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ORIGINAL PAPER

Glutamate restores growth but not motility in the absence of chloride in the moderate halophile *Halobacillus halophilus*

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Abstract *Halobacillus halophilus* is a strictly chloride-dependent, moderately halophilic bacterium that synthesizes glutamate and glutamine as the major compatible solutes at intermediate NaCl concentrations. The key enzyme in production of the compatible solutes glutamine and glutamate, glutamine synthetase, is dependent on chloride on a transcriptional and activity level. This led us to ask whether exogenous supply of glutamate may relief the chloride dependence of growth of *H. halophilus*. Growth of *H. halophilus* in minimal medium at 1 M NaCl was stimulated by exogenous glutamate and transport experiments revealed a chloride-independent glutamate uptake by whole cells. Growth was largely impaired in the absence of chloride and, at the same time, glutamate and glutamine pools were reduced by 90%. Exogenous glutamate fully restored growth, and cellular glutamate and glutamine pools were refilled. Although glutamate could restore growth in the absence of chloride, another chloride-dependent process, flagellum synthesis and motility, was not restored by glutamate. The differential effect of glutamate on the two chloride-dependent processes, growth and motility, indicates that glutamate can not substitute chloride in general but apparently bypasses one function of the chloride regulon, the adjustment of pool sizes of compatible solutes.

Keywords Chloride · Glutamate · Halophile · Motility · Solutes

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Introduction

Chloride is an abundant anion on earth but studies addressing a possible function of chloride in prokaryotes are scarce. The moderately halophilic, aerobic bacterium *Halobacillus halophilus* is so far the only bacterial species for which a strict chloride dependence of growth was reported (Roeßler and Müller 1998). Apart from growth, endospore germination, activation of transport of the compatible solute glycine betaine, and motility and flagella production were identified as Cl^--dependent processes (Dohrmann and Müller 1999; Roeßler et al. 2000; Roeßler and Müller 2001). In addition, two-dimensional gel electrophoresis revealed five more chloride-induced proteins (Roeßler and Müller 2002). Molecular studies revealed that FliC, the structural component of the flagellum, as well as LuxS, an enzyme involved in the ''activated methyl cycle'' and the production of the autoinducer-2 are produced only in the presence of chloride (Roeßler and Müller 2002; Sewald et al. 2007). Based on these data, the idea arose that *H. halophilus* ''senses'' salinity via the chloride concentration of its environment and thus, chloride may be a

signaling compound for a complex regulatory network, the chloride regulon (Müller and Saum 2005). However, these experiments did not reveal the basis for the strict chloride dependence of growth of *H. halophilus*.

Like other organisms (Galinski and Trüper 1994; da Costa et al. 1998; Ventosa et al. 1998) *H. halophilus* copes with the elevated salt concentration in its environment by accumulation of different compatible solutes such as glycine betaine, N^{ε}-acetyl lysine, N^{δ}-acetyl ornithine, alanine, citrulline, glutamate, glutamine, ectoine and proline (Severin 1993). The quantitative and qualitative adjustment of the pool size of compatible solutes is one of the greatest challenges in moderate halophiles and clearly a process that is absolutely essential for growth (Sleator and Hill 2002; Grant 2004). The finding that the uptake of the compatible solute glycine betaine is strictly Cl^--dependent (Roeßler et al. 2002) in *H. halophilus* indicates a role of chloride in adjusting pool sizes of compatible solutes. Very recently, we demonstrated that the biosynthesis of glutamate and glutamine, the major compatible solutes at external salinities of around 1 M NaCl, is salt dependent (Saum et al. 2006). The glutamine synthetase was identified not only to be upregulated in response to salt and chloride, but also to exhibit a strictly chloride dependent enzymatic activity. These data demonstrate that the chloride regulon is involved in the regulation of the pool size of compatible solutes. If this is true, then the absence of chloride should result in a decrease of the concentration of compatible solutes. Vice versa, addition of compatible solutes to the medium that are transported in the absence of chloride should relieve the growth inhibition. To test this hypothesis, we asked whether exogenous supply of the compatible solute glutamate could restore growth of *H. halophilus* and/or other chloride - dependent processes in the absence of chloride.

Materials and methods

Organism and cultivation

Stock cultures of *H. halophilus* (DSMZ 2266) were maintained on complex medium (5 g/l peptone, 3 g/l beef extract, 0.05 M $MgSO_4$, 1 M NaCl) or minimal medium [1% glucose, NH_4Cl (2 g/l), $FeSO_4 \times 7\ H_2O$ (10 mg/l), Tris base (12 g/l), K_2HPO_4 (0.49 g/l), yeast extract (0.1 g/l), vitamin solution DSM 141 (1 ml/l), and artificial sea water DSM 79 (250 ml/l)]. For growth experiments, NaCl, $NaNO_3$, Na gluconate, Na_2SO_4 or Na glutamate were added as indicated. Growth experiments were done in 15 ml tubes filled with 5 ml of complex or minimal medium. After inoculation (5%) the cultures were incubated on a rotary shaker at 30°C. The optical density was determined in an Eppendorf photometer at 578 nm. All data points given reflect the means of duplicate tubes from one experiment, and experiments were performed at least three times.

Motile cells were selected and maintained by several passages over swarm-agar plates (0.3% agar) prepared with complex medium as described (Roeßler et al. 2000).

Preparation of concentrated cell suspensions

For preparation of concentrated cell suspensions, complex medium was inoculated (1%) from cultures maintained in the same medium. Fresh cell suspensions were prepared for each experiment. Cells in the late exponential growth phase were harvested by centrifugation and washed once with 50 mM Tris–HCl, pH 7.5, containing 50 mM $MgSO_4$ and 1 M of the salt indicated. The cell pellet was resuspended in the same buffer to a protein concentration of 12–18 mg ml^{-1} and stored on ice until use. The protein concentration of the cell suspension was determined as described (Schmidt et al. 1963), with bovine serum albumine as a standard.

Transport studies

Hundred microliters of the concentrated cell suspension were centrifuged in Eppendorf tubes and resuspended in assay buffer (0.05 M Tris–HCl buffer, pH 7.5, containing 0.05 M $MgSO_4$, salts as indicated, and 1 mM unlabelled and 0.5 µCi [^{14}C]-labelled glutamate). The experiment was started by resuspending the cells in the assay buffer. Fifty-microliter samples were taken at the time points indicated, filtered through nitrocellulose filters (25 mm in diameter; pore size 0.45 µm; Sartorius, Göttingen, Germany), and washed three times with the corresponding buffer. Sampling and washing were done in less than 15 s. All values were corrected for unspecific binding of glutamate to the filters. The filters were air-dried, and radioactivity was determined in a liquid scintillation counter type Tri-Carb 2100 TR (Packard, Dreieich, Germany) using Rotiszint eco plus (Roth, Karlsruhe, Germany) as the scintillation cocktail. The internal glutamate concentration was calculated on the basis of the internal volume published earlier (Roeßler and Müller 1998).

Determination of compatible solutes

Cells were grown up to an OD_{578} of about 0.8, harvested, and freeze dried. A 10 mg of lyophilized cells were then extracted with 570 µl extraction mixture (methanol/chloroform/water 10:5:4, by vol.) by vigorous shaking for 5 min. Then, 170 µl chloroform and 170 µl water were added and the solution was shaken again for 10 min. The aqueous supernatant was recovered and dried. The pellet was then resuspended in 500 µl water.

In order to remove residual proteins and other high-molecular-weight compounds, the solution was then filtered through a Microcon YM-3 filter. The sample was then derivatized with 9-Fluorenylmethyl chloroformate (FMOC) according to the method of Kunte et al. (1993). One hundred and fifty microliters of FMOC reagent (1.5 mM in acetone) was added to 150 µl sodium borate buffer (0.5 M, pH 7.7) and 150 µl sample or standard solution and then mixed by vortexing. Thereafter, 200 µl amantadine hydrochloride reagent (12 mM in borate buffer) was added to remove excess FMOC reagent. In order to make ectoine accessible to FMOC it has to be hydrolyzed to N^{γ}- acetyldiaminobutyric acid and N^{α}-acetyldiaminobutyric acid. This was accomplished by incubating the sample solution with NaOH (0.1 M final conc.) at 50°C for 20 h. The sample was neutralized with perchloric acid before FMOC derivatization. FMOC-derivatized amino acids were separated by reversed phase chromatography (4 µm Supersphere 60 RP-8 endc.; Merck, Germany). The solvent system used comprised solvent A [20% acetonitrile, and 0.5% tetrahydrofuran in 50 mM sodium acetate buffer (pH 4.2)] and solvent B [80% acetonitrile in 50 mM sodium acetate buffer (pH 4.2)]. The gradient used was as follows: 0–2 min: 100% A; 2–8 min: 80% A; 8–16 min: 73% A; 16–29 min: 46% A and 29–31 min: 0% A. Chromatography was carried out at a flow rate of 1.25 ml/min at 45°C. FMOC-labeled derivatives were monitored with a RF 2000 Fluorescence Detector (Dionex, Idstein, Germany) with an excitation wavelength of 254 nm and an emission wavelength of 315 nm.

SDS-PAGE and immunoblots

Cells were harvested by centrifugation (10,000×*g*, 3 min), resuspended in denaturing buffer as described previously (Schägger and von Jagow 1987), and boiled for 10 min. Proteins were separated by SDS-PAGE on 12.5% gels and transferred to nitrocellulose membranes (Schleicher and Schuell) using a semidry blotting chamber (Bio-Rad, Munich, Germany). Membranes were blocked with 0.5% skim-milk powder in PBST (140 mM NaCl, 10 mM KCl, 6.4 mM Na_2HPO_4, 2 mM KH_2PO_4, 0.05% Tween 20) for 1 h, washed three times in PBST for 10 min, and incubated with antisera for 12 h at room temperature. The membranes were washed again three times with PBST for 30 min and then incubated for 1 h with protein A-horseradish peroxidase conjugate. After three additional washing steps (10 min), luminescence was detected using the chemiluminescence blotting substrate from Roche Molecular Biochemicals (Mannheim, Germany), and signals were detected by autoradiography with Kodak X-OMAT-AR film.

Results and discussion

Glutamate stimulates growth in minimal medium

A first hint for the accumulation of exogenous glutamate as compatible solute was obtained by examining growth of *H. halophilus* in minimal medium at 1 M NaCl. In the absence of yeast extract, *H. halophilus* displayed a growth rate of 0.1 h^{-1} and a final OD_{578} of 1.06 (Fig. 1). Upon addition of yeast extract, growth was stimulated by 200% and the final OD_{578} increased by 164%. Addition of betaine at a concentration of 1 mM also stimulated growth to the same extent as yeast extract, whereas the growth rate only increased by 155%. Glutamate also stimulated growth but to a lesser extent and at higher concentrations than betaine (Fig. 1). Interestingly, glutamine inhibited growth at a concentration of 10 mM or higher (at 10 mM glutamine only 62% of the max. growth rate and 67% of the final optical density were observed compared with cells grown without yeast extract). At a concentration of 500 mM, glutamine nearly completely inhibited growth. However, growth inhibition could be fully abolished at 1 and 10 mM or partially at 500 mM by glutamate or glutamate plus betaine. This inhibition of growth by glutamine can not be explained easily. It could result from a competition of glutamine with growth essential amino acids at the transport level or from a negative effect of glutamine on the accumulation of compatible solutes. The latter seems plausible since growth inhibition could be overcome by glutamate and glutamate plus betaine. However, the site of glutamine action remains unknown.

Glutamate uptake in *H. halophilus* is chloride independent

The experiments described above demonstrate a growth supportive effect of glutamate, indicating the presence of a glutamate transport system. To test for glutamate uptake, resting cells were prepared. Upon addition of glutamate to these cell suspensions, glutamate was taken up with a rate of 14.6 nmol/min· mg protein. Uptake rates and final accumulation factors were the same at 1.0 M NaCl and Na_2SO_4, respectively (Fig. 2). These experiments demonstrated not only an uptake system for glutamate in *H. halophilus*, but also that uptake of glutamate is chloride independent.

Glutamate restores growth of *H. halophilus* in the absence of chloride

Next, we asked whether glutamate could replace chloride in growing cells. To test this, complex media containing 1 M NaCl, Na_2SO_4, $NaNO_3$, Na-gluconate or Na-gluta-

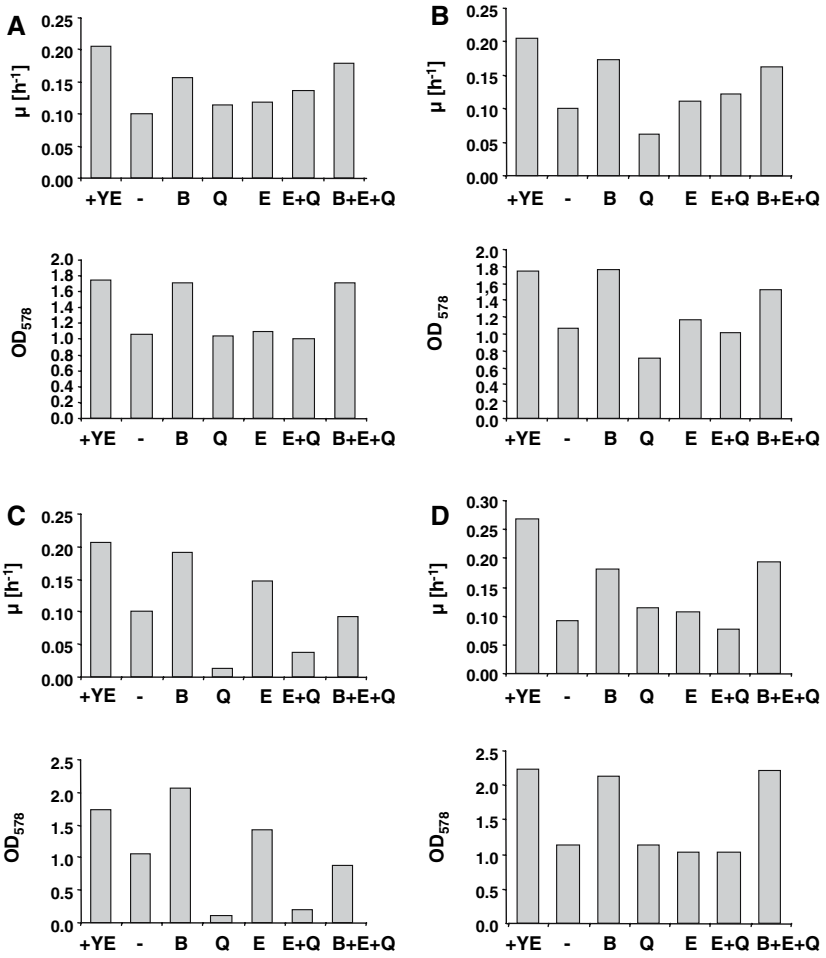

Fig. 1 Growth of *H. halophilus* in the absence or presence of different solutes. To test the influence of different solutes *H. halophilus* was cultivated in mineral medium containing 1 M NaCl (**a**–**c**) or 2 M NaCl (**d**) in the presence of yeast extract (*YE*) as a positive control, glycine betaine (*B*), glutamine (*Q*) or glutamate (*E*). To check for synergistic effects also mixtures of solutes like glutamine and glutamate (*E* + *Q*) or glycine betaine, glutamine and glutamate (*B* + *E* + *Q*) were tested. As a negative control cells were cultivated without any additive (–). The solutes have been supplied to a concentration of 1 mM (**a**, **d**), 10 mM (**b**) or 500 mM (**c**). For each condition the growth rate and final OD_{578} are given

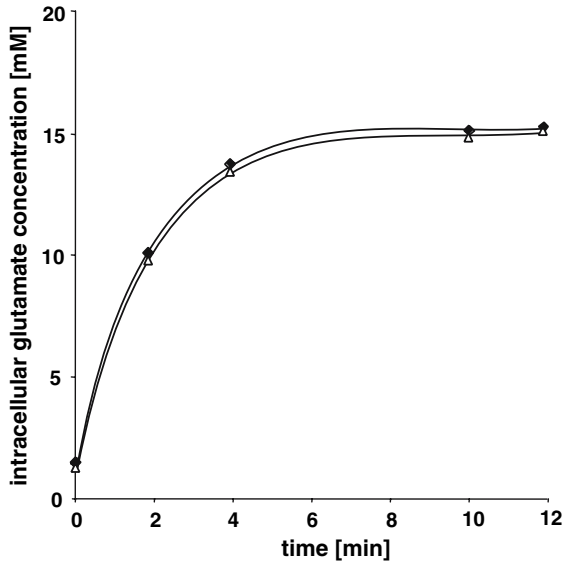

Fig. 2 Uptake of glutamate by *H. halophilus*. Hundred microliters of a *H. halophilus* cell suspension were sedimented and the reaction was started by resuspension in 1 ml Tris-buffer (pH 7.5) containing 1 M NaCl (*filled diamond*) or Na_2SO_4 (*open triangle*), 1 mM unlabelled and 0.5 μCi [^{14}C]-labelled glutamate. Fifty-microliter samples were taken and the intracellular glutamate concentration was determined

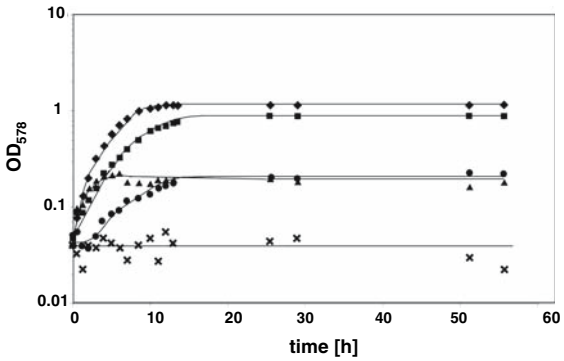

Fig. 3 Growth of *H. halophilus* in the presence of different sodium salts. *H. halophilus* was grown in complex medium in the presence of different sodium salts at a final concentration of 1 M. *Filled diamond* NaCl, *filled square* Na-glutamate, *filled triangle* $NaNO_3$, *filled circle* Na-gluconate, *multi symbol* Na_2SO_4

mate were inoculated with a culture pregrown in the presence of 1 M NaCl. Growth was completely impaired in the presence of Na_2SO_4 (Fig. 3). In the presence of $NaNO_3$ growth was largely impaired. The optical density increased by only a factor of 4, which is equivalent to only two

doublings; thereafter, the optical density stayed constant. However, after a prolonged incubation, cells adapted to nitrate (data not shown), as seen before (Roeßler and Müller 1998). The same, small increase in OD was observed in the presence of sodium gluconate, but the growth rate was decreased by about 45% compared to nitrate. In contrast, growth in the presence of 1 M sodium glutamate and 1 M sodium chloride was nearly identical, indicating that glutamate can replace chloride in growing cells.

To determine the glutamate concentration required for optimal growth, the sodium glutamate concentration was varied from 0 to 1.0 M while the Na^+ and anion concentration was kept constant at 1 M by appropriate addition of sodium gluconate. As can be seen from Fig. 4, growth was impaired in the absence of glutamate, at 0.2 M glutamate growth rates and final optical densities were about 56 and 48%, respectively, compared to the values at 1 M glutamate, but increasing glutamate concentrations led to increasing growth rates. Interestingly, the final cell yield was also strictly dependent on the glutamate concentration (Fig. 4).

The pool size of compatible solutes is dependent on the anion

The experiments described above revealed that (unphysiologically) high concentrations of glutamate restored growth of *H. halophilus* in the absence of chloride. A possible explanation may be that cells that are incubated in the absence of chloride but in the presence of high concentrations of glutamate, may accumulate glutamate as (main) compatible solute. To test this hypothesis, the cellular concentration of compatible solutes was analyzed. Therefore, cells were grown in minimal medium at 1 M NaCl, 1 M $NaNO_3$, 1 M Na-gluconate or 1 M Na-glutamate. Cells grown in the presence of NaCl accumulated glutamate, glutamine, and proline as main compatible solutes (Fig. 5). Alanine, *N*-acetyl lysine and citrulline were found in minor amounts, and ectoine was not found (data not shown). *N*-acetyl ornithine was not measured routinely in this study since a standard is not commercially

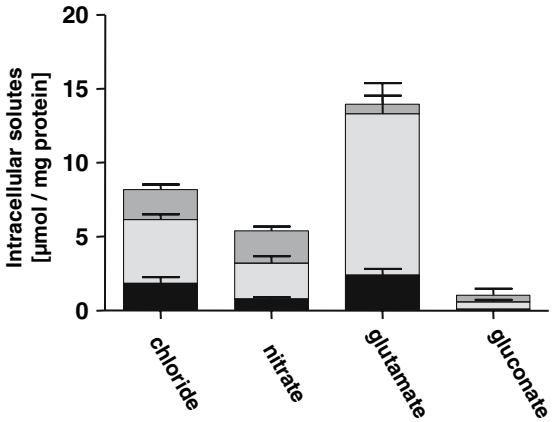

Fig. 5 Compatible solutes in *Halobacillus halophilus* grown in the presence of different anions. Cells were grown in glucose mineral salt medium containing 1 M of the compound indicated as sodium salt. Compatible solutes were extracted and analyzed by HPLC. The height of the bars reflect the total amount of solutes in the respective cell culture. *Black bar* glutamine, *light shaded bar* glutamate, *dark shaded bar* proline

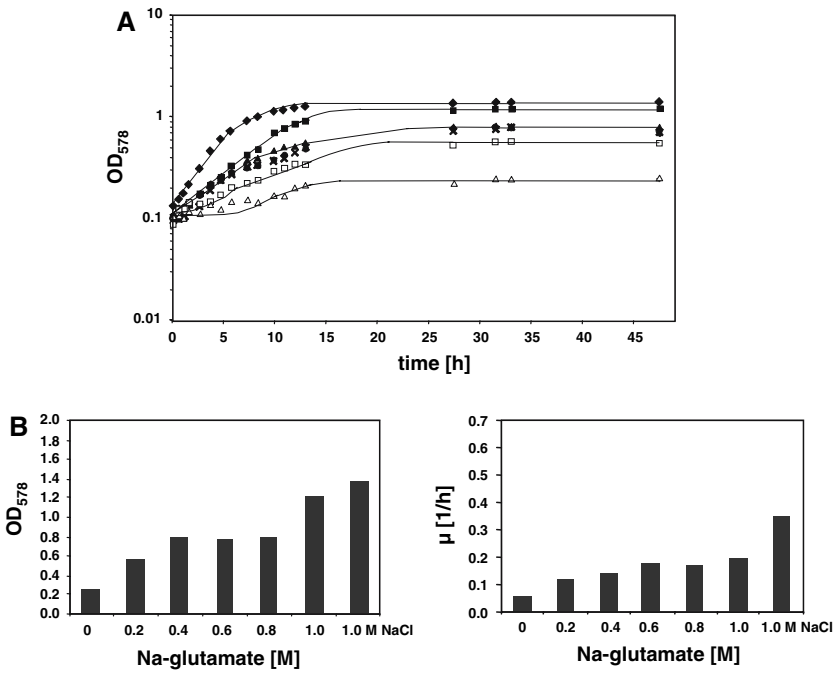

Fig. 4 Growth (**a**), final optical densities and growth rates (**b**) of *H. halophilus* in the presence of different glutamate concentrations. *H. halophilus* was grown in complex medium in the presence of different glutamate concentrations. The salinity was kept constant at 1 M by adding appropriate amounts of gluconate. *Filled diamond* 1 M NaCl, *filled square* 1 M Na-glutamate, *filled triangle* 0.8 M Na-glutamate, *multi symbol* 0.6 M Na-glutamate, *filled circle* 0.4 M Na-glutamate, *open square* 0.2 M Na-glutamate, *open triangle* 0 M Na-glutamate

available. Glutamate was the predominant compatible solute (47%), whereas proline and glutamine made only 26 and 18%, respectively. The amount of compatible solutes accumulated by nitrate-adapted cells grown in nitrate-containing media was only about 83%, but again glutamate (38%), proline (34%) and glutamine (12%) were the predominant solutes. However, it should be remembered that cells adapted to nitrate grow with rather high rates. In contrast, growth in the presence of Na-gluconate is largely impaired. Here, the total concentration of compatible solutes decreases to only 27% of the value of chloride-grown cells. In contrast, cells grown in the presence of 1 M glutamate increased their pool size to 203% compared to chloride, and especially the concentrations of glutamate and glutamine were increased to 299 and 168%, respectively. The reason for the two-fold increase in pool size of compatible solutes is unknown, but it apparently has no effect on growth rates. The proline concentration decreased to only 32% compared to cells grown in the presence of chloride. The concentration of all three major compatible solutes is drastically reduced in cells grown in the presence of gluconate but glutamate and glutamine concentrations were restored or even exceeded in cells grown in the presence of 1 M glutamate. Apparently, exogenous glutamate was taken up and used as compatible solute. The nature of this transport (primary or secondary) is not elucidated to date but in the case of an active import one would have to argue for a high specificity and the ability to accumulate a given solute in the medium up to a very high concentration within the cell (Poolman and Glaasker 1998; Krämer and Morbach 2004). The apparent absence of a salinity/osmo-dependent glutamate transport system reaching high accumulation factors under these conditions may be the reason for the high concentrations of glutamate required to fill the solute pool and restore growth.

Glutamate does not restore motility in the absence of chloride

The experiments described above are fully compatible with a function of the chloride regulon in the regulation of the poolsize of compatible solutes in *H. halophilus*. Growth inhibition caused by the absence of chloride is relieved by uptake of glutamate from the medium. To determine whether glutamate can restore other chloride-dependent processes in the absence of chloride, the effect of glutamate on motility was analyzed. Expression of the gene encoding the flagellum, the cellular pool of flagellin and thus motility were identified before as strictly Cl^- dependent processes (Roeßler and Müller 2002). When motile cells of *H. halophilus* were spotted onto swarm agar plates containing 1 M NaCl, the motility was visible by the diffuse zone of bacteria around the colonies (Fig. 6). It should be noted

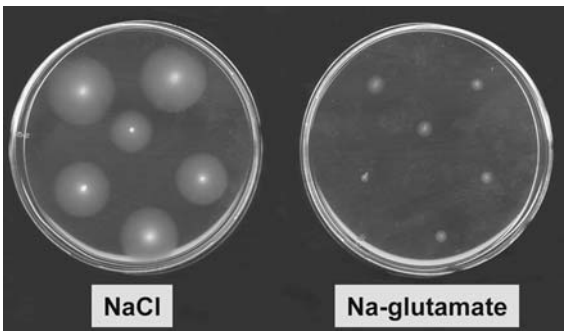

Fig. 6 Chloride-dependent motility of *H. halophilus*. *H. halophilus* cells were applied on swarm agar plates containing complex medium with 1 M NaCl or Na-glutamate, respectively. The plates were incubated at 30°C and high humidity for 40 h

that motility of *H. halophilus* is much slower than that of other bacteria, and, therefore, true chemotacting rings were not observed. In contrast, cells spotted onto swarm agar containing 1 M Na-glutamate did not swarm (Fig. 6). Correspondingly, flagella were not detected by electron microscopy (data not shown), and flagellin could not be detected immunologically in glutamate grown cells (Fig. 7). These experiments showed that glutamate could not restore flagellin production and thus motility in the absence of Cl^-. This makes it unlikely that chloride and glutamate act on the same ''salinity'' sensor in the chloride regulon. Moreover, motility seems not to be influenced by

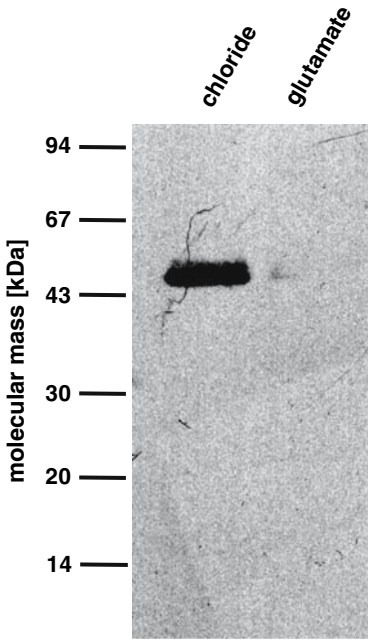

Fig. 7 Synthesis of flagellin can not be stimulated by glutamate. Cells have been cultivated in complex medium in the presence of 1 M NaCl or Na-glutamate. 25 µg of crude extract were separated on a SDS-gel. Analysis was done by Western-Blot analysis with anti-flagellin antiserum

the cell turgor per se since the cells had even higher solute concentrations when grown in the presence of Na-glutamate compared to NaCl but were immotile. Therefore, a glutamate-independent but chloride-dependent signal transduction chain leads to the synthesis of flagellin. Furthermore, the differential effect of glutamate on growth and motility underlines the conclusion that Na-glutamate bypasses the chloride regulon in *H. halophilus*.

Concluding remarks

For a moderate halophile, accumulation of compatible solutes is clearly essential to life (Sleator and Hill 2002; Grant 2004). *H. halophilus* uses a cocktail of different solutes for osmoadaptation and responds to changing salinities in its environment by adjusting the pool size of compatible solutes. Glutamate and glutamine are the major solutes accumulated by *H. halophilus* at an external salinity of 1 M NaCl. Biosynthesis and activity of the glutamine synthetase is chloride dependent (Saum et al. 2006). When grown in the absence of chloride, intracellular glutamate and glutamine levels were drastically reduced but growth can be restored by uptake of the solute glutamate from the medium. These data strengthen our conclusion that the chloride regulon is involved in salt perception and signal transduction leading to accumulation of compatible solutes. The bypass of the chloride regulon by Na-glutamate demonstrated here may enable a genetic screen to identify mutants defect in salt/chloride signaling.

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References

da Costa MS, Santos H, Galinski EA (1998) An overview of the role and diversity of compatible solutes in Bacteria and Archaea. Adv Biochem Eng Biotechnol 61:117–153

Dohrmann AB, Müller V (1999) Chloride dependence of endospore germination in *Halobacillus halophilus*. Arch Microbiol 172:264–267

Galinski EA, Trüper HG (1994) Microbial behaviour in salt-stressed ecosystems. FEMS Microbiol Rev 15:95–108

Grant WD (2004) Life at low water activity. Philos Trans R Soc Lond B Biol Sci 359:1249–1266

Krämer R, Morbach S (2004) BetP of *Corynebacterium glutamicum*, a transporter with three different functions: betaine transport, osmosensing, and osmoregulation. Biochim Biophys Acta 1658:31–36

Kunte HJ, Galinski EA, Trüper HG (1993) A modified FMOC-method for the detection of amino acid-type osmolytes and tetrahydropyrimidines (ectoines). J Microbiol Meth 17:129–136

Müller V, Saum SH (2005) The chloride regulon of *Halobacillus halophilus*: a novel regulatory network for salt perception and signal transduction in bacteria. In: Gunde-Cimerman N, Oren A, Plemenitas A (eds) Adaption to life at hight salt concentrations in Archaea, Bacteria, and Eukarya. Springer, Heidelberg, pp 303–310

Poolman B, Glaasker E (1998) Regulation of compatible solute accumulation in bacteria. Mol Microbiol 29:397–407

Roeßler M, Müller V (1998) Quantitative and physiological analyses of chloride dependence of growth of *Halobacillus halophilus*. Appl Environ Microbiol 64:3813–3817

Roeßler M, Müller V (2001) Chloride dependence of glycine betaine transport in *Halobacillus halophilus*. FEBS Lett 489:125–128

Roeßler M, Müller V (2002) Chloride, a new environmental signal molecule involved in gene regulation in a moderately halophilic bacterium, *Halobacillus halophilus*. J Bacteriol 184:6207–6215

Roeßler M, Pflüger K, Flach H, Lienard T, Gottschalk G, Müller V (2002) Identification of a salt-induced primary transporter for glycine betaine in the methanogen *Methanosarcina mazei* Gö1. Appl Environ Microbiol 68:2133–2139

Roeßler M, Wanner G, Müller V (2000) Motility and flagellum synthesis in *Halobacillus halophilus* are chloride dependent. J Bacteriol 182:532–535

Saum SH, Sydow JF, Palm P, Pfeiffer F, Oesterhelt D, Müller V (2006) Biochemical and molecular characterization of the biosynthesis of glutamine and glutamate, two major compatible solutes in the moderately halophilic bacterium *Halobacillus halophilus*. J Bacteriol 188:6808–6815

Schägger H, von Jagow G (1987) Tricine-sodium dodecylsulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. Anal Biochem 166:369–379

Schmidt K, Liaanen-Jensen S, Schlegel HG (1963) Die Carotinoide der *Thiorodaceae*. Arch Mikrobiol 46:117–126

Severin J (1993) Kompatible Solute und Wachstumskinetik bei halophilen aeroben heterotrophen Eubakterien. University of Bonn, Germany

Sewald X, Saum SH, Palm P, Pfeiffer F, Oesterhelt D, Müller V (2007) Autoinducer-2-producing protein LuxS, a novel salt- and chloride-induced protein in the moderately halophilic bacterium *Halobacillus halophilus*. Appl Environ Microbiol 73:371–379

Sleator RD, Hill C (2002) Bacterial osmoadaptation: the role of osmolytes in bacterial stress and virulence. FEMS Microbiol Rev 26:49–71

Ventosa A, Nieto JJ, Oren A (1998) Biology of moderately halophilic aerobic bacteria. Microbiol Mol Biol Rev 62:504–544