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Redox stress in geobacilli from geothermal springs: Phenomenon and membrane-associated response mechanisms

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ABSTRACT

Geobacillus toebii ArzA-8, from Armenian geothermal springs, grew well in nutrient broth. During its growth, changes in pH in opposite directions were observed depending on glucose supplementation. Accordingly, the decrease in the redox potential was determined using titanium-silicate (E_h) and platinum (E_h') electrodes: E_h decreased to -150 ± 3 mV and E_h' to -350 ± 2 mV without glucose; the decrease in these potentials was smaller with glucose. Redox stress due to an oxidizer, $K_3[Fe(CN)_6]$, or a reducer, DL-dithiothreitol (DTT), inhibited bacterial growth. However, a stimulatory effect of $K_3[Fe(CN)_6]$ or DTT was observed with or without glucose, respectively. With glucose, the H^+ efflux was sensitive to *N,N′*-dicyclohexylcarbodiimide (DCCD), an inhibitor of $FoF1F_OF_1$-ATPase and other H^+ translocation mechanisms, but the addition of an oxidizer or reducer suppressed the H^+ efflux. The ATPase activity of membrane vesicles was ~1.3-fold higher in cells grown with glucose compared with cells grown without glucose. DCCD and DTT suppressed ATPase activity in cells grown without glucose, whereas DTT stimulated F_OF_1-ATPase activity in cells grown with glucose. Thus, *G. toebii* senses redox stress; this thermophile likely presents specific membrane-associated response mechanisms involving F_OF_1-ATPase to overcome redox stress and survive; these mechanisms are important for adaptation to extreme environments.

1. Introduction

Bacteria exhibit oxidation–reduction properties which are important for their metabolism and adaptation to different environments, especially to high temperature [1–3]. The pathways and regulation of redox processes in bacteria may affect the environmental oxidation–reduction (redox) potential (ORP). The ORP has been determined for mesophilic *Escherichia coli*, *Enterococcus hirae* and other bacteria in liquid media during sugar (glucose) or glycerol fermentation [4–8]. Ultimately, a reduction of the ORP in parallel with a decrease of the pH is observed during bacterial growth. Hence, a relationship between the ORP and pH is suggested; however, it appears to be complex. The decreased ORP might be due to the secretion of redox active metabolites into the culture medium, consequently lowering the external pH. However, changes in redox proteins on the bacterial surface might result in disturbance of the ORP [9,10]. Moreover, there is likely a relationship between the reduction of ORP and the proton motive force ($\Delta\mu_{H+}$) of bacteria. In addition, a role of the H^+-translocating F_OF_1-ATPase (the key membrane-associated enzyme of bioenergetics relevance, responsible for maintaining $\Delta\mu_{H+}$ and for ATP synthesis under certain conditions) in redox sensing by bacteria under fermentation has been proposed [5,10,11]. Moreover, recent studies indicate that the ORP can even be applied to discriminate among bacterial species [8,12]. Interestingly, the effects of the ORP on bacteria do not depend on dissolved oxygen [10].

The ORP is suggested to be a factor determining bacterial growth that can be controlled by oxidizers and reducers or mediated by redox stress. Therefore, an oxidizer such as ferricyanide (at a low concentration) expresses positive ORP values, suppresses *E. coli* growth and decreases the acidification of the medium [5]. Alternatively, the environment restores conditions through adjustment with DL-dithiothreitol (DTT), inhibiting bacterial growth and leading to increased formation of formic acid by *E. coli* [13]. The effect of DTT is unusual. Therefore, further study is required. Using thermophilic *Clostridium thermosuccinogenes* it was demonstrated that this bacterium can ferment sugars (inulin) producing different end products: the yield of succinate was the highest at intermediate values of the ORP (−275 mV), while ethanol yield was the lowest [14]. The role of ORP in generating biomass and controlling enzymatic and transport activity in different thermophilic bacteria during the bioleaching process has also been demonstrated [15]. Therefore, the ORP could be considered for use as a tool for monitoring the growth and the changes in the metabolic state of aerobic bacterial cultures and for optimization of the yield of aerobic and anaerobic fermentation end products. However, the pathways and mechanisms involved in redox sensing by bacteria and the regulation of the bacterial metabolism are still not clear for many bacteria and are completely unknown for others, especially for thermophiles. Many metabolic redox reactions have been

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identified in different thermophiles, and energy changes have been calculated for some simple reactions, depending on the composition and temperature of the medium [1,2]. At the same time, little attention has been paid to bacterial energetics and ATPase reactions. Importantly, a different membrane composition and structure are suggested for thermophilic bacteria [1] those can modify the action of redox reagents and subsequent stress responses.

Geobacillus has been described as a new genus of obligate thermophiles with an optimal temperature of 55–65 °C and growth pH of 6.0–8.5 [16]. They carry out aerobic or facultative anaerobic metabolism using oxygen or, in some species, nitrate as the terminal electron acceptor [17]. Geobacilli have a potential worldwide distribution, as they are able to grow in various environments where redox processes are performed. However, nothing is known about redox processes and ATPase activity or redox stress in *Geobacillus*.

In the present work, the growth, pH changes and ORP kinetics of *Geobacillus toebii* as well as H^+ translocation in whole cells and ATPase activity of its membrane vesicles were first studied under environment redox stress. Bacterial growth was suppressed upon redox stress by both oxidizers and reducers. However, some redox agents stimulated bacterial growth at the beginning of the lag growth phase, overcoming the stress. Moreover, low H^+ extrusion and different F_OF_1-ATPase activities as well as their inhibition by *N,N′*-dicyclohexylcarbodiimide (DCCD) were observed under glucose availability; the changes in these membrane-associated properties induced by oxidizers and reducers were determined to be involved in overcoming redox stress.

2. Materials and methods

2.1. Bacterial strain and growth, pH

The *G. toebii* strain ArzA-8 isolated from the Arzakan geothermal mineral spring in Armenia (temperature > 44 °C, pH 7.0–7.2) [18] was used in this study.

Bacteria were grown under aerobic conditions at 55 °C with shaking with 250 rpm in nutrient broth (NB) containing 5 g/l peptone, 1.5 g/l beef extract, 1.5 g/l yeast extract and 5 g/l NaCl, either without glucose or with glucose at different concentrations (5, 11 and 22 mM) at pH 7.5 or pH 6.5. The pH of NB medium was adjusted with 0.1 M NaOH or 0.1 N HCl. The pH was measured using a pH-meter with a pH-electrode (HJ1131B, HANNA Instruments, Portugal). The change in the pH of the bacterial culture was monitored during bacterial growth. Growth was estimated from the increasing absorbance (A) of the bacterial culture, measured using a spectrophotometer (Scanning UV–VIS Auto, LaboMed Inc., USA) at a wavelength of 600 nm.

2.2. ORP assays

The growth medium ORP was measured using a pair of platinum (Pt) (EPB-1, Measuring Instruments Enterprise, Gomel, Belarus, or PT42BNC, HANNA Instruments, Portugal) and titanium-silicate (Ti–Si) (EO-02, Measuring Instruments Enterprise, Gomel, Belarus) glass redox electrodes, as described elsewhere [4–8,19]. In contrast to the Pt electrode (E_h'), which is sensitive to O_2 and H_2 in the medium, the Ti–Si electrode (E_h) measures the overall ORP and is not affected by the presence of O_2 and H_2.

Prior to the assays, E_h and E_h' readings were checked in a test solution composed of a mixture of 0.049 M potassium ferricyanide ($K_3[Fe(CN)_6]$) and 0.05 M potassium ferrocyanide ($K_4[Fe(CN)_6]\cdot 3H_2O$) (pH 6.86). The E_h and E_h' readings in the solution at 25 °C were $+245 \pm 10$ mV. It was determined that the ORP was not affected by cell numbers (not shown). According to the instructions of the manufacturer, the Pt and Ti–Si electrodes can be employed at high temperatures of up to 60 °C and 150 °C, respectively.

2.3. Determination of ion fluxes across the cell membrane

H^+ and K^+ fluxes across the cell membrane of whole cells were determined based on the changes in the external activities of ions using appropriate selective electrodes (Cole Parmer Instrument Co., USA) as described previously [4,6,20]. Bacteria were grown in the absence or presence of glucose, then harvested, washed with distilled water and added to the assay medium (50 mM Tris–phosphate buffer (pH 7.5), containing 0.4 mM $MgSO_4$, 1 mM NaCl and 1 mM KCl). This preparation made the cells suitable for measurements of ion fluxes.

The electrode readings were outputted automatically by the LABVIEW program (National Instruments Co., USA). They were calibrated through titration of the medium with small quantities of 0.01 N HCl or 0.1 M KCl. Ion fluxes were expressed in mMol/min per the number of cells in a unit of volume.

2.4. Preparation of membrane vesicles and ATPase assay

Membrane vesicles were isolated from cells treated with 0.5 mg/ml lysozyme and 20 mM ethylenediaminetetraacetic acid, as described by Konings and Kaback [21] and detailed previously [22]. The ATPase activity of vesicles was assayed based on the amount of liberated inorganic phosphate (P_i) in the reaction with ATP using a spectrophotometer, as described previously [19,22]. The reaction was initiated by adding 3 mM ATP (Tris salt) to vesicles in the assay mixture (50 mM Tris–HCl buffer (pH 7.5) containing 0.4 mM $MgSO_4$ and 1 mM KCl) and stopped with 10% trichloroacetic acid. The detected activity was expressed in nMol P_i per min and mg of protein.

2.5. Others, reagents and data processing

The formation of endospores in bacteria was detected using a microscope (HumanScope light, Germany). Cell numbers in the assays were determined based on the A, calibrated according to bacterial colony numbers grown on plates with NB and agar. Protein contents were measured by the Lowry method using bovine serum albumin as a standard [20]. For DCCD inhibition experiments, whole cells or vesicles were incubated with 0.5 mM DCCD for 10 min. DCCD is highly soluble in ethanol but insoluble in water [23]. Therefore, a DCCD solution in ethanol was used; ethanol at the applied concentrations had no effect on ATPase activity (not shown). All assays were conducted at 55 °C.

ATP (Tris salt), DCCD, DTT, NB, $K_3[Fe(CN)_6]$ and $K_4[Fe(CN)_6]\cdot 3H_2O$ were obtained from Sigma (USA). Peptone, beef and yeast extracts were purchased from Roth (Germany). Glucose was obtained from the Borisov Plant of Medicinal Preparations (Belarus). The other reagents used were of analytical grade.

Data were averaged from duplicate or, mostly, triplicate measurements, with the standard errors of the data being calculated [5,7,19,24], which did not exceed 3% (if not indicated). Student's t test was employed to validate the differences in the average data between various series of experiments, as described previously [5,7,19,24] ($p < 0.5$ if not indicated otherwise).

3. Results and discussion

3.1. G. toebii growth and ORP kinetics: effect of glucose

The maximal growth yield of *G. toebii* ArzA-8 (OD ~ 0.9) was observed in NB medium rich in organic substrates (see Materials and methods) without glucose supplementation (Fig. 1). Some differences were detected between added glucose concentrations of 5, 11 and 22 mM (see Fig. 1), possibly due to different glucose uptake rates in the bacteria, different amounts of end product secretion, or different changes in pH. Therefore, the changes in pH were studied. Simultaneously, alkalization of the growth medium was also observed in the absence of glucose: after 24 h of growth, the pH was 8.4 (not shown).

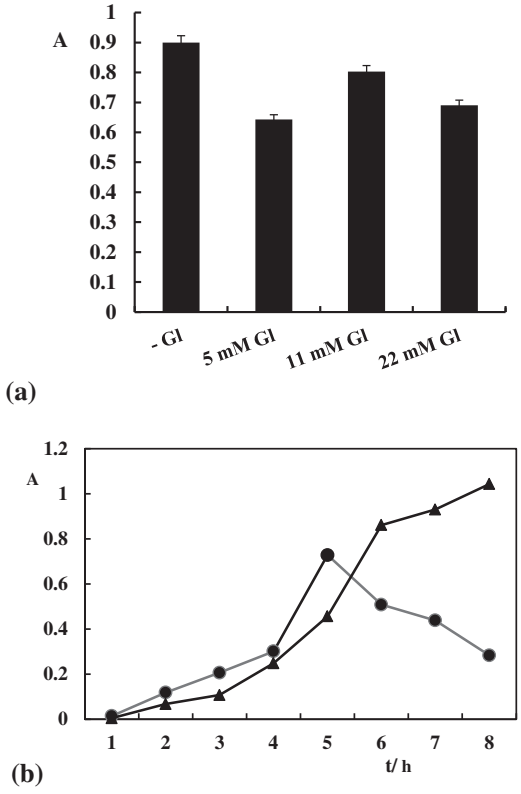

Fig. 1. The changes in absorbance (A) during *G. toebii* ArzA-8 growth. a) In the absence of glucose (−Gl) and presence of glucose (Gl) at different concentrations, pH 7.5. Glucose concentration supplemented was 5, 11 and 22 mM. A_{600} was presented after 5 h of bacterial growth. b) *G. toebii* ArzA-8 growth in the presence of 11 mM glucose (-▲-) and absence of its supplementation (-●-). The experiments were performed in triplicate, average data are presented and the standard errors are within the designations (b). For others, see the Materials and methods section.

However, an acidification effect was detected in the presence of 5, 11 or 22 mM glucose at the end of the log growth phase: the growth medium pH decreased to 5.9, 5.7 or 5.4, respectively (Fig. 2). These results probably reflect the major differences in metabolic end-products depending on the availability of the carbon source (glucose): the growth medium alkalization effect may be due to the secreted basic end products or the utilized amino acids present in NB, which cause the pH to increase. Moreover, glucose utilization can be followed by acid secretion, leading to acidification of the medium, as observed [17]. However, the pathways involved in this variation are not completely clear. At the beginning of

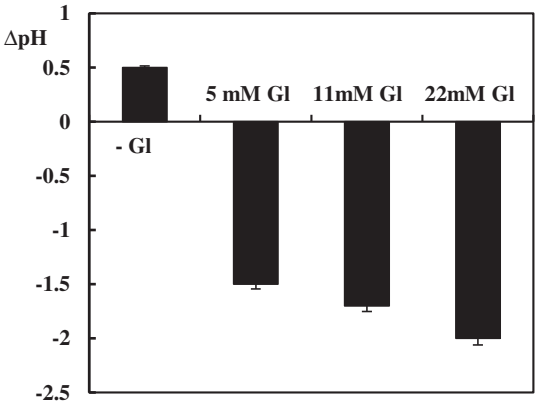

Fig. 2. The changes in the medium pH during *G. toebii* ArzA-8 growth in the absence and presence of glucose at different concentrations. ΔpH is a difference between initial pH of growth medium and pH after 8 h of growth. For the others, see legends to Fig. 1.

the stationary phase bacterial growth on glucose, a decrease in the A was detected (Fig. 1, b): endospore formations were observed through microscopy in the samples grown on glucose (not shown). This sporulation could be induced by dramatic acidification of the medium, as suggested [17]. All of these results were attributed to using a different approach involving geobacilli, including the evaluation of ORP kinetics by the bacteria during their growth in NB medium in the absence and presence of glucose and the effects of various oxidizers and reducers at different pHs.

A decrease in the ORP was observed during the bacterial log growth phase, as measured by the redox electrodes (see Materials and methods) at both pH 6.5 and pH 7.5. As was presented in Fig. 3, ORP kinetics had not been affected by initial pH of growth medium. The investigation of the ORP kinetics revealed remarkable differences: E_h decreased to a negative value of -150 ± 5 mV and E_h' to -350 ± 4 mV with an increase of pH without glucose but increased to positive values of $+111 \pm 3$ mV and $+80 \pm 3$ mV with a decrease in pH when glucose was added at pH 7.5 (see Fig. 3). It is worth mentioning that the supplementation of glucose at different concentrations (5, 11 or 22 mM) inhibited the decrease in the ORP but did not affect the characteristics in the kinetics of the ORP (not shown). The smaller decrease of the ORP observed when *G. toebii* was grown in the presence of glucose was significantly different from what was observed with other bacteria. A significant decrease of the ORP to low negative values has been reported for *E. coli*, *E. hirae* and other mesophilic bacteria [4–9]. The decrease of the ORP indicates strengthening of redox processes during bacterial growth. Interestingly it has been shown by different groups

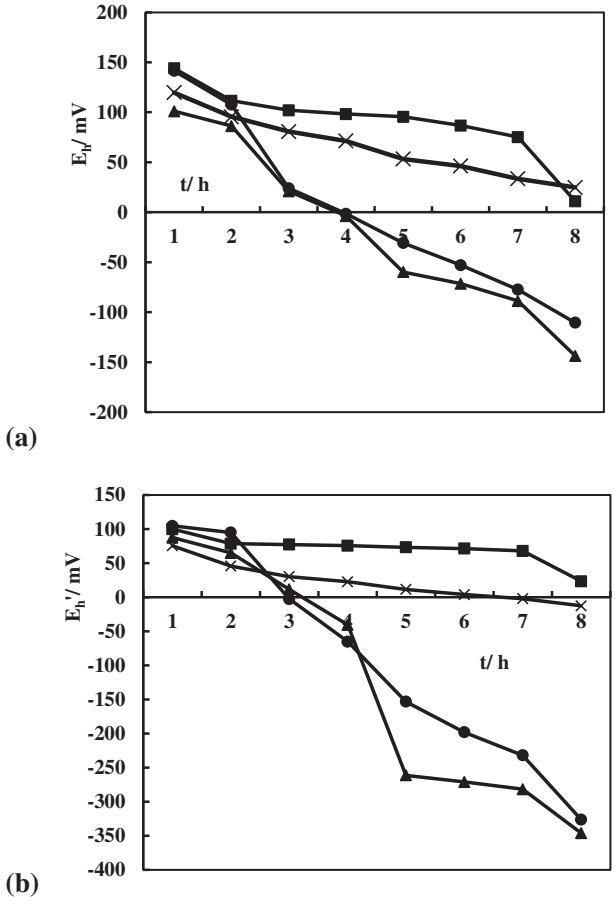

Fig. 3. The oxidation–reduction potential (ORP) kinetics during *G. toebii* ArzA-8 growth in the absence and presence of glucose (5 mM) at pH 6.5 and pH 7.5. The ORP was measured by a) E_h (Ti–Si electrode) and b) E_h' (Pt electrode). The symbols are -■- in the presence of glucose, pH 6.5; -●- in the absence of glucose, pH 6.5; -×- in the presence of glucose, pH 7.5; and -▲- in the absence of glucose, pH 7.5. For the others, see the legends to Fig. 1.

[4,5,8,10,25] that duplication of bacterial cells begins after a decrease in the ORP in various bacteria. Moreover, Oktyabrski and Smirnova [9] have proposed that the decrease in the ORP might determine a transition of the bacterial culture from logarithmic into stationary growth phase.

3.2. The effects of redox reagents on G. toebii growth and ORP kinetics

A determinant role of the ORP for bacterial growth means that various oxidizers and reducers affecting the ORP can mediate the growth of bacteria. The oxidizer $K_3[Fe(CN)_6]$ and the reducer DTT were used to obtain initial positive (~+250 mV) and negative (~−300 mV) ORP values in bacteria, respectively. These redox reagents were used because their effects have been studied in detail in *E. coli* and other bacteria; the effective concentrations were determined [5–7,9–11,25]. Moreover, these agents are of interest because they are applied in various biotechnology processes, to mediate bacterial growth and for the storage or preservation of foodstuffs [5,10]. Indeed, both reagents inhibited the growth of *G. toebii* (Figs. 4, 5): in medium without glucose supplementation, 1 or 2 mM $K_3[Fe(CN)_6]$ stimulated growth at the beginning of the log growth phase (not shown) but inhibited (~1.3-fold) subsequent bacterial growth (see Fig. 4), also resulting in a decrease of the ORP to -20 ± 5 mV. Interestingly it has been suggested that positive or less negative ORP values following the addition of $K_3[Fe(CN)_6]$ might oxidize thiol groups on the surface of *E. coli* cells and affect membrane transport proteins and enzymes leading to instability of metabolism [5]. However, in conditions without glucose, the inhibitory effect of DTT (3 mM) on *G. toebii* growth was stronger (~4.4-fold) (see Fig. 4).

For bacteria to carry out metabolic processes efficiently, it is important to maintain the intracellular environment in a reducing state [9,10]. However, in the medium with glucose supplementation, DTT also markedly repressed *G. toebii* bacterial growth, but initial stimulation (~1.3-fold) by DTT was observed (see Figs. 4, 5). These results were contrasted with the data obtained in the absence of glucose supplementation. After 8 h of growth, the ORP remained at negative values of -280 ± 3 mV in the medium with DTT in the presence of glucose supplementation (not shown). Interestingly, it was shown that supplementation of the reducer L-cysteine in the culture broth reduced the extended lag time of *Symbiobacterium toebii*, and it was assumed that the reducing reagent could reduce the presence of reactive oxygen species in the initial growth stage [26].

The difference in the effects of the oxidizer $K_3[Fe(CN)_6]$ and the reducer DTT on *G. toebii* may be due to the difference in their permeability through the bacterial plasma membrane, as $K_3[Fe(CN)_6]$ is generally considered as non-permeable in mesophile bacteria while DTT is a membrane-penetrating agent [5–7,9–11,25]. However, these peculiarities might be different for thermophilic bacteria, those require further study.

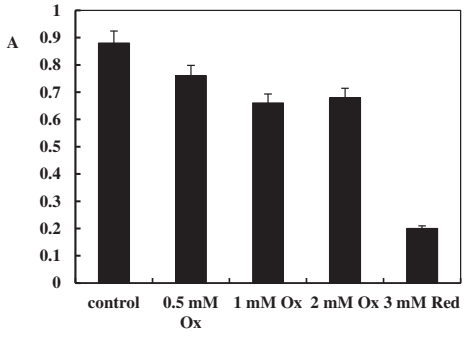

Fig. 4. The changes in absorbance (A) during *G. toebii* ArzA-8 growth upon oxidizer or reducer supplementation at different concentrations. The concentrations of the oxidizer $K_3[Fe(CN)_6]$ (Ox) were 0.5, 1 and 2 mM and of the reducer DTT (Red) was 3 mM. A_{600} was presented after 5 h of bacterial growth. Control was without oxidizer or reducer supplementation. For the others, see the legends to Fig. 1.

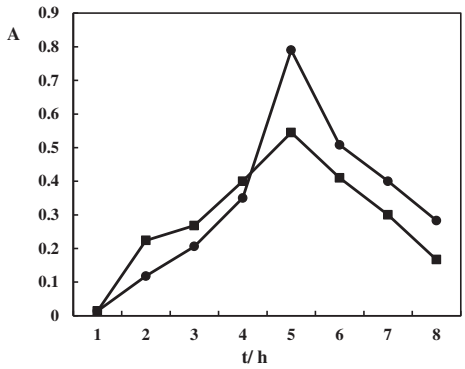

Fig. 5. *G. toebii* ArzA-8 growth kinetics in the medium in the presence of glucose and/or DTT at pH 7.5. 11 mM glucose (-●-) and/or 3 mM DTT (-■-) were supplemented. For the others, see the legends to Fig. 1.

3.3. Proton translocation in whole cells and the ATPase activity of G. toebii membrane vesicles

To understand the mechanisms underlying the changes in the ORP and bacterial responses to redox stresses, H^+ translocation and ATPase activity were studied in *G. toebii*. Indeed, a low H^+ efflux (0.32 mM/min·10^{10} cells) was observed upon the addition of 11 mM glucose (Table 1). The addition of DCCD, an inhibitor of F_OF_1-ATPase and other H^+ translocating mechanisms [18,19,21], at the concentration of 0.5 mM suppressed the H^+ efflux ~1.7-fold ($p < 0.01$, see Table 1). This effect of DCCD might be explained by affecting the H^+ translocation mechanism differently from FoF1-ATPase [10] because under aerobic conditions F_OF_1-ATPsynthase should function in the H^+ influx mode resulting in ATP synthesis. The addition of an oxidizer (1 mM $K_3[Fe(CN)_6]$) and reducer (3 mm DTT) almost completely inhibited H^+ extrusion (see Table 1). However, K^+ transport across the plasma membrane was not observed in the prepared cells (not shown); roles of different ions in the thermophile may be suggested.

The overall ATPase activity of *G. toebii* membrane vesicles was ~532 ± 0.05 nMol P_i/min·μg protein for cells grown in the absence of glucose, which was ~1.3-fold higher compared with cells grown on glucose (Fig. 6a). To determine FoF_1-ATPase activity, membrane vesicles were incubated with 0.5 mM DCCD for 10 min. DCCD markedly inhibited total ATPase activity (~3-fold, $p < 0.005$) (Fig. 6a). FoF_1-ATPase activity was then calculated based on the difference in total and DCCD-inhibited activities. The FoF_1-ATPase activity of membrane vesicles was again ~1.23-fold higher for cells grown in the absence of glucose compared with those grown on glucose (Fig. 6b). This result correlated well with the DCCD inhibition of F_OF_1-ATPase activity, as observed in many bacteria [19,20,22]. It is likely that different mechanisms, including F_OF_1-ATPase activity, can be inhibited by DCCD in *G. toebii*, though F_OF_1-ATPase is the most important mechanism in this context. In other thermophile bacteria an F-type ATPase is suggested [27].

DTT at the concentration of 3 mM repressed total ATPase activity by ~1.23-fold and the DCCD-inhibited activity by ~5-fold ($p < 0.001$) in cells grown in the absence of glucose (see Fig. 6a) compared with the control, without reagent supplementation. In contrast, DTT stimulated total ATPase activity negligibly (~1.1-fold) in cells grown on glucose compared with cells grown with DTT in the absence of glucose supplementation, and DCCD again inhibited total activity ~4-fold ($p < 0.001$) (see Fig. 6a). It is significant that DTT stimulated FoF_1-ATPase activity ~1.24-fold in the cells grown on glucose compared with the control, without reagent supplementation (Fig. 6b). Thus, the DTT stimulation effect could be attributed to F_OF_1-ATPase.

Unfortunately, ATPase activity could not be investigated under oxidizing conditions because $K_3[Fe(CN)_6]$ interfered with the enzyme assay.

Table 1
H^+ fluxes by *G. toebii* ArzA-8 grown in the absence and presence of glucose at pH 7.5. 11 mM glucose, 0.5 mM DCCD, 1 mM $K_3[Fe(CN)_6]$ or 3 mM DTT was added. Control was without inhibitor or reducer supplementation.

Conditions	H^+ efflux (mM/min·10^{10} cells)			
	Total	DCCD-inhibited[a]	$K_3[Fe(CN)_6]$	DTT
In the absence of glucose	0.01 ± 0.01	0.01 ± 0.01	ND[b]	ND
In the presence of glucose	0.32 ± 0.02	0.14 ± 0.02 ($p < 0.01$)[c]	0.02 ± 0.01 ($p < 0.001$)	0.01 ± 0.01 ($p < 0.001$)

[a] The difference between H^+ fluxes in the parallel assays without and with DCCD.
[b] ND, not determined because of residual total and DCCD-inhibited H^+ fluxes.
[c] For the difference between the value mentioned and the control one (see Materials and methods).

The increased ATPase activity observed during bacterial growth without glucose supplementation could be attributed to the medium pH: it is possible that FoF1-ATPsynthase operates optimally under an alkaline pH. In contrast, upon glucose utilization acidification of the medium was observed (see Fig. 2), which may affect F_OF_1-ATPase activity.

4. Concluding remarks

In the present study, novel data on the redox properties of *G. toebii* during growth at pH 6.5 and pH 7.5 were first described. Remarkable differences were observed in both ORP and pH kinetics in relation to the available carbon source, such as under glucose availability: during growth of the bacteria under glucose supplementation, with acidification of the medium, the ORP remains positive, while when glucose is not present with alkalization of the medium, low negative values are observed (see Figs. 3–5). These changes might have resulted from a relationship between pH and the ORP, likely indicating the existence of different metabolic redox pathways in this bacterium, compared with other bacteria, as suggested [1,2]. Moreover, during *G. toebii* growth, ORP kinetics doesn't depend on the initial pH variation in the medium. The optimal ORP for different bacteria is identified based on growth regulation upon redox stress. The fact that the addition of an oxidizer or reducer stimulated bacterial growth in the initial phase without and with the addition of glucose represents a complex phenomenon, which might be explained by an altered ORP [5,7,10] or other pathways, as suggested for *E. coli* or *E. hirae* [6,9,10], and further study will be required to address this issue.

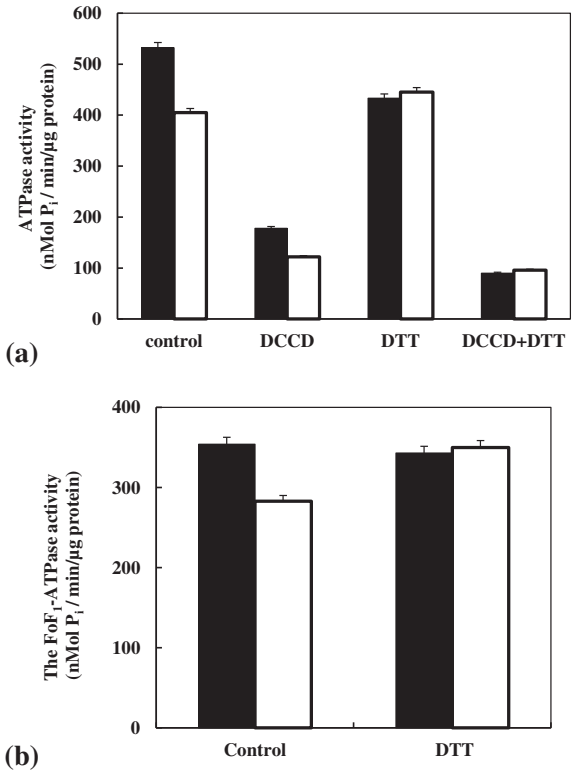

Fig. 6. Membrane vesicle ATPase activity of *G. toebii* ArzA-8. a) Total ATPase activity; b) the FoF_1-ATPase activity. Bacteria were grown in the absence (-■-) and in the presence (-□-) of glucose (11 mM) at pH 7.5. For initiation of ATPase reaction 3 mM ATP was added. Membrane vesicles were incubated with 0.5 mM DCCD or 3 mM DTT when indicated. FoF_1-ATPase activity was the DCCD-inhibited one, which was calculated as the difference between total ATPase activity and ATPase activity in the presence of DCCD. Control was without inhibitor or reducer supplementation. For the others, see the legends to Fig. 1.

The DCCD-sensitive H^+ efflux and F_OF_1-ATPase activity were also first investigated in geobacilli (see Table 1 and Fig. 6). ATPase activity was quite high in *G. toebii*. It is of interest that different levels of ATPase activity were observed with cells in relation to glucose availability: reducing conditions suppressed total ATPase activity, but not F_OF_1-ATPase activity without glucose supplementation. Reducing conditions induced by DTT stimulated F_OF_1-ATPase activity in bacteria upon glucose supplementation. These results indicate a role of F_OF_1-ATPsynthase in bacterial physiology and ecology, especially in redox sensing by this bacterium. It is possible that the observed effects of redox reagents during bacterial growth could be attributed to F_OF_1-ATPsynthase. This enzyme has not been well studied in thermophilic bacteria [27], and these results should stimulate further study. In general, the energetics of metabolic pathways of carbon sources and phosphates in thermophiles is problematic, because the energy changes observed for this class of compounds as a function of temperature currently prohibit quantitative evaluation of the energetics of many stepwise reactions in metabolic pathways [2]. It should be noted that these findings for the thermophile *G. toebii* are quite different from those obtained for *E. coli* [11,19].

Thus, the results of this study lead to new insights into the physiology and bioenergetics in *Geobacillus* and thermophilic bacteria regarding the basic characteristics of redox processes. Furthermore, they contribute to the understanding of some ecological aspects and adaptation mechanisms of microorganisms under redox stresses in extreme environments.

It is known that in nature, with the limited conditions for microbial growth, thermophiles with harboring unique enzymes exhibit different metabolic pathways and metabolize crude oil hydrocarbons. Thus, these organisms could be productively used in the treatment of hot wastewater as well as in the bioremediation and bio-augmentation of desert or tropical soils [3,15,28,29]. The ORP can clearly modify metabolic fluxes and might be a further environmental physicochemical parameter to be taken into account for the optimization of metabolic processes and the application of thermophiles in biotechnology to generate biomass and various valuable products.

Conflict of interest

The authors have no conflict of interest.

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