# Comparative Studies on Intracellular Potassium- and Sodium Concentrations of Wild-Type and a Macrotetrolide Negative Mutant of *Streptomyces griseus**

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The studied macrotetrolide negative mutant of *Streptomyces griseus* showed a different specificity of potassium accumulation than the macrotetrolide producing wild-type, indicating, that the macrotetrolides may be part of the physiological potassium carrier.

Most living cells require potassium for growth and maintain a high intracellular concentration of that ion over a wide range of extracellular concentration. The selectivity for potassium over sodium is determined by the transport systems which reside in the cytoplasmic membrane.

It is a well established fact by now that the permeability of natural and artifical membranes for potassium is increased by antibiotics such as valinomycin, the macrotetrolides, the gramicidines, and enniatines [1-3]. This information has led to the hypothesis that ionophores are components of the physiological potassium transport systems [4, 5], responsible for cation selectivity for entry into the cell. If this view is correct, strains which do not produce such ionophores should have lost the specificity for potassium. Furthermore, potassium transport should be inhibited by sodium. In a previous paper [6] a mutant was described producing no detectable amounts of macrotetrolides, while the parent strain (*Streptomyces griseus* Tü 10; ETH 7796) produces several grams of these ionophores per liter under optimal conditions [7]. We were able to show, that growth of this mutant is inhibited by sodium in media with low extracellular potassium. This inhibition can be restored by adding back potassium. The experiments described in this paper constitute an attempt to analyse if this difference between wild type and mutant can be due to an un-

specific alkalimetal transport system in the macrotetrolide-negative mutant. The methods for growing the cells and measurement of potassium and sodium concentrations are already described [6].

If cells are grown in medium without any addition of sodium and potassium, the mutant accumulates potassium as well as the wild-type (Fig. 1). Cellular potassium concentration is high in cells in early log-phase of growth, lower in late log-phase, and again high during stationary phase. Both the mutant and the wild-type are similar in this respect, though, probably because of its slower growth, the time of maximal potassium concentration occurs later in the mutant than in the wild-type.
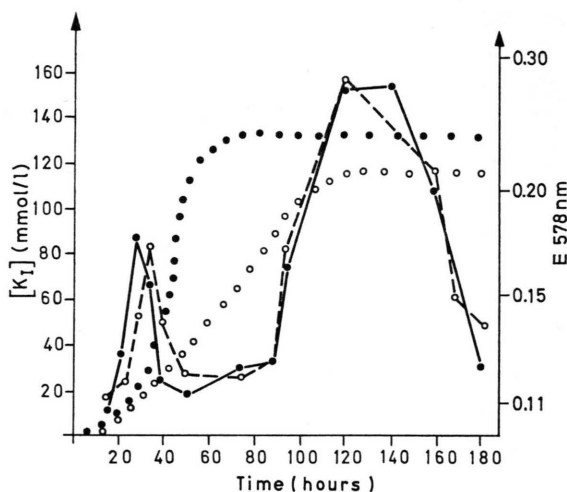


Fig. 1. Intracellular potassium concentrations [K_I] of wild-type ●—● and mutant ○———○ grown in medium with minimum amount of sodium and potassium; ● ● ●, growth of wild-type; ○ ○ ○, growth of mutant.

Changes in intracellular potassium concentration during fermentation are not due to low extracellular concentration of potassium (Fig. 2). The amount of potassium uptake following a rise in extracellular concentration depends on the age of



Fig. 2. Changes of intracellular potassium concentrations due to the age of culture. Addition of 10 mм K+/l after 75 or 95 hours, respectively. + — +, wild-type; ● – – – ●, mutant.

the culture. For example, late log-phase cells show only a small, reversible net influx of potassium, but stationary phase cells exhibit a pronounced influx.

Both the mutant strain and the wild-type take up potassium in a similar fashion in the absence of sodium. Fig. 3, however, shows that potassium accumulation by the mutant is completely blocked in



Fig. 3. Net potassium uptake of wild-type (a) and mutant (b) cells after addition of 0,2 mм K+/l and no sodium (1) or 0,2 mм K+/l and 200 mм Na+/l (2).

the presence of sodium; the accumulation by the wild-type is virtually unchanged. Neither strain loses potassium if it is grown in potassium-rich medium and then transferred into medium without addition of potassium and sodium (Fig. 4). If, however, the resuspension medium contains high
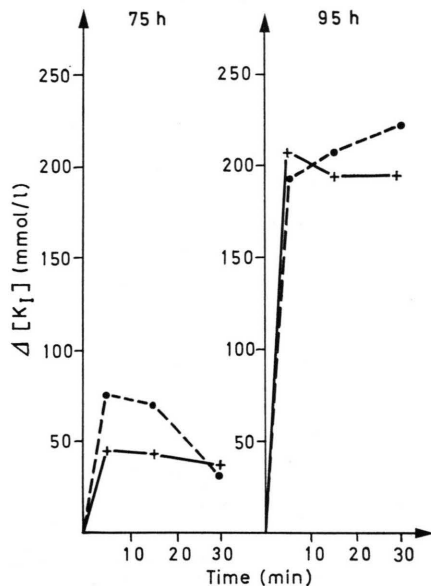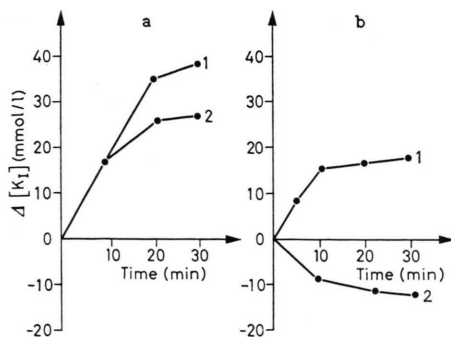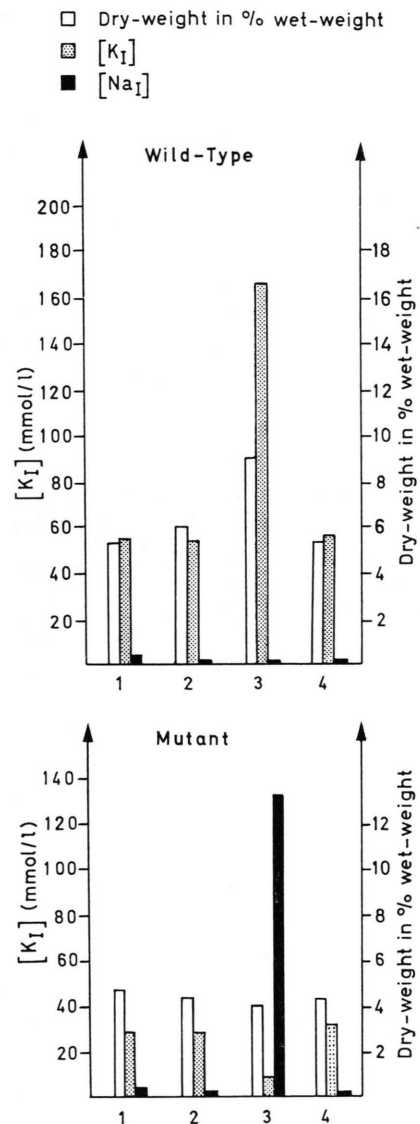


Fig. 4. Intracellular potassium- and sodium concentrations of cells grown in rich medium for 48 hours (1). Cells were centrifuged and the pellet was resuspended in medium containing minimum amounts of sodium and potassium (<0,02 mм/l). The cells were kept in this medium for 3 hours (2). Cells were than transfered into medium with 200 mм Na+/l and minimum amount of potassium (3). Finally these cells were resuspended in medium with minimum amount of sodium and potassium (4).

sodium levels, the mutant takes up sodium and loses potassium, while the wild-type takes up potassium.

The accumulation of potassium shown by the wild-type may be at least partially accounted for by a concomitant decrease in cell volume, as is shown in Fig. 4; the cell volume of the mutant remains constant. Suspending the cells again in medium of low sodium concentration the intracellular potassium and sodium levels can be restored.

The specificity of certain ionophores for potassium has raised the question as to the role of such compounds in biological systems. One possibility could be that ionophores are an integral part of the physiological transport system responsible for specificity. As a result of that prediction cells which have lost the ability to produce such antibiotics by genetic manipulation should have an altered response to potassium and sodium in comparison to the wild-type. The present studies indicate that cells producing no macrotetrolides have a decreased specificity for potassium. Therefore, in medium with a high sodium level such cells transport sodium preferentially. This abnormal increase in intracellular sodium concentration causes inhibition of growth. In contrast, mutant cells exhibit normal potassium accumulation and a normal growth pattern in medium with low concentration of sodium. In agreement with this view are observations obtained from potassium transport mutants of *Streptococcus faecalis* [8]. From these studies it was deduced, that cation exchange is mediated by a transport system which has two specific sites; one controls cation selection for entry, the other for exit [9]. In agreement with this

Table I. Characteristics of potassium deficient mutants and the macrotetrolide minus mutant of *Streptomyces griseus*.

| Characteristic | $Cn_{K6}^-$-type | $Tr_{K8}^-$-type | Macrotetrolide-minus mutant |
|---|---|---|---|
| Growth in inhibited by sodium | − | + | + |
| Accumulation of potassium is inhibited by extracellular sodium | − | + | + |
| Elevated levels of potassium are required for growth | + | − | − |
| Retention of intracellucar potassium at low extracellular concentration of sodium and potassium | − | + | + |

model two classes of potassium transport mutants were isolated, called $Tr_{K8}^-$ and $Cn_{K6}^-$ [8]. Some characteristics of these mutants are compared with our findings obtained from the macrotetrolide-minus mutant of *Streptomyces griseus* (Table I). There is a striking similarity between the macrotetrolide-minus mutant and the $Tr_{K8}^-$-mutants. Therefore, it is conceivable the mutants of the $Tr_{K8}^-$-type have lost the ability to produce a transport component of ionophoric nature responsible for cation selectivity.

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