SHORT COMMUNICATIONS

The Nutrition, Resistance to Antibiotics and Ultrastructure of *Prototheca wickerhamii*

By N. J. PATNI AND S. AARONSON

Biology Department, Queens College, City University of New York,
Flushing, New York 11367, U.S.A.

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INTRODUCTION

The colourless chlorophytan algal genus, *Prototheca*, has been implicated in human and animal disease (Fetter, Klintworth & Nielsen, 1971) but to the best of our knowledge, the nutrition of no species or strain isolated from humans has been studied in detail. We here report the nutrition and ultrastructure of a strain of *Prototheca wickerhamii* isolated from a lesion on a human in Johannesburg, South Africa and kindly supplied to us by Dr R. E. Lee of the University of Witwatersrand, South Africa.

METHODS

P. wickerhamii was maintained on BBL nutrient agar or broth. It also multiplied well on *Ochromonas danica* medium (Aaronson & Baker, 1959) at pH 5.0 and on the medium of Anderson (1945) at pH 7.0. Anderson's medium (NH_4Cl , 0.1 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.02 g; KH_2PO_4 , 0.1 g; Na_2HPO_4 , 0.1 g; distilled water, 100 ml) was used for nutritional studies. Growth was measured as extinction at 600 nm in a Beckman DB spectrophotometer.

The algae were fixed, stained, embedded and sectioned for electron microscopy as described by Aaronson, Behrens, Orner & Haines (1971) and examined with a Jeolco T7 electron microscope.

All experiments were done at least in triplicate, with almost identical results.

RESULTS

The strain of *Prototheca wickerhamii* isolated from a human grew better at 25 °C than at 37 °C during the first week of growth, and equally well at both temperatures by the second week. Supplementation of Anderson's medium with glycerol and then comparing growth with thiamine (0.0001 to 0.1 mg/100 ml) and/or biotin (1 µg/100 ml) or yeast extract (0.2 %) revealed a requirement for thiamine only. A number of organic compounds (at 0.5 %) were examined for their ability to serve as carbon and energy sources. Glycerol was the best energy source, followed by sucrose, cellobiose, mannose, lactose and galactose in that order. The following were not good energy sources: arabinose, fructose, glucose, xylose or sodium salts of acetate, pyruvate or succinate. The source of nitrogen, NH_4Cl (0.1 %) or glutamic acid (0.1 %), did not affect carbon utilization. Glutamic acid, ammonium chloride and urea were good sources of nitrogen at 0.1 %, but NaNO_3 , L-alanine, L-arginine-HCl, L-aspartic acid, L-cystine, glycine, L-leucine, L-methionine, L-proline, L-serine and

Fig. 1. (a) *Prototheca wickerhamii* immature cell. N, nucleus; NO, nucleolus; M, mitochondrion; P, plasma membrane; D, dense bodies. (b) Autospores within a common cell wall. (c) Enlargement of wall and cell membrane area in (a), to show details of wall structure. Note layers in wall and rough outer layer.

DL-valine were not. Complex nitrogen sources, such as peptone and yeast extract, were no better as nitrogen sources than glutamic acid, ammonium chloride or urea.

Multiplication of *P. wickerhamii* in liquid medium was inhibited by cycloheximide and SKF 525-A (β -diethylaminoethylphenylpropylacetate-HCl, by courtesy of Smith, Kline & French) but not by chloramphenicol, bacitracin, gramicidin, penicillin, or streptomycin to a concentration of 150 μ g/ml. Cycloheximide and SKF 525-A gave a 50 % inhibition of multiplication at about 25 μ g/ml and 100 % inhibition at about 60 μ g/ml.

Prototheca wickerhamii contained the usual organelles described for the immature form of *Prototheca* (Klintworth, Fetter & Nielsen, 1968): plastids, dense bodies, peripheral cytoplasm with ribosomes, nucleus and nucleolus, Golgi body, endoplasmic reticulum, mitochondria, dense wall and plasma membrane (Fig. 1*a*). At several places the plasma membrane was invaginated and pulled away from the wall leaving a space filled with what we assumed to be the remains of cytoplasm including ribosomes (Fig. 1*a, b*). This invagination continued until the wall enclosed several distinct autospores (Fig. 1*b*), each containing its own nucleus and limited by a plasma membrane and separated by a space in which there were granules presumably representing the remains of cytoplasm and ribosomes. The wall of *P. wickerhamii* was made up of what seemed to be several layers with a rough outer layer separated from the bulk of the wall by an electron-lucent layer (Fig. 1*c*).

DISCUSSION

The strain of *P. wickerhamii* isolated from a South African has typical *Prototheca* nutrition as described by Pore (1972); it requires thiamine, uses ammonium and urea but cannot use nitrate as a nitrogen source. Of the several amino acids tested, *P. wickerhamii*, unlike *P. zopfii* and *P. chlorelloides* (Casselton & Stacey, 1969), can use only glutamic acid as a N-source. Some species, for example *P. zopfii* and *P. ciferrii*, use many carbohydrates, including monosaccharides, disaccharides, and polysaccharides, as carbon and energy sources; *P. portoricensis* and *P. chlorelloides* use fewer carbohydrates (Ciferri, 1957). *Prototheca wickerhamii*, however, grew best with glycerol and only half as well with sucrose, irrespective of inorganic or organic nitrogen source. Our N-source results differ from those of Tubaki & Soneda (1959); they obtained results with several species of *Prototheca*, including *P. wickerhamii*, that were markedly different from those of Pore (1972) and Casselton & Stacey (1969) in that they are the only authors to report the use of nitrate as an N-source by several *Prototheca* species. Our carbon and energy data for *P. wickerhamii* are markedly different from those of Tubaki & Soneda (1959), which we attribute to differences in basic media.

Multiplication of *P. wickerhamii*, like that of other eukaryotes, was inhibited by the inhibitor of eukaryotic protein synthesis, cycloheximide; inhibitors of prokaryotic protein synthesis such as chloramphenicol, streptomycin and penicillin were without effect. The hypocholesteremic compound SKF 525-A was an effective inhibitor of *P. wickerhamii* as it is of other micro-organisms (Aaronson, 1966).

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