and that formation of insoluble collagen increases as the animal approaches maturity.

D. J. CARMICHAEL

Food Science Department,
School of Agriculture,
University of Nottingham.

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Utilization of Adenine but not Nitrate as Nitrogen Source by *Prototheca zopfii*

CULLINMORE[1] has recently reported that *Prototheca zopfii* (Cambridge Collection strain No. 263/5) will utilize butyrate as the sole carbon source for growth, but only after a lag period of 3 days. It is presumed that this represents a period of enzyme induction. We have been investigating some aspects of the nitrogen metabolism of this strain of *Prototheca* and have observed that while adenine can be used as the sole nitrogen source there is again a lag period before growth commences. In this case, however, the lag is only about 30 h. (Fig. 1). The increase in dry weight obtained suggests that ring nitrogen of the adenine molecule is utilized in addition to the amino group.

Pure cultures of *Prototheca zopfii* were grown in 100-ml. Erlenmeyer flasks containing 20-ml. of the medium used previously[2], but with the ammonium nitrate omitted and with the addition of 1 per cent glucose and 10^{-5} M thiamine[3]. 140 mg nitrogen/l. was then supplied either as ammonium sulphate, potassium nitrate or adenine. After autoclaving, the *p*H of the medium was 6·1. The flasks were inoculated under aseptic conditions and were shaken in darkness at 25° C. The dry weight of algae in cultures was determined by means of sintered glass filter crucibles dried in an oven at 80° C.

Inorganic nitrogen assimilation by *Chlorella vulgaris* has been extensively studied and it was shown that the activity of the nitrate reductase is inhibited by ammonium assimilation[4] and further that the synthesis of the enzyme is repressed by ammonium[5]. We assumed that this situation might apply to *Prototheca zopfii*, which is generally considered to be a colourless *Chlorella*[6], and were interested to determine whether adenine as well as ammonium would repress the production of nitrate reductase. However, a preliminary experiment showed that the alga is unable to utilize nitrate as sole nitrogen source as is shown in Fig. 1. We are not fully convinced that this observation is previously unrecorded but in the investigations known to us the alga was grown in media which contained either ammonium chloride or ammonium sulphate as nitrogen source. This derives from Barker's[7] original work in which a medium containing ammonium chloride was used and the significant observation was made that growth was still dependent on the addition of small quantities of yeast extract owing to the requirement for thiamine[8].

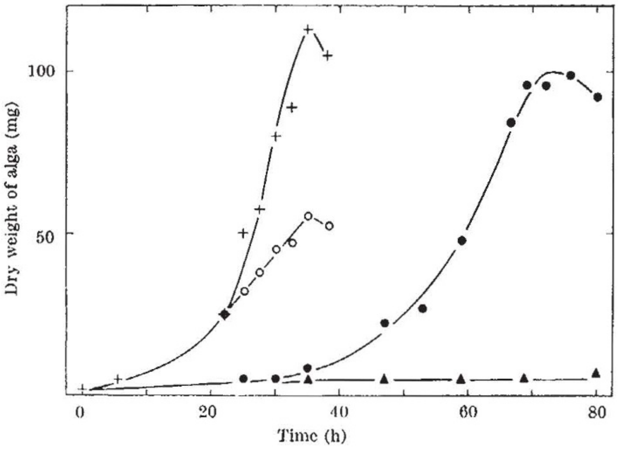

Fig. 1. Growth of *Prototheca zopfii* with various nitrogen sources in the medium. +, 140 mg N/l. as ammonium sulphate; ○, 28 mg N/l. as ammonium sulphate; ●, 140 mg N/l. as adenine; ▲, 140 mg N/l. as potassium nitrate

It has been suggested that the fact that *Prototheca zopfii* contains a definite plastid[9,10] implies that it arose from a mutation rendering it incapable of synthesizing chlorophyll[10]. At present it is not known whether the alga is unable to synthesize nitrate reductase, as in the case of the *Chlorella* mutant isolated by Shafer *et al.*[11], or whether nitrate assimilation is actually blocked at the stage of nitrite reductase. In *Ankistrodesmus*[12] the nitrate reductase requires as coenzyme reduced nicotinamide adenine dinucleotide ($NADH_2$) and the nitrite reductase reduced nicotinamide adenine dinucleotide phosphate ($NADPH_2$). The latter enzyme is particulate. Similarly, a nitrite reductase requiring ferredoxin has been associated with chloroplasts isolated from several higher plants[13]. Thus there seems to be a definite possibility that the inability of *Prototheca* to utilize nitrate is associated with the absence of a functional chloroplast. If this is indeed the case the biochemical differences between *Prototheca* and *Chlorella* are more complex than the inability to synthesize chlorophyll, and as a consequence carbohydrate by photosynthesis, would indicate. The assimilation of ammonium by yeast leads to a stimulation of the pentose phosphate pathway of glucose oxidation to satisfy the requirement of the glutamic dehydrogenase for $NADPH_2$ (ref. 14). Although it is quite clear that *Prototheca zopfii* is capable of degrading glucose by this pathway[15] a comparable coupling to nitrogen metabolism may not have developed.

J. L. STACEY
P. J. CASSELTON

Department of Botany,
Birkbeck College,
London, W.C.1.

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AGRICULTURE

Incidence of *Rhizoctonia* in a Cultivated and a Fallow Soil in Hong Kong

THE arable land available in Hong Kong is very limited and farming is so intensive that the annual production of 8–9 vegetable crops, often on the same land, is common. For many reasons, especially from an accumulation of soil-borne pathogens, this might be expected to have a deleterious effect on the soil.

A comparison[1] of the fungi present in a cultivated and in a fallow soil using Warcup's soil-plate method[2] indicated that at least two genera of fungi which are known to be causal agents of soil-borne diseases were more prevalent in the cultivated soil. *Pythium* sp. was only isolated from the cultivated soil. Whereas the occurrence of *Fusarium dimerum* was high in this soil, it was seldom found in the fallow soil.