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Ammonium-dependent transports of amino acids and glucose in a facultatively anaerobic alkalophile

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It was found that the growth of a facultatively anaerobic alkalophile depends on the presence of NH_4^+. The poor growth in the absence of NH_4^+ did not seem to be ascribed to a lack of nitrogen sources. The bacterium was suggested to require specifically NH_4^+ for the acceleration of transports of amino acids and glucose, which is likely to contribute to the optimum growth.

Alkalophile; Amino acid transport; Glucose transport; Ammonium

1. INTRODUCTION

Although it seems difficult for the organisms to adapt to an alkaline environment, there exist many alkalophilic bacteria (alkalophiles) which exhibit an optimum growth in an alkaline medium [1–7]. The aerobic alkalophiles have been suggested to contain much higher contents of cytochrome components for the respiratory chain as compared with the aerobic neutrophilic bacteria (neutrophiles), suggesting special energy demands for the growth in an alkaline medium [7,8]. Recently, a facultatively anaerobic alkalophile which exhibits an optimum growth in a pH range of 9.5–10.0 irrespective of the presence of oxygen was isolated [9]. In spite of the cytochrome component deficiency, the growth rate of the bacterium was comparable to those of aerobic bacteria [9]. It was suggested that the bacterium exhibited an extremely low magnitude of proton electrochemical potential [10]. These results may suggest that the growth of the bacterium depends on a unique energy metabolism. Thus, we are interested in the energy coupling system like the solute transport of the bacterium. In the present study, the bacterium was suggested to exhibit a unique transport system of amino acids and glucose, which is accelerated specifically by NH_4^+.

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2. MATERIALS AND METHODS

2.1. *Culture*

The facultatively anaerobic alkalophile, Ep01, was grown at 39°C as described previously [10]. The bacteria collected at a late logarithmic phase were washed with 20 mM Tris-HCl (pH 8) containing 100 mM KCl and suspended in the same buffer for the application to the experiments.

2.2. *Assays*

For leucine and phenylalanine uptake, the reaction was started by the addition of 50 μl of the bacterial suspension to 0.95 ml of 20 mM Tris-HCl (pH 9) containing 100 mM NaCl, 10 mM glucose and 10 μM [^{14}C]leucine (16 Ci/mol, Amersham International) or [^{3}H]phenylalanine (600 Ci/mol, NEN) in the absence and presence of 20 mM NH_4Cl at 30°C, respectively. After an appropriate time of the reaction, 5 ml of the ice-cold incubation buffer without the labelled amino acids was added to the reaction mixture. The bacteria were collected on a membrane filter (Advantec, 0.45 μm) by filtration and assayed for radioactivity as described previously [11,12]. Glucose uptake was measured by the same procedure as that for leucine uptake except that cold glucose (10 mM) was replaced by 1 mM [^{14}C]glucose (0.17 Ci/mol, ICN Radiochemicals) and addition of leucine was omitted.

Protein concentration was determined according to [13].

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3. RESULTS AND DISCUSSION

The bacterium is able to grow over a pH region from 8.0 to 10.5 with an optimum range of 9.5–10.0 [9]. Fig.1 shows the growth pattern of the bacterium in the standard medium at pH 9.7. We found that the growth rate decreased remarkably, when the addition of NH_4NO_3 to the medium was omitted. Even though the concentration of polypeptone was increased from 0.03 to 0.5% without the addition of NH_4NO_3, the growth rate was not accelerated (fig.1). The result suggests that the poor growth in the absence of NH_4NO_3 was not ascribed to a lack of nitrogen sources. The replacement of NH_4NO_3 by the same concentration (25 mM) of NH_4Cl did not decrease the growth rate, suggesting that the bacterium required NH_4^+ for optimum growth.

To search for the role of NH_4^+ in the growth of the bacterium, the effect of NH_4^+ on the uptake of amino acids and glucose was determined. Although the bacterium exhibited a considerable leucine uptake in a first minute after initiation of the reaction in the absence of NH_4Cl, the amount of leucine accumulated in the first minute of the reaction was nearly three times greater in the presence of NH_4Cl (fig.2). Leucine accumulation in the presence of NH_4Cl continued to increase beyond 8 min after the initiation of the reaction, whereas that in the absence of NH_4Cl ceased to increase around 2 min after the initiation of the reaction. Similar acceleration by the addition of NH_4Cl was shown in the accumulation of glucose (fig.2) and phenylalanine (data not shown). These results suggest that the requirement of NH_4^+ for the optimum growth is ascribed to the stimulatory effect of NH_4^+ on the accumulation of amino acids and glucose. Monovalent cations such as Na^+, Li^+ and K^+ have been suggested to activate the transport system across the membrane in bacterial and eukaryotic cells [11,14–20]. None of the chloride salts of these cations substituted for NH_4Cl in the acceleration of transport of leucine, phenylalanine and glucose in this bacterium. The bacterium is likely to possess a unique transport system, which is activated specifically by NH_4^+. Further studies will be needed to clarify how NH_4^+ contributes to the transport system.

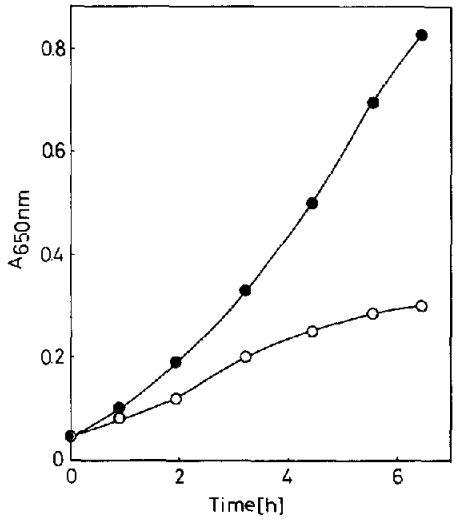

Fig.1. Effect of NH_4NO_3 on the growth rate. The growth was followed by the measurement of absorbance at 650 nm of the medium. (●) Growth at pH 9.7 in the standard medium (the composition (g/l) was: polypeptone, 0.3; yeast extract, 3.0; glucose, 10.0; NH_4NO_3, 2.0; K_2PO_4, 1.0 and trace amounts of salts of divalent cations [10]). (○) Growth under the same conditions except that the medium contained 5.0 g/l of polypeptone and the addition of NH_4NO_3 was omitted.

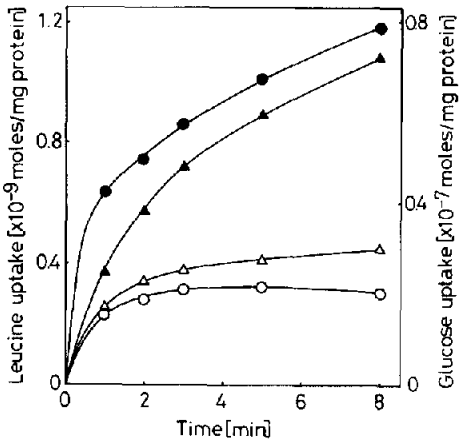

Fig.2. Effect of NH_4Cl on the uptake of leucine and glucose. (○,●) Leucine uptake in the absence and presence of NH_4Cl, respectively. (△, ▲) Glucose uptake in the absence and presence of NH_4Cl, respectively.

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