theoretical and applied biology we have examined strains of *Pseudomonas* and *Achromobacter* that had been isolated under non-selective conditions.

Using the sensitive tracer nitrogen-15 technique as well as the more conventional Kjeldahl method, we have demonstrated aerobic fixation in six strains of *Pseudomonas* spp. and eight strains of *Achromobacter* spp. All the pseudomonads except *Ps. azotogensis*[3] also fixed anaerobically, but only Jensen's strain of the *Achromobacter* fixed in the absence of oxygen. The fixation process in both genera appears to be controlled by an inducible enzyme system.

The basal medium used throughout contained sucrose 2·0 per cent, disodium hydrogen phosphate 1·25 per cent, potassium dihydrogen phosphate 0·15 per cent, calcium chloride dihydrate 0·01 per cent, magnesium sulphate heptahydrate 0·01 per cent, ferric iron 0·0001 per cent and molybdenum (as molybdate) 0·00001 per cent. Ammonium nitrogen was added as required. Total carbohydrates were determined by the method of Morris[7], aeration values by the method of Cooper *et al.*[8].

Aerobic fixation and growth were studied in 100 ml. cultures in 500 ml. flasks, at 30° C., shaking at 350 r.p.m. on a Brunswick shaker with $\frac{3}{4}$-in. rotor; these conditions resulted in an aeration value of 0·315 mmoles of oxygen per litre per min. Initial and final aliquots were analysed for nitrogen and total carbohydrates. For the pseudomonads fixation varied from 3·0 to 50·0 μgm./ml. in 4 days, and the efficiencies of fixation from 1·1 to 4·3 μgm. of nitrogen per mgm. of carbohydrate used. Three phases of growth were evident: (*a*) an initial growth; (*b*) a lag phase; (*c*) a second growth phase. The extent of the initial growth depends on the quantity of ammonium nitrogen added to the medium; the lag phase probably reflects the synthesis of the enzyme system responsible for fixation; and the third phase is growth on molecular nitrogen. Analysis of the total increase of nitrogen in 25 ml. shake cultures indicated fixation of from 0·20 to 0·55 mgm. in 14 days. Incorporation of heavy nitrogen was demonstrated using concentrated suspensions of organisms harvested in the second growth phase. Exposure to an atmosphere of 10 per cent nitrogen containing approximately 36 atoms per cent excess nitrogen-15, 20 per cent oxygen and 70 per cent helium was made for 4 hr. at 30° C. in a well-shaken Warburg vessel; analyses showed a final incorporation of greater than 0·1 atoms per cent excess, for all cultures.

Achromobacter spp. showed the same type of growth characteristics; fixation was in the range 3·0–17·0 μgm./ml. in four days. The efficiency was of the order of 1·25 μgm./mgm. of carbohydrate used; nitrogen-15 incorporation ranged from 0·02 to 0·16 atoms per cent excess.

Anaerobically, fixation by the pseudomonads was of the order of 40 μgm./ml.; incorporation of heavy nitrogen was approximately 0·15 atoms per cent excess. The sole strain of *Achromobacter* that fixed anaerobically assimilated 39 μgm. nitrogen/ml., and contained 0·10 atoms per cent excess nitrogen-15.

In common with the nitrogen-fixing systems of other organisms, those in *Pseudomonas* and *Achromobacter* required a greater quantity of iron for the fixation of nitrogen than for growth upon ammonium-nitrogen. When fixing nitrogen, the strains examined possessed a hydrogenase demonstrable by the hydrogen–deuterium exchange reaction but not detectable by the evolution of hydrogen from reduced methyl viologen, or by the uptake of hydrogen on methylene blue.

This demonstration of nitrogen fixation by an apparently inducible enzyme system in a 'random' selection of strains of *Pseudomonas* and *Achromobacter* confirms and extends the investigations from the other laboratories. In the pseudomonads it provides yet another example of the adaptive versatility of the genus. Because of the widespread occurrence of both genera in soil and surface waters, their importance in nitrogen fixation may be considerable.

This research was supported in part by grant *E*-1417 (*C*4) from the National Institutes of Health.

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