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ORIGINAL PAPER

NO_3^-/NO_2^- assimilation in halophilic archaea: physiological analysis, *nasA* and *nasD* expressions

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Abstract The haloarchaeon *Haloferax mediterranei* is able to assimilate nitrate or nitrite using the assimilatory nitrate pathway. An assimilatory nitrate reductase (Nas) and an assimilatory nitrite reductase (NiR) catalyze the first and second reactions, respectively. The genes involved in this process are transcribed as two messengers, one polycistronic (*nasABC*; *nasA* encodes Nas) and one monocistronic (*nasD;* codes for NiR). Here we report the *Hfx mediterranei* growth as well as the Nas and NiR activities in presence of high nitrate, nitrite and salt concentrations, using different approaches such as physiological experiments and enzymatic activities assays. The *nasA* and *nasD* expression profiles are also analysed by real-time quantitative PCR. The results presented reveal that the assimilatory nitrate/nitrite pathway in *Hfx mediterranei* takes place even if the salt concentration is higher than those usually present in the environments where this microorganism inhabits. This haloarchaeon grows in presence of 2 M nitrate or 50 mM nitrite, which are the highest nitrate and nitrite concentrations described from a prokaryotic microorganism. Therefore, it could be attractive for bioremediation applications in sewage plants where high salt, nitrate and nitrite concentrations are detected in wastewaters and brines.

Keywords Halophile · Archaeon · Assimilatory nitrate reductase · Assimilatory nitrite reductase · RT-PCR

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Abbreviations

NiR	Assimilatory nitrite reductase
Nas	Assimilatory nitrate reductase
O.D.	Optical density

Introduction

The nitrate assimilation is a main process of the N-cycle carried out by higher plants, algae, yeast and bacteria (Hageman and Redd 1980; Lin and Stewart 1998; Richardson and Watmough 1999). This process starts when nitrate is transported into the cells by an active transport system and then reduced to ammonium by the sequential action of assimilatory nitrate reductase (Nas) (Rubio et al. 1996; Moreno-Vivián et al. 1999) and assimilatory nitrite reductase (NiR) (Sengupta et al. 1996; Moreno-Vivián et al. 1999). Ammonium produced by NiR is incorporated into carbon skeletons by glutamate dehydrogenase or glutamine synthetase–glutamate synthase pathway (Flores et al. 1983; Kanamori et al. 1987).

Nitrate and nitrite have important agricultural, environmental and public health implications. The manufacturing of chemicals such as pesticides, herbicides, explosives and dyes usually generates effluents containing complex mixtures of salts and nitrate or nitrite (Moreno-Vivián et al. 1999; Schlesinger 2009). The capacity of microorganisms to degrade organic pollutants is severely limited by their ability to survive or proliferate in these salted wastewaters (Blasco et al. 2001). The increase in salinity and nitrate/nitrite concentrations in soils and ground waters in the last few decades has focused much attention on the physiological and molecular mechanisms

involved in salt-stress tolerance and nitrate metabolism by microorganisms (Moreno-Vivián et al. 1986). Microorganisms are in general sensitive to low nitrate and nitrite concentrations. The negative effect of these nitrogen compounds is mainly due to the extreme toxicity of nitrite and nitric oxide produced upon nitrate reduction. The last compound is produced through respiratory nitrate reduction (Carr and Ferguson 1990; Bonete et al. 2008).

Haloferax mediterranei is an *Halophilic archaeon* able to assimilate nitrate and nitrite, thanks to Nas and NiR, enzymes previously characterized in our research group (Martínez-Espinosa et al. 2001a, b). With the objective to analyse if *Hfx mediterranei* could be applied in wastewater or brines bioremediation techniques, we have analysed the assimilatory nitrate pathway in *Hfx mediterranei* cells grown in cultures containing high nitrate or nitrite concentrations. We prove that this strain is resistant to very high nitrate and nitrite concentrations, and the influence of nitrate/nitrite concentrations on growth and enzymatic activities is also discussed.

Materials and methods

Growth conditions

Haloferax mediterranei (ATCC 33500) cultures were grown in a 25% (w/v) mixture of inorganic salts (25% SW) as described before (Martínez-Espinosa et al. 2001a, b). This minimal mineral medium was supplemented with 0.005 g/l $FeCl_3$; 0.5 g/l KH_2PO_4; 5 g/l glucose and 0.5 mM–2 M KNO_3 or 0.5–50 mM KNO_2. In all cases, high pure nitrate and nitrite salts were used to avoid the effect of trace amounts of nitrate in the nitrite preparations or vice versa.

The pH value of the culture media was adjusted to 7.3 using KOH or HCl. The media were inoculated with a seed culture grown for 5 days. 0.5 l batch cultures of *Hfx mediterranei* were grown aerobically at 37°C in 1 l Erlenmeyer flasks on a rotary shaker at 200 rpm. Cultures with KNO_2 concentrations higher than 10 mM were also grown in a 2-l flask in a Biostat® B fermenter (B. Braun Biotech International, Melsungen, Germany) with continuous pH control system. Growth was monitored by measuring the optical density (O.D.) at 600 nm. All the cultures cited above were carried out in quadruplicate, and the data points plotted are the average of the results obtained from them.

Cell-free extracts

Aliquots (12 ml) of cell suspensions were harvested at different times during the cultures growth and washed by centrifugation at 30000×*g* for 20 min at 4°C in a Sigma 2K15 centrifuge. Then, the pellet was washed with 25% SW followed by centrifugation at the conditions cited above. The freshly harvested cells were resuspended in a 50 mM phosphate buffer, pH 7.3 containing 2.5 M $(NH_4)_2SO_4$. The cells were disrupted by sonication at 150 W for eight periods of 3 min each at 4°C. The suspension was centrifuged at 30000×*g* for 20 min.

Determination of nitrate and nitrite

The nitrate disappearance of the medium was estimated by UV method after a 50-fold dilution of 1 ml of the medium (Martínez-Espinosa 2004). The nitrite concentration within the media was determined after a 40-fold dilution of 25 µl of the medium using the diazo-coupling method (Snell and Snell 1949). Nitrate and nitrite concentrations were measured in crude extracts or in culture media at the times shown in the figures. Data presented in "Results" correspond to the average of four separate experiments yielding essentially the same results.

Nitrate reductase and nitrite reductase assays

Nas and NiR activities were measured by colorimetric determination of nitrite produced or consumed as previously described by using the diazo-coupling method (Martínez-Espinosa et al. 2001a, b). All the activity assays were carried out in triplicate and against a control assay without enzyme. The enzymatic activity units are described as nmol nitrite produced per min or nmol nitrite consumed per min for Nas and NiR activities, respectively. The concentration of the protein was quantified by means of Bradford method.

RNA isolation and cDNA synthesis

Total RNA was extracted using the High Pure RNA Isolation Kit (Roche) from *Hfx mediterranei* cells grown in ammonium (control) and nitrate media. Cells were harvested at different O.D. The reaction mixture for reverse transcription contained: 60 U of MultiScribe Reverse Transcriptase with the supplied 10xRT Buffer, 8 U of RNase ribonuclease inhibitor, 2.5 µM of random hexamers, 2 mM dNTPs, 5.5 mM $MgCl_2$ and 0.5 µg of a RNA template.

To estimate the genomic DNA contamination in isolated RNA, cDNA synthesis was performed in duplicate omitting RTase from the reaction mixture. The reaction conditions were as follows: incubation for 10 min at 25°C, followed by 50 min at 37°C, enzyme inactivation for 5 min at 95°C and rapid cooling to 4°C. cDNA samples were stored at −20°C before PCR analysis.

Table 1 Primers and TaqMan probes

Gene	Forward primer (5′ → 3′)	TaqMan probe (5′ → 3′)	Reverse primer (5′ → 3′)	Position (PCR product size)
Ala-t-RNA	GGC TAACAC GGG CCC ATA G	**FAM** CTC AGT GGT AGA GTG CCT C **TAMRA**	TGG GCA TCC TCC TTG CAA AG	108–166 (58 pb)
nas	ACC GCG ACG ACG CT	**FAM** TCC CGC CAG GAC TC **TAMRA**	CAT TGG GTG GTG AAT CGA AAG C	1935–1991 (56 pb)
nir	ACG AAG AGC GTG CTT ACG A	**FAM** TTC GCG GAT TCG TC **TAMRA**	CCC GTG GTC GTG GTA GAG	731–793 (62 pb)

FAM reporter dye, *TAMRA* quencher dye

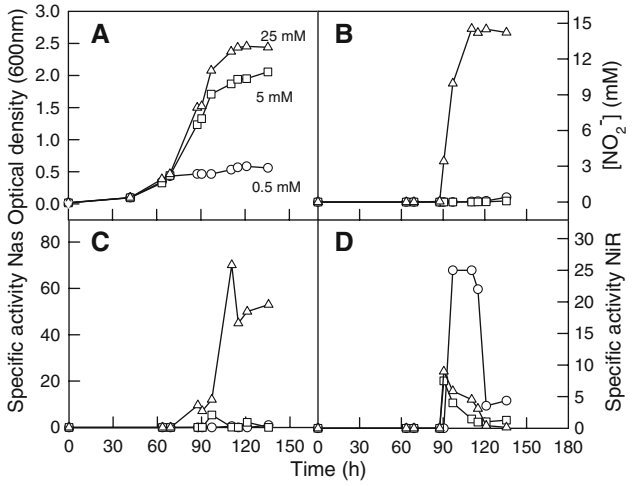

Fig. 1 Analysis of *Hfx mediterranei* growth, Nas and NiR activities in minimal culture media with nitrate as sole nitrogen source. **a** O.D. (600 nm); **b** nitrite concentration within the media; **c** Nas activity (mU/mg prot.); **d** NiR activity (mU/mg prot.). (*circle*) 0.5 mM, (*square*) 5 mM, (*triangle*) 25 mM nitrate media

TaqMan quantitative PCR

The cDNA quantification was performed on an ABI PRISM 7700 Sequence Detection System (PE Applied Biosystems, Foster City, CA, USA). TaqMan probes and primers used in this study (Table 1) were designed using the Primer Express 1.5 Software (PE Applied Biosystems) according to assimilative nitrate reductase (*nasA*, AJ621498), assimilative nitrite reductase (*nasD*, AJ621501) and Ala-tRNA (AF093412) gene sequences from *Hfx mediterranei*. The probes were labelled with a reporter dye (FAM) at their 5′ end and a Quencher dye (TAMRA) at its 3′ end. Quantitative PCR was performed with 1 µl of cDNA template, 10 µl of 2× TaqMan PCR Master Mix (PE Applied Biosystems), 900 nM final concentration of each primer and 250 nM final concentration of each TaqMan probe. Primers and probes were synthesized by PE Applied Biosystems.

Thermal cycling conditions for real-time PCR runs were as follows: 2 min at 50°C, 10 min at 95°C, 40 cycles of 15 s at 95°C, and 1 min at 60°C. The data collection was performed during each annealing step. Two negative controls' reaction without template and two genomic DNA contamination controls were included in each real-time PCR run. For each time point of the growth curve, three replicates were analysed. Data acquisition was performed with the SDS 1.7 software (PE Applied Biosystems). The comparative $2^{-}\Delta\Delta C_T$ method (Livak and Schmittgen 2001) was used to quantify the *nas* and *nir* mRNA content relative to the Ala-t-RNA content. The Ala-t-RNA is the 16S/23S spacer intervening sequence, which is a common feature in *Euryarchaeota* (Briones and Amils 2000). Calibrator was the sample grown in ammonium medium since in presence of ammonium assimilatory nitrate pathway is kept out (Martinez-Espinosa et al. 2007). The mRNA quantities in all other samples are expressed as an *n*-fold difference relative to the calibrator.

Results

Nitrate assimilation by *Hfx mediterranei*

Hfx mediterranei growth, enzymatic activities (Nas and NiR) as well as the expression profile of *nasA* and *nasD* genes were analysed from minimal culture medium under aerobic conditions supplemented with 0.5 mM–2 M KNO_3.

In those cultures with initial nitrate concentrations <5 mM (nitrogen starvation), nitrate was completely consumed during the first 60 h and consequently, the final O.D. of these cultures did not reach the optimal O.D. at stationary phase of growth (O.D. = 2.4), nevertheless all cultures with initial nitrate concentrations >20 mM showed O.D. around 2.4 at stationary phase (Fig. 1a). All growth curves obtained from cultures with high nitrate concentrations (20 mM–2 M) showed the same pattern that those observed from 25 mM nitrate cultures.

Concerning the specific Nas and NiR activities' measurements during the cells growth in 0.5–25 mM nitrate media (Fig. 1c, d), it can be observed that in the presence of nitrate concentrations <5 mM, Nas activity was detected

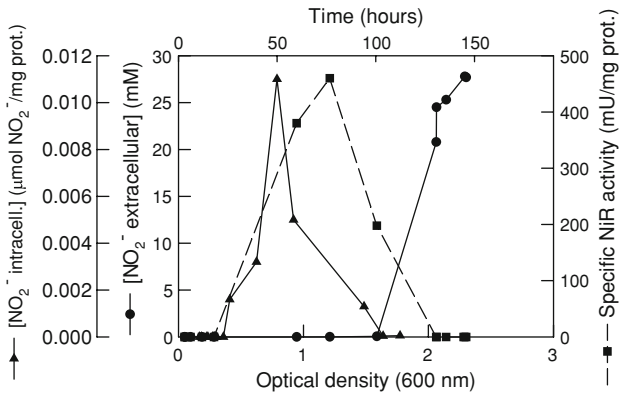

Fig. 2 Relationship between NiR activity and intracellular nitrite concentration in *Hfx mediterranei* cells grown in presence of 100 mM nitrate. (*filled circle*) Nitrite concentration within the medium (mM), (*filled triangle*) intracellular nitrite concentration (µmol NO_2^-/mg prot.) and (*filled square*) specific NiR activity (mU/mg prot.)

concurrently or even slightly after NiR activity. However, when nitrate concentrations were higher than 5 mM, Nas activity was detected at O.D. values between 0.4 and 2.5, whereas NiR activity appeared at mid-exponential phase of growth (O.D. = 1.2).

Nas activity in crude extracts from cells grown in low nitrate concentration media (5 mM) was smaller than those activity values obtained from cells grown in presence of nitrate concentrations >5 mM. NiR activity showed the same pattern described for Nas; however, in low nitrate concentration media (0.5 mM) specific NiR activity was higher than in media with 5–25 mM nitrate. From these results it is possible to think that this fact could be a physiological adaptation to facilitate the utilization of nitrogen in minimal medium with nitrate/nitrite depletion. Nevertheless, specific NiR activity becomes much higher in 100 mM nitrate cultures (500 mU/mg) (Fig. 2)

When nitrate concentration was higher than 20 mM, nitrite was quantified at high concentrations (10–30 mM) within the media at stationary phase of growth. This pattern has also been previously described from different microorganisms (Martínez-Luque et al. 1991) and it has been analysed in detail in 100 mM nitrate culture media, conditions previously used to purify Nas and NiR (Martínez-Espinosa et al. 2001a, b). As it is shown in Fig. 2, when the O.D. is around 0.4 (which corresponds to the beginning of the exponential phase of growth) we detected nitrite accumulation within the cells. The intracellular nitrite concentration reached the higher value when the O.D. of the cultures was around 0.78–0.81 and shortly after that, high NiR activity levels can be detected.

The behaviour of the Nas and NiR activities described above by physiological approaches was in agreement with the results obtained from real-time quantitative PCR experiments. In first place, real-time PCR validation was

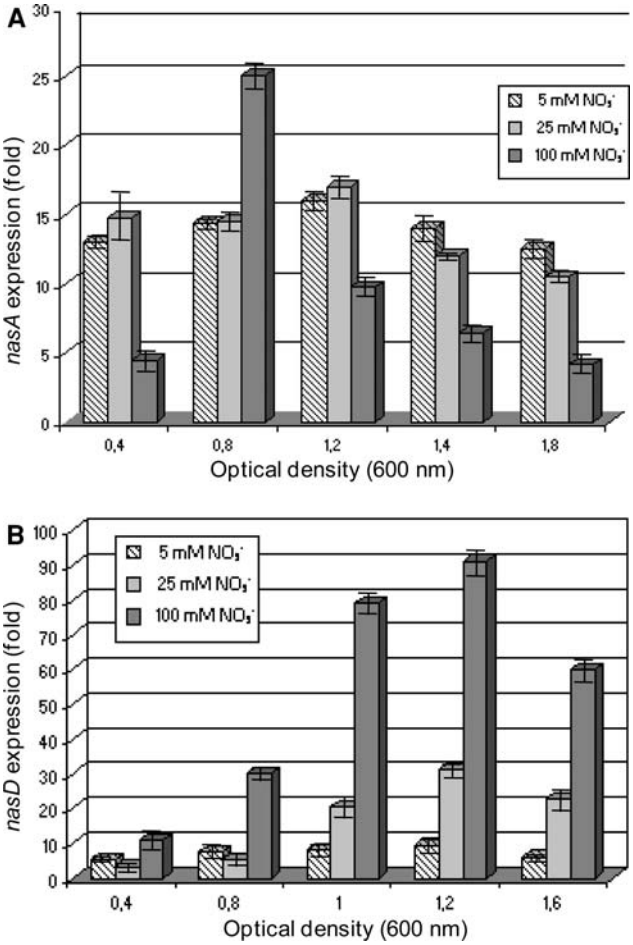

Fig. 3 *nasA* and *nasD* mRNA expression levels under different growth conditions during different O.D. Comparisons between results obtained from cultures grown in 5 mM NO_3^- (*hatched bar*), 25 mM NO_3^- (*light shaded bar*) and 100 mM NO_3^- (*dark shaded bar*) as sole nitrogen source. Data plotted are the average of the results obtained from quadruplicate experiments. Bars represent the standard deviations

carried out as previously described (Ginzinger 2002). A dilution series over four orders of magnitude of cDNA amount was used for real-time PCR and plotted the C_T as a function of $\log_{10}$ concentration of template; the slope of the resulting trend line was a function of the PCR efficiency. The amplification efficiencies for *nas*, *nir* and Ala-t-RNA were 97, 99 and 99%, respectively, suggesting that $\Delta\Delta C_T$ method could be used for relative quantification.

As can be seen in Fig. 3, the *nasA* (Fig. 3a) and *nasD* (Fig. 3b) mRNA contents steadily increased during exponential phase and then decreased at the beginning of the stationary phase (O.D. around 1.8). The maximum *nasA* (codes for Nas) and *nasD* (encodes NiR) mRNA expressions were detected before the maximum enzyme activity in all conditions. We observed that *nasA* mRNA expression depends on NO_3^- concentration. When NO_3^- concentration in cultures was 100 mM, we detected the maximum

expression level, 24-fold. In contrast, no significant differences were detected between 5 and 25 mM NO_3^- cultures. Moreover, we could observe that *nasA* mRNA expression decreased faster after the maximum expression in those media containing 100 mM NO_3^-. This fact suggests that the product of nitrate assimilation repress the expression of *nasA* mRNA. The *nasD* mRNA levels monitored from culture media with 5, 25 and 100 mM NO_3^- show a clear induction (Fig. 3b). In all experiments, the maxima *nasD* expressions were detected after the maximum Nas activity (coded for *nasA*), suggesting that NO_2^- could act as an inductor of the gene expression.

Finally, it has been observed that there is a relationship between the initial nitrate concentrations of the unbuffered cultures and pH changes. When initial nitrate concentration in the medium was >25 mM, initial pH value (7.3) increased up to 9, which did not caused growth limitation. To eliminate the effect of pH modification on growth, pH was continuously controlled. Thus, we observed that *Hfx mediterranei* is able to grow in minimal medium in presence of high concentrations of nitrate (up to 2 M).

Nitrite assimilation by *Hfx mediterranei*

To analyse if *Hfx mediterranei* is able to use nitrite as sole nitrogen source for growth, two different assays were carried out. On one hand, 0.5–10 mM KNO_2 minimal media without continuous pH control were studied. On the other hand, 0.5–50 mM KNO_2 with continuous pH control were prepared as explained in "Material and methods". Figure 4 illustrates *Hfx mediterranei* growth, nitrite consumption and NiR activity obtained from media without constant pH control. Gene expression assays by means of real-time quantitative PCR were also carried out to quantify the expression of *nasD* (Fig. 5).

Under all conditions assayed, *nasA* mRNA was observed in minimal medium supplemented with nitrite, indicating that although the *nasA* expression is higher in nitrate medium (nitrate has positive effect on *nasA*), basal *nasA* expression take place in presence of nitrite as sole nitrogen source. The specific activity of this basal Nas activity was around 0.1 mU/mg protein. The highest NiR activity levels were detected in minimal medium with 2 mM nitrite (Fig. 4c). As shown in Fig. 5, *nasD* mRNA expression depends on NO_2^- concentration and on the O.D. of the culture. The maximum *nasD* expression was 75-fold in medium with 2 mM NO_2^-. No significant differences were detected in media with 0.5 or 1 mM, probably because of nitrogen depletion.

In minimal medium with nitrite concentrations <2 mM, all nitrite were consumed by cells and the stationary phase was reached at a relatively low O.D. (cell growth was limited by low nitrite concentration) (Fig. 4a, b). However,

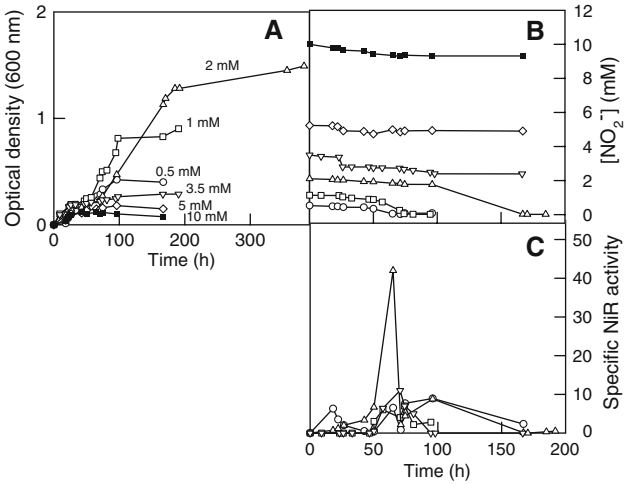

Fig. 4 Analysis of *Hfx mediterranei* growth, Nas and NiR activities, in minimal culture media with low nitrite concentrations as sole nitrogen source. **a** O.D. (600 nm); **b** nitrite concentration within the medium; **c** NiR activity (mU/mg prot.). (*circle*) 0.5 mM, (*square*) 1 mM, (*triangle*) 2 mM, (*inverted triangle*) 3.5 mM, (*diamond*) 5 mM, (*filled square*) 10 mM nitrite media

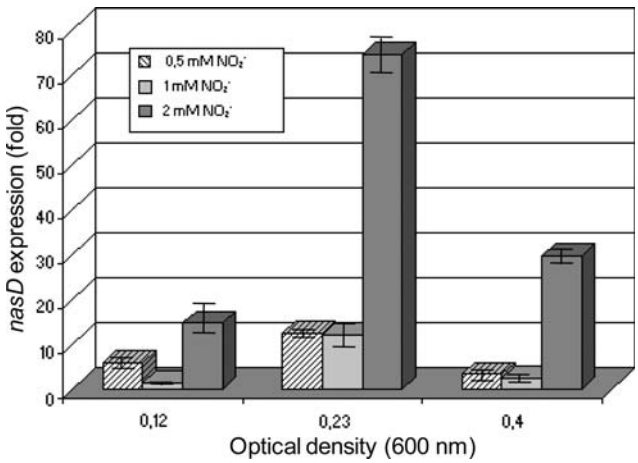

Fig. 5 *nasD* mRNA expression level under different growth conditions during different O.D. Comparisons between results obtained from cultures grown in 0.5 mM NO_2^- (*hatched bar*), 25 mM NO_3^- (*light shaded bar*) and 100 mM NO_3^- (*dark shaded bar*) as sole nitrogen source. Data plotted are the average of the results obtained from quadruplicate experiments. Bars represent the standard deviations

cell growth was not improved by increasing nitrite concentration. Besides, in all the media with high nitrite concentrations, the pH value decreased from 7.3 up to 4 after 50 h of incubation. In presence of 10 mM nitrite, for example, the O.D. reached by the culture was lower than that observed in 2 mM nitrite cultures. These results seemed to point towards: (i) nitrite toxicity or (ii) negative effect of the culture acidification on the cells growth.

To determine if *Hfx mediterranei* is able to tolerate higher nitrite concentrations under pH control, cells were grown in

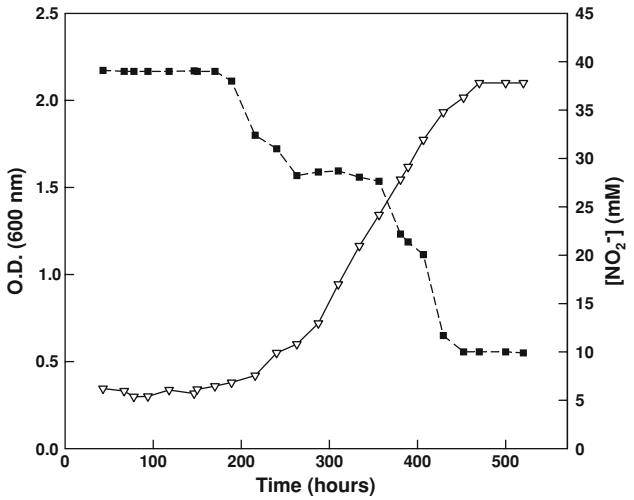

Fig. 6 *Hfx mediterranei* growth in minimal culture media with 40 mM nitrite as sole nitrogen source. Cultures were grown in a Biostat® B fermentor with continuos control of pH. (*inverted triangle*) O.D. (600 nm), (*filled square*) nitrite concentration in the culture media

nitrite cultures loaded in the Biostat® B fermenter with continuous pH control system. In this kind of experiments, *Hfx mediterranei* grew optimally in presence of 50 mM nitrite. The growth of *Hfx mediterranei* under these conditions makes possible the removal of most of nitrite present within the culture media; in 40 mM nitrite minimal media, for example, only 25% of the nitrite added to the culture was still present at the stationary phase of growth (Fig. 6).

Discussion

The *Hfx mediterranei* growth, as well as assimilatory nitrate and nitrite reductases activities were studied by adding 0.5 mM–2 M KNO_3 or 0.5–50 mM NO_2^- to minimal culture media under aerobic conditions. In parallel, real-time quantitative PCR experiments were carried out to quantify the expression of two of the genes involved in nitrate and nitrite assimilations: *nasA*, which encodes the Nas (Lledó et al. 2005) and *nasD* (codes for NiR) (Lledó et al. 2005).

Using physiological, biochemical and molecular biology approaches, we have observed that during the cell growth in culture media with nitrate concentrations >5 mM, the induction of *nasA* takes place first followed by the induction of *nasD*. This profile correlates with the enzymatic activity profile. The intracellular nitrite concentrations increased shortly before we detected the highest NiR activities, which suggest that the nitrite accumulation within the cells could be acting as a signal for the nitrite reductase activity increase.

Nitrate has a positive effect on *nasA* induction as well as nitrite has positive effect on *nasD* induction, but basal Nas activity is also detected in those media with nitrite as sole nitrogen source. This is not an expected result, because Nas activity would not be detected if nitrate is the only signal for assimilatory nitrate reduction pathway as it has been previously described (Chai and Stewart 1998). So, this result suggests that in *Hfx mediterranei* there are other signals promoting the induction of the assimilatory nitrate reduction pathway. In this way studies on regulation of the genes involved in carbon and nitrogen assimilation should be addressed as it has been reported from cyanobacterium such us *Synechococcus* (Suzuki et al. 1996).

Several pH changes were observed in all the cultures during the experiments development. The highest nitrate concentrations, the highest pH values were detected in the culture media at stationary phase of growth. The pH increase in nitrate media is an expected result since an acid is consumed by the cells and converted to an amine. Besides, it is also possible a pH increase because of the nitrate transport systems. This effect has also been previously analysed in cyanobacteria, where pH changes in media with NO_3^- were caused by uptake of this anion associating with proton pumps (Flores et al. 1983). In all unbuffered nitrite cultures, a dramatic drop of the initial pH value was observed (from 7.3 up to 4) after 50 h of incubation. This pH drop was more pronounced if higher NO_2^- concentrations were used. It has been described that in unbuffered medium supplemented with carbohydrates, the growth of some halophilic archaea is accompanied by a decrease in pH (Oren and Gurevich 1994). The decrease in the pH in culture media containing different carbon sources has been associated so far with the dissimilatory modified Entner–Doudoroff pathway, and the excretion of acetate and pyruvate (Tomlinson and Hochstein 1972). The greatest decrease in pH occurred in presence of glucose, galactose or lactose; although the final pH in these media depended both on the substrate and on the archaea strain (Tomlinson and Hochstein 1972). By HPLC analysis, we have demonstrated that organic acids excretion is not enough to generate pH changes from 7.3 to 4 (results not shown). So, we propose that the organic acid production plus nitrite transport mechanisms (proton pumps) could be associated with the drop of pH observed. Future study on nitrate/nitrite transporters will shed light on nitrite/proton pumps in *Hfx mediterranei*.

Nitrite is toxic, at concentrations <5 mM, for most of the microbiological systems analysed (Shen et al. 2003; Alonso and Camargo 2006), and it is also toxic for aquatic animals and even for humans. Related to this, it has been reported that nitrite strongly inhibits bacterial metabolism by inactivating Fe–S proteins (Moreno-Vivián et al. 1986) and some aerobic enzyme such as cytochrome oxidase, aconitase and fumarase (Martínez-Luque et al. 1991). However, on the basis of the results presented in Fig. 6, the toxic

effect of low nitrite concentrations on *Hfx mediterranei* cells did not turn out to be the cause of the growth changes. The highest nitrite concentration used in this study (50 mM) is one of the highest concentrations described for a prokaryotic microorganism with the exception of *Rhodococcus* sp. RB1, which is able to grow in presence of 60 mM nitrite (Blasco et al. 2001). The *Hfx mediterranei* growth under these conditions, which are toxic for almost all of the microorganisms studied up to now, makes possible the nitrite removal from the culture media. Even without pH control, *Hfx mediterranei* is able to grow in presence of nitrite concentrations up to 3 mM, which is higher than the nitrite concentration tolerated by the majority of microorganisms described up to now. The nitrate and nitrite concentrations usually present in wastewaters are smaller than the values mentioned throughout this study, so it is possible to think that this haloarchaeon could be an excellent model for salted wastewater bioremediation techniques. Although, several anthropogenic activities are contributing to the water pollution, for now, it is unlikely to find wastewaters containing nitrate or nitrite concentrations around 2 M or 50 mM, respectively (Schlesinger 2009). Nevertheless, we have explored the tolerance limits for nitrate and nitrite using a *Halophilic archaeon* as a model, to point out their possible role in extreme bioremediation processes, in terms of salts concentrations.

In summary, the results reported here indicate that *Hfx mediterranei* is able to grow in presence of salt concentrations that are even higher than those detected in their natural environments and it tolerates high nitrate or nitrite concentrations. During the cells' growth, nitrate and nitrite are removed from the culture media. There is worldwide concern over the excessive use of fertilizers in agricultural activities, leading to nitrate and nitrite accumulation in groundwater. These studies should make significant contributions to future understanding of the assimilatory nitrate pathway regulation in archaea and reveal that *Hfx mediterranei* could be a microorganism very interesting for bioremediation applications.

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