ADAPTATION OF *SULFOLOBUS SOLFATARICUS* ON MINIMAL MEDIA.
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SUMMARY

An economic method to grow the thermoacidophilic archaebacterium *Sulfolobus solfataricus* is reported.

INTRODUCTION

Thermophilic archaebacteria are a group of microorganisms of interest both in fundamental and applied research. In particular for biotechnological point of view, thermophiles are of interest as sources of unique enzymes with unusual properties (Bu'Lock and Kristiansen, 1987). In recent years, a large number of new interesting thermophilic archaebacteria has been isolated and characterized. These organisms can be cultured readily but expensively and with low growth yield in large scale fermenters (Brock, 1986).

This work reported the adaptation of *Sulfolobus solfataricus*, a thermoacidophilic archaebacterium, to different minimal culture media to establish a convenient method in growing this microorganism.

MATERIALS AND METHODS

Microorganism and culture conditions.

*Sulfolobus solfataricus* strain MT4 (ATCC N 49155) was isolated from an acid hot spring in Agnano, Naples. The organism was grown at 88°C in a 90 liter fermenter (Terzano, Milano, Italy) with low mechanical agitation and an aeration flux of 30 ml/min/l of broth. The standard culture medium contained: KH₂PO₄ (3.00 g/l), (NH₄)₂SO₄ (2.00), MgSO₄.7H₂O (0.20), CaCl₂.2H₂O (0.25), Yeast extract (2.00). The pH was adjusted to 3.5 with H₂SO₄ (De Rosa et al. 1984). *S.solfataricus* was also grown under these standard conditions varying the salt concentration. The minimal culture medium had the same standard salt composition with the sole difference that yeast extract was replaced by one of the following carbon sources: Glucose (0.20 %), Fructose (0.10), Galactose (0.20), Lactose (0.20), Xylose (0.15), Citric acid
(0.30), Arabinose (0.13), Malic acid (0.10), Rhamnose (0.20) and Glutamic acid (0.15; (NH₄)₂SO₄ was decreased to 0.20 g/l). The growth on Glucose, as sole carbon source, was also performed varying the temperatures between 75 and 91°C. The Glucose concentration during growth was tested enzymatically by the glucose oxidase-peroxidase (Bergmeyer and Bernt, 1974). The pH was also monitored during the growth. Cell growth was quantified turbidimetrically at 540 nm, an absorbance of 0.6 corresponding to 0.2 gr/l of lyophilized cells. Cells were harvested in the early stationary phase of growth by continuous flow centrifugation on an alfa-laval model Lab 102-20 centrifuge and then stored at -20°C.

RESULTS AND DISCUSSION

Although biotechnological applications of thermophiles seem promising, the large scale uses are to date rather modest.

The industrial potential of thermophilic microorganisms has not yet been fully realized also for culturing problems such as increased evaporation of medium, lower solubility of gases with increasing temperatures, instability of agar at temperatures above 65 to 70°C, faster caramelization of sugars, especially under aerobic conditions and in the presence of high phosphate concentrations.

Sulfolobus solfataricus is able to grow under the culture conditions listed in Table 1. In particular this microorganism utilized Glucose, as sole carbon source, a cheap and available sugar that also after three days at temperature above 85°C does not undergo degradation. In addition was able to grow on Rhamnose, Galactose, Xylose and Arabinose; Malic, Glutamnic and Citric acid also support growth, while moderate growth was obtained with Fructose and Lactose. No effect on the growth yield was observed changing salt composition (Table 1).

The doubling time on glucose at optimal temperature and pH is 6 h, value similar to that obtained when S. solfataricus was grown on complex medium (4 h).

A general observation is that S. solfataricus grown on glucose medium can be stored longer (5-6 years) at -20°C than cultures grown on complex media. The frozen cells, after this period, were capable to support new cultures.

S. solfataricus is easily adaptable to grown on glucose as sole carbon source having doubling time, optimal temperatures and pH comparable with those shown when the microorganism was grown on complex medium. In addition the growth yield of S. solfataricus grown on glucose is higher than that obtained with yeast extract.