The Influence of Different Carbon Sources and Medium Osmolarity on the Potassium Requirements of *Candida utilis* NCYC 321, Growing in Continuous Culture

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**Abstract.** In order to study the influence of different carbon sources on the K\(^+\)-requirements of *Candida utilis* NCYC 321, this yeast was grown at several different dilution rates in potassium-limited continuous cultures with either glucose, glycerol, ethanol, citrate or lactate serving as the carbon and energy source.

It was found that the nature of the carbon source profoundly influenced the cellular potassium content, especially at low dilution rates, but that these differences could not be correlated with any differences in relative growth rate (i.e., \(\mu/\mu_{\text{max}}\)). And although small amounts of potassium seemingly were needed to serve in osmoregulation and in the cotransport of some acidic carbon sources (lactate and citrate), these requirements were negligible.

Independent of carbon source, a strong correlation existed between the intracellular potassium concentration and the yield value on oxygen (\(Y_o\)), and between cellular potassium concentration and growth rate. From these two correlations it was concluded that potassium probably was involved mainly in processes associated with ATP synthesis in this yeast.

Finally the effect of the addition of NaCl to the medium was tested with glucose-containing cultures that were either carbon- or potassium-limited. Up to a concentration of 20 g/l, NaCl was without influence on \(Y_o\), \(Y_{\text{glucose}}\) and \(g_{\text{O}_2}\), but effected a slight increase in the cellular potassium content of the potassium-limited cells and a decrease in that of the glucose-limited cells.

**Key words:** *Candida utilis* — Potassium-limitation — Continuous culture — Oxidative phosphorylation — Yield values — Sodium chloride.
The alternative carbon-substrates that were used (ethanol, glycerol, lactate and citrate) were chosen as representatives of different classes of metabolites—that is, an alcohol, a polyol, a monobasic acid and a tricarboxylic acid. But since the use of acid substrates required the concomitant addition of much Na⁺, and since, at least with prokaryotic (Gram-negative) organisms, medium osmolarity affects the cellular potassium requirement (Tempest and Meers, 1968), the influence of NaCl concentration on the requirement of C. utilis for potassium also was studied separately.

MATERIALS AND METHODS

Organism. Candida utilisNCYC 321 was maintained by monthly subculture on a yeast extract-peptone-glucose medium (pH 5.5) solidified with 2% (w/v) agar

Media. Media were as described previously (Aiking and Tempest, 1976) or, in the case of the experiments detailed in Tables 3 and 4, slightly modified (Aiking et al., 1977a). The main difference was that the former contained 25 mM (NH₄)₂SO₄ whereas the latter contained instead 45 mM NH₄Cl plus 2.5 mM (NH₄)₂SO₄. As carbon sources either 150 mM glucose, 300 mM glycerol, 110 mM ethanol, 120 mM citrate or 330 mM lactate, was added. The K⁺-requirement increased rapidly with increasing dilution rate. In the experiments from Tables 3 and 4, NaCl was added as a sterile 250 g/l solution, thus producing a dilution of the medium to the extent of 4.3% per 1% (w/v) NaCl added. The steady state dry weight was not corrected for this dilution, but the Yglucose value was.

Culture Conditions. A Porton-type chemostat of 0.5 l working volume (Herbert et al., 1965) was used throughout. The temperature was controlled at 30°C and the pH value at 5.5 as previously (Aiking and Tempest, 1976).

Analyses. Cells were collected and analysed for potassium and phosphate as described previously (Aiking and Tempest, 1976). Glucose was determined by the 'oxidase' assay (Glox novum, Kabi AG, Sweden).

Oxygen consumption was measured by passing the effluent gas from the fermentor through an oxygen analyser (Taylor Servomex Type OA 272; Crowborough, Sussex, England); carbon dioxide was determined in the same way with an Irga 10 (Grubb Parsons & Co., Ltd., Walkergate, Newcastle upon Tyne, England).

RESULTS

When the potassium content of potassium-limited organisms that had been grown in a glucose-containing medium is compared with that of similarly-limited organisms that had been grown in media containing other carbon and energy sources, marked differences are immediately obvious (Table 1). And since organisms that had been limited in their growth by the availability of potassium may be expected to contain the minimum amount of this cation necessary for them to function (that is, to grow) at the imposed rate in the prescribed environment, then it follows that the clear differences in cellular potassium content expressed at each growth rate must have some precise physiological significance.

With each carbon substrate, the minimum potassium requirement was markedly growth rate-dependent, but the maximum growth rate value at which steady state conditions could be maintained was markedly substrate-dependent. Hence, the difference between the minimum potassium requirements of, say, glucose-grown and citrate-grown cells, expressed at a dilution rate of, say, 0.1 h⁻¹ might be due to the fact that their relative growth rates (i.e., μ/μₘₐₓ; see Tempest, 1977) were substantially different (that is, 0.18 and 0.29 for growth on glucose and on citrate, respectively). But if we compare the potassium content of glucose- and citrate-grown cells at a comparable relative growth rate, for instance 0.29 (i.e., D = 0.16 h⁻¹ and D = 0.1 h⁻¹ for the glucose-grown and the citrate-grown cells, respectively), then still there is a difference. Also, the potassium contents of cells grown in the presence of either ethanol or citrate show different relationships with dilution rate, although the two cultures express the same maximum growth rate. So we must conclude that there is, indeed, an effect of the carbon source on the cellular potassium requirement that needs to be explained.

In this connection, previous results with potassium-limited, glucose-containing, cultures showed a strong correlation to exist between the cellular K⁺-content and the Y₀ value, from which it could be reasonably concluded that potassium was stoichiometrically implicated in oxidative phosphorylation (Aiking and Tempest, 1976). In the present investigation this correlation was found to be closely similar to that found with potassium-limited ethanol- or lactate-containing cultures (the dashed line in Fig. 1). However, a line passing through the points obtained with a similarly-limited citrate-containing culture seemed to be displaced such as to indicate a consistently higher requirement for potassium in order to obtain the same yield value on oxygen (the unbroken line in Fig. 1). These results thus tended to indicate that, although, in all cultures, potassium was mainly involved in oxidative phosphorylation, at least in the citrate-grown cells one or more additional roles might be served by this cation, which demanded a constant additional amount of potassium to be present.

Yet other differences can be seen between potassium-limited cultures grown with the different carbon sources (Table 2). This table clearly shows a cellular phosphorus content of more than 4% (w/w) in potassium-limited cultures with either glucose, glycero...