J Ind Microbiol Biotechnol (2008) 35:867–873
DOI 10.1007/s10295-008-0359-0

ORIGINAL PAPER

Enterobacter cloacae SLD1a-1 gains a selective advantage from selenate reduction when growing in nitrate-depleted anaerobic environments

James T. Leaver · David J. Richardson · Clive S. Butler

Received: 11 December 2007 / Accepted: 4 April 2008 / Published online: 1 May 2008

Abstract *Enterobacter cloacae* SLD1a-1 is capable of the complete reduction of selenate to selenium and the initial reaction is catalysed by a membrane-bound selenate reductase. In the present study, continuous culture experiments were employed to investigate the possibility that selenate reduction, via the selenate reductase, might provide sufficient energy to maintain cell viability when deprived of the preferred anaerobic terminal electron acceptor nitrate. The evidence presented indicates that the selenate reductase supports slow growth that retards the wash-out of the culture when switching to nitrate-depleted selenate-rich medium, and provides a proton motive force for sustained cell maintenance. In contrast, a strain of *E. cloacae* (sub sp. *cloacae*) that does not readily reduce selenate, cannot sustain cell maintenance when switching to a selenate-rich medium. This work demonstrates for the first time that respiratory linked selenate reduction gives *E. cloacae* SLD1a-1 a selective advantage when inhabiting selenate-contaminated environments and highlights the suitability of utilising *E. cloacae* SLD1a-1 when developing selenium remediation strategies.

J. T. Leaver
Institute for Cell and Molecular Biosciences,
University of Newcastle,
Newcastle upon Tyne NE2 4HH, UK

D. J. Richardson
School of Biological Sciences,
University of East Anglia,
Norwich NR4 7TJ, UK

C. S. Butler (✉)
School of Biosciences, Centre for Biocatalysis,
University of Exeter, Stocker Road,
Exeter EX4 4QD, UK
e-mail: c.s.butler@exeter.ac.uk

Keywords Selenate · Nitrate · Electron transport chain · Terminal electron acceptor · Bioreactor

Introduction

Respiratory flexibility permits microbes to populate a diverse range of habitats [21] and facilitates their use in many bioremediation applications. It is the potential to use a number of different terminal electron acceptors that gives them the selective advantage to survive and colonize many of the most extreme and hostile anoxic environments. One such adaptation is the ability of some bacteria to utilise toxic metalloid oxyanions such as selenate and arsenate as terminal electron acceptors [25]. Selenate (SeO_4^{2-}) is the most oxidised form of selenium (Se); it is highly soluble and can present a significant hazard to health and the environment [7, 19]. Naturally high levels of selenate are found in America and Canada, where for example, selenate-contaminated drainage water from the seleniferous rich soils of California's San Joaquin Valley has been a major cause of death and deformities in the inhabiting wildlife [14, 15]. However, it is more commonly human activities such as petroleum refining, mining and fossil fuel combustion that result in generating selenate contamination [7]. These soluble oxyanions (selenate and selenite) are the primary forms of selenium in aerated environments and their transformation to elemental selenium and selenide-containing compounds occurs primarily by microbial processes [26]. Consequently, microbes capable of the precipitation of non-toxic elemental selenium are of considerable interest to those developing environmental cleansing and remediation strategies.

The micro-organisms that have been reported to reduce selenium oxyanions are not restricted to any particular

group/sub group of prokaryotes and examples are found throughout the microbial hierarchy [8, 11, 16–18, 26]. It is becoming increasingly evident that a number of biochemically distinct systems are expressed to reduce/respire these Se-oxyanions and both periplasmic and membrane-bound selenate reductases have been purified and characterised from *Thauera selenatis* [3, 9, 12, 20, 24] and *Enterobacter cloacae* SLD1a-1 [22, 27], respectively. Under anaerobic conditions, *T. selenatis* can respire with selenate as the sole terminal electron acceptor and provide sufficient energy to readily grow in batch culture. The selenate reductase (SER) from *T. selenatis* is a periplasmic trimeric enzyme with an apparent molecular mass of ~180 kDa [24]. The three subunits consist of SerA (96 kDa), SerB (40 kDa) and SerC (23 kDa). The SerA subunit has an N-terminal cysteine-rich motif, possibly co-ordinating a [4Fe–4S] cluster and also contains the molybdenum (Mo) active site in the form of the molybdopterin guanine dinucleotide (bis-MGD) cofactor [5, 13, 24]. The SerB subunit also has a number of cysteine-rich motifs [9], which co-ordinate a number of iron–sulphur clusters [5]. The selenate reductase complex also contains a *b*-type cytochrome [5, 24], which is presumed to be co-ordinated to the SerC subunit. A membrane-bound component analogous to NapC or NarI in the nitrate reductase systems has not yet been identified so the process by which SerABC receives electrons from the quinol pool remains to be established.

E. cloacae SLD1a-1 was isolated from Se-contaminated drainage water in the San Luis drain (SLD), Joaquin Valley, CA, USA. It also readily reduces Se-oxyanions to elemental selenium [4, 11, 22, 27, 28], but cannot grow using selenate as the sole electron acceptor when cultured anaerobically on non-fermentable carbon sources in batch [27]. However, *E. cloacae* SLD1a-1 is capable of reducing selenate to elemental selenium under both aerobic and anaerobic growth conditions. The initial reductive step is the two electron reduction of selenate to selenite and is catalysed by a multi-component membrane-bound molybdoenzyme orientated in the cytoplasmic membrane such that its active site faces the periplasmic compartment [22, 27]. The enzyme is a hetero-trimeric ($\alpha\beta\gamma$) complex with an apparent M_r of ~600 kDa. The individual subunit sizes are α ~100 kDa, β ~55 kDa and γ ~36 kDa, with a predicted overall subunit composition of $\alpha_3\beta_3\gamma_3$. The membrane bound selenate reductase also contains molybdenum, heme and non-heme iron as prosthetic constituents. The apparent K_m for selenate, when determined using the artificial electron donor methyl viologen, was shown to be ~2 mM with an observed V_{max} of 500 nmol SeO_4^{2-} min^{-1} mg^{-1} (k_{cat} ~ 5.0 s^{-1}) [22].

The physiological function of the membrane-bound selenate reductase remains to be established but it appears to be regulated under anaerobic conditions by FNR [30]. The fact that the selenate reductase cannot support growth in batch culture using selenate as the sole electron acceptor, combined with the apparent low substrate affinity, would seem to indicate a role dedicated solely to selenate detoxification. However, the location of the selenate reductase in the cytoplasmic membrane indicates a direct link to the Q-pool and under certain conditions may help to energise the cytoplasmic membrane, if for example proton translocation is coupled to the primary Q-reductase activity (i.e., through an NADH dehydrogenase or formate dehydrogenase). In the present study we have investigated whether selenate reduction can maintain a stable energy level when *E. cloacae* SLD1a-1, grown in continuous culture, is deprived of nitrate as an electron acceptor and thus might confer a selective advantage when growing in a selenate-enriched (or contaminated) environment.

Methods

Bacterial strains and batch cultivation

E. cloacae SLD1a-1 was purchased from the American Type Culture Collection (ATCC 700258). Wild-type *E. cloacae* sub sp. *cloacae* (ATCC 13047) was purchased from DSMZ (DSM No: 30054). All chemicals used were purchased from Sigma-Aldrich. For anaerobic growth in batch culture, bacteria were inoculated into basal salt medium (BSM) [11] containing 1% (v/v) vitamin and 1% (v/v) mineral solution [29] and further supplemented with glycerol, formate and nitrate, all at a final concentration of 15 mM. Cells were grown at 37 °C and after ~17 h harvested by centrifugation (6,000×*g*) for 20 min, washed and resuspended (~20 mg ml^{-1}) in 50 mM potassium phosphate buffer pH 7.5.

Continuous cultures

Continuous culture experiments were performed in accordance with Ellington et al. [6] using a New Brunswick Bioflow 110 fermenter containing 1.5 l of BSM supplemented with 1% (v/v) vitamin/mineral solutions, and 15 mM glycerol/formate as a carbon and energy source and 7 mM nitrate or selenate as the terminal electron acceptor as appropriate. Prior to continuous culture the bioreactor was inoculated with ~0.20 g wet weight of cells from an overnight starter culture. The vessel was initially operated in batch mode until culture turbidity reached an OD_{600nm} of 0.45 ± 0.05. Once at this turbidity the vessel was switched to continuous-culture mode at a dilution rate of D ~ 0.14 h^{-1}. The culture was allowed to equilibrate for ~24 h prior to nitrate wash-out or selenate wash-in. Culture cell wash-out was monitored by measuring turbidity at OD_{600nm} at 30-min intervals and allowed to

proceed to a change in OD_{600nm} of about ~0.3 U. At the end of the wash-out period, cultures were supplemented with nitrate (7 mM), switched to batch mode and allowed to recover to the starting turbidity. For nitrate wash-out, growth media was replaced using BSM depleted in nitrate and for selenate wash-in growth media was replaced with BSM depleted in nitrate but supplemented with sodium selenate (7 mM). During selenate wash-in the total oxyanion [$NO_3^- + SeO_4^{2-}$] concentration remained constant at 7 mM throughout the experiment. Anaerobic culture conditions were maintained throughout the experiment by sparging oxygen-free nitrogen through the vessel at a rate of ~1.5 l min^{-1}. The vessel was stirred at 200 rpm to ensure constant mixing of the growth medium. The pH of the spent media was routinely monitored and pH values between 7.5 and 8.5 were observed during operation. The culture was checked for contamination and cell viability by spreading serial dilutions (1/10 in triplicate) on enrichment agar plates and observing colony morphology and determining the number of colony-forming units (c.f.u.). Confirmation that the colonies formed were *E. cloacae* was obtained by 16S rDNA typing [10]. DNA sequences were PCR amplified using one forward primer (27f) and 2 alternative reverse primers (1525r and 1495r) according to the method of Lane [10]. To check that the bioreactor was operating under nitrate-limited conditions, a 10-mM nitrate addition was made to a culture at steady state, resulting in a rapid increase in cell turbidity to a value typically double that of the steady state culture. Each continuous culture experiment has been repeated in triplicate.

Analytical procedures

Cell growth was monitored by measuring the culture turbidity at OD_{600nm} using a Hitachi U2001 spectrophotometer. To determine cell viability, serial dilutions of culture samples were spread on LB agar plates and the number of c.f.u.'s per ml were counted. Samples of ~2 ml were taken from the fermenter using the sample port and placed on ice for immediate serial dilution. Typically, sample dilutions of 10^{-5} and 10^{-6} were plated in 10-µl aliquots, and incubated at 37 °C for ~14 h. Across replicate plates and different serial dilutions the variation in the number of c.f.u.'s was determined to be <10%. Each experiment has been repeated in triplicate.

Enzyme activity assays

Reductase activity in cell fractions was measured at 20 °C by following the oxidation of reduced methyl viologen spectrophotometrically at 600 nm coupled to the reduction of sodium selenate as described by Craske and Ferguson [2].

Data analysis and wash-out simulations

Analysis of experimental data and the simulation of non-growing cultures were done using Origin 7.5 (Origin Lab software) in accordance with the washout Eq. (1) [6].

$$x = x_0 \times e^{-Dt} \quad (1)$$

where x is the biomass at time t and x_0 is the initial biomass at time $t = 0$, and D is the dilution rate, calculated from $D = F/V$, where V volume and F flow rate.

Results

Selenate does not support anaerobic growth in batch culture on non-fermentable carbon substrates

E. cloacae SLD1a-1 readily grows in batch culture under anaerobic conditions using nitrate as the sole terminal electron acceptor, using either glycerol or a combination of glycerol and formate as the source of carbon and electrons (Fig. 1). In cultures where nitrate is absent or replaced with selenate no growth was observed, demonstrating that selenate reduction cannot provide sufficient energy for cell proliferation. Cultures grown in the presence of both nitrate and selenate (both at 7 mM) showed no difference in growth when compared to cultures grown on nitrate alone, demonstrating that 7 mM selenate was not toxic to the cells (not shown).

Selenate retards wash-out in anaerobic continuous cultures deprived of nitrate

Continuous culture experiments were employed to explore the possibility that selenate reduction may provide sufficient energy to maintain cell viability in growing cultures of *E. cloacae* when deprived of the preferred anaerobic terminal electron acceptor nitrate. In these experiments, steady-state cultures ($\delta x/\delta t = 0$) were maintained for ~24 h under strict nitrate limited anaerobic conditions [Fig. 2, region (a)]. Typical cell density was maintained at OD_{600nm} = ~0.4 U by keeping dilution rate constant at $D = 0.14\ h^{-1}$. Cultures were then fed with BSM either depleted in nitrate only or depleted in nitrate but containing 7 mM selenate and subsequent wash-out of cell density and cell viability was monitored [an example of washout is shown in Fig. 2, region (b)]. Cultures that were switched to growth medium depleted in either the respiratory or carbon substrates resulted in non-growing cultures with a concomitant decrease in cell density and cell viability (washout). The rate and profile of the culture wash-out when switching from a nitrate to selenate-containing medium could, therefore, be used to assess if the presence

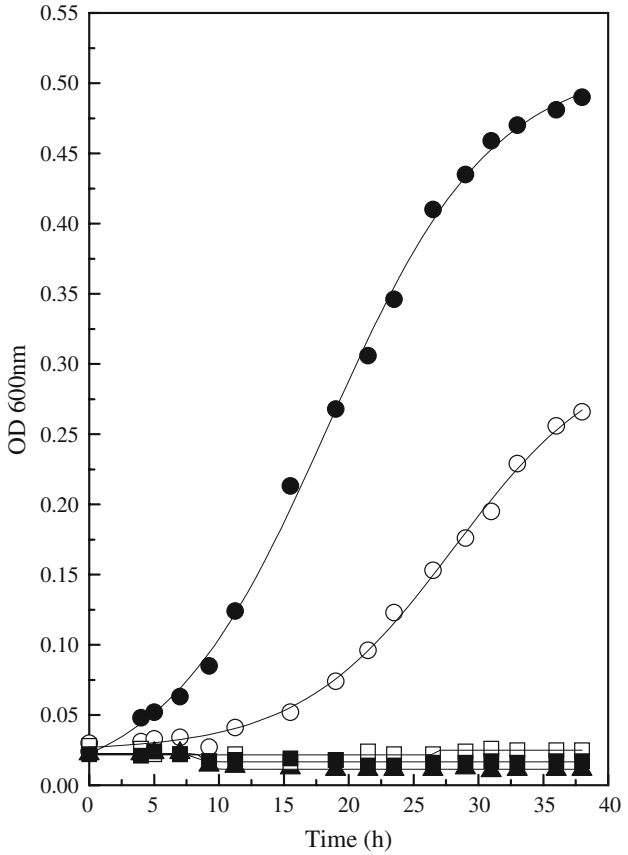

Fig. 1 Growth profile of *E. cloacae* SLD1a-1 during batch culture. All cultures were grown anaerobically in 500 ml BSM containing the following supplements: *filled circle* glycerol (15 mM), formate (15 mM) and nitrate (7 mM); *open circle* glycerol (15 mM) and nitrate (7 mM); *open square* glycerol (15 mM), formate (15 mM) and selenate (7 mM); *filled square* glycerol (15 mM) and selenate (7 mM); *filled triangle* glycerol (15 mM) and formate (15 mM) with no selenate or nitrate

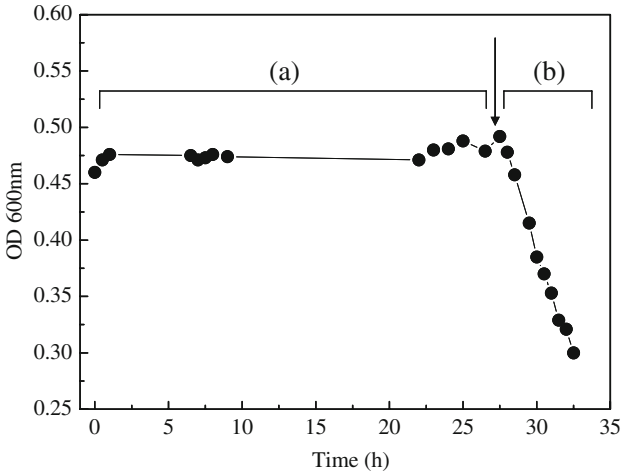

Fig. 2 Growth profile of *E. cloacae* SLD1a-1 during continuous culture. *E. cloacae* SLD1a-1 was grown anaerobically on BSM supplemented with glycerol/formate (15 mM) and nitrate (7 mM) as the terminal electron acceptor. Growth state *a* shows the culture in steady state ($\delta x/\delta t = 0$) maintained at a dilution rate of $D = 0.14\ h^{-1}$. Growth state *b* shows the culture in wash-out. The *arrow* indicates a switch to BSM either depleted in nitrate or depleted in nitrate but containing 7 mM selenate

of selenate in the growth medium could sustain growth and retard the rate of cell wash-out.

Figure 3 shows the wash-out of a continuous culture of *E. cloacae* SLD1a-1 as it switched from nitrate-rich to nitrate-depleted BSM. Immediately following the switch to nitrate-depleted conditions the culture cell turbidity starts to decrease and after 1 h the rate of wash-out is consistent with the simulation for a non-growing culture (Fig. 3a). In comparison, cultures that were switched from a nitrate-rich medium to a selenate-rich medium also showed a decrease in cell turbidity following the onset of washout. However, after a period of approximately 4 h, at selenate concentrations >2 mM, the rate of wash-out of the culture was significantly reduced and shown to deviate from the theoretical wash-out simulation (Fig. 3a, Table 1). It is apparent that at selenate concentrations above ~2 mM, a new steady state was achieved at approximately 0.3 OD units, which was maintained for a number of hours (>4 h). Attempts to maintain prolonged steady state growth (~20 h) by lowering D to a level $<D_{crit}$ was unsuccessful primarily because maintaining low-dilution rates resulted in highly irregular medium flow. During the wash-out phase no obvious red colour indicative of selenium production was seen, but as it is well documented that *E. cloacae* SLD1a-1 excretes insoluble elemental selenium into the culture medium when growing in the presence of selenate or selenite, it was appreciated that even low levels of elemental selenium produced could create additional scattering at OD_{600nm}, thus giving rise to the observed increases in turbidity. Furthermore, cell culture turbidity can be a poor measure of culture growth since changes in cell morphology can occur when switching to different growth conditions. Consequently, samples were also taken and cells plated to analyse cell morphology and cell viability. In order to confirm that the bioreactor was not contaminated colonies were also subjected to 16S rDNA typing. Figure 3b shows the cell viability during the wash-out phase for cultures depleted in nitrate and cultures depleted in nitrate but in the presence of increasing selenate. Nitrate-depleted cultures show that cell viability decreases in line with the predicted pattern for a non-growing culture (average rate of viability decrease $\sim 4.4 \times 10^8$ Δc.f.u. $ml^{-1}\ h^{-1}$) (Fig. 3b, Table 1), whereas, cultures switched to a selenate-rich medium again show that cell viability is maintained at a level higher than would be expected for a non-growing culture (average rate of viability decrease $\sim 1.2 \times 10^8$ Δc.f.u. $ml^{-1}\ h^{-1}$) (Fig. 3b, Table 1). These combined results indicate that the membrane-bound selenate reductase supports slow growth that retards the

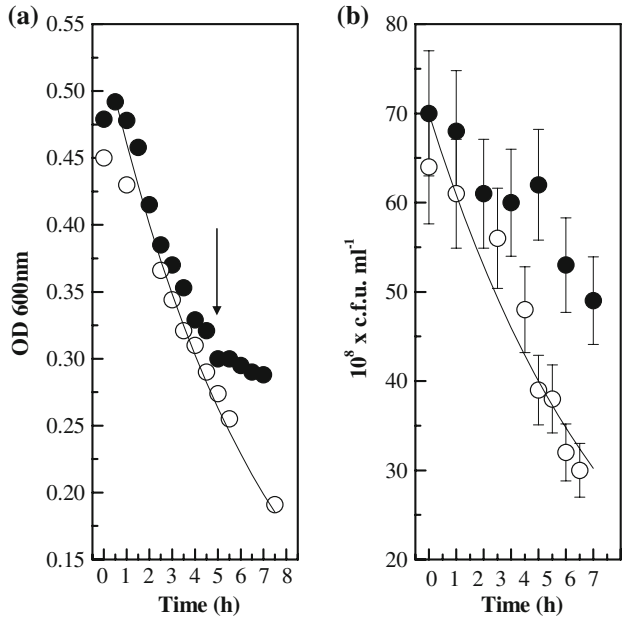

Fig. 3 Response of the growth profile of *E. cloacae* SLD1a-1 to nitrate wash-out and selenate wash-in conditions. **a** Decrease in culture turbidity (OD_{600nm}) and **b** decrease in cell viability (c.f.u. ml^{-1}) following the switch to BSM depleted in nitrate (*open circle*) and BSM depleted in nitrate, but containing 7 mM selenate (*filled circle*). The *solid line* in each case shows the theoretically predicted wash-out profile for a non-growing culture based upon the Eq. (1). The *arrow* in **a** indicates the point at which the selenate concentration is approximately 2 mM. Cultures were maintained at a dilution rate of $D = 0.14\ h^{-1}$. Experiments were repeated in triplicate and the same trend was observed in each case. A typical data set is presented

wash-out of the culture when switching to a nitrate-depleted selenate-rich medium, and provides sufficient proton motive force for sustained cell maintenance.

Wild-type *E. cloacae* (sub sp. *cloacae*) cannot reduce selenate and has no selective advantage when switching to a selenate-rich medium

The wild-type strain of *E. cloacae* (sub sp. *cloacae*) (ATCC 13047) when grown in batch culture either aerobically or anaerobically in the presence of selenate (7 mM) failed to reduce the selenate to elemental selenium (Fig. 4a). Furthermore, attempts to detect viologen-driven selenate reductase activity in isolated membrane fractions from the wild-type strain were unsuccessful. Nitrate reductase activity was, however, readily detected ($\sim$40 μmol [NO_3^-] min^{-1} g ww $cells^{-1}$) and anaerobic growth on BSM containing glycerol, formate and nitrate was observed. Consequently, this strain was utilised in continuous culture experiments to demonstrate that the retardation of wash-out seen with *E. cloacae* SLD1a-1 was due to the functional membrane-bound selenate reductase and not just dual substrate utilization by the nitrate-reducing systems. A typical nitrate wash-out and selenate wash-in experiment is shown in Fig. 4b. These data show that in the presence of increasing [selenate], the wild-type strain displays the wash-out kinetics of a non-growing culture as determined by comparison with the non-growing simulation. Similarly, analysis of the cell viability (Fig. 4c) again shows that there is no significant increase in viability as cells are switched to media containing selenate as the sole electron acceptor. This work strongly suggests that respiratory linked selenate reduction by the membrane-bound selenate reductase does give *E. cloacae* SLD1a-1 a selective advantage when growing in selenate-contaminated environments and thus assigns a physiological function to the novel membrane-bound selenate reductase.

Discussion

The physiological function of selenate reduction by *E. cloacae* SLD1a-1 has been the cause of some debate. An early study by Losi and Frankenberger [11] reported a true respiratory function for the selenate reductase, demonstrating anaerobic growth in the presence of selenate as the sole electron acceptor. However, these cultures were grown in the presence of glucose and subsequent attempts to reproduce selenate-dependant growth in batch culture using non-fermentable carbon substrates have been unsuccessful [27]. Consequently, we have previously suggested that the

Table 1 Rates of culture change for *E. cloacae* SLD1a-1 during nitrate wash-out and selenate wash-in conditions

Parameter measured	Nitrate wash-out				Selenate wash-in			
	Replicate experiments			Av	Replicate experiments			Av
	1	2	3		1	2	3	
Culture turbidity ($\Delta OD_{600nm}\ h^{-1}$)[a]	0.027	0.023	0.023	0.024	0.013	0.016	0.013	0.014*
Cell viability (Δc.f.u. $ml^{-1}\ h^{-1}$)[a] $\times 10^8$	3.3	4.0	6.0	4.4	2.5	0.5	0.5	1.2[†]

A P value of less than 0.05 is considered significant

* $P = 0.036$ and [†] $P = 0.045$ when compared to the nitrate washout conditions without the addition of selenate

[a] Rates are calculated from data obtained post 3 h from the onset of nitrate wash-out or selenate wash-in. Data are analysed using a two-sample paired Student's t test (Origin 7.5 software)

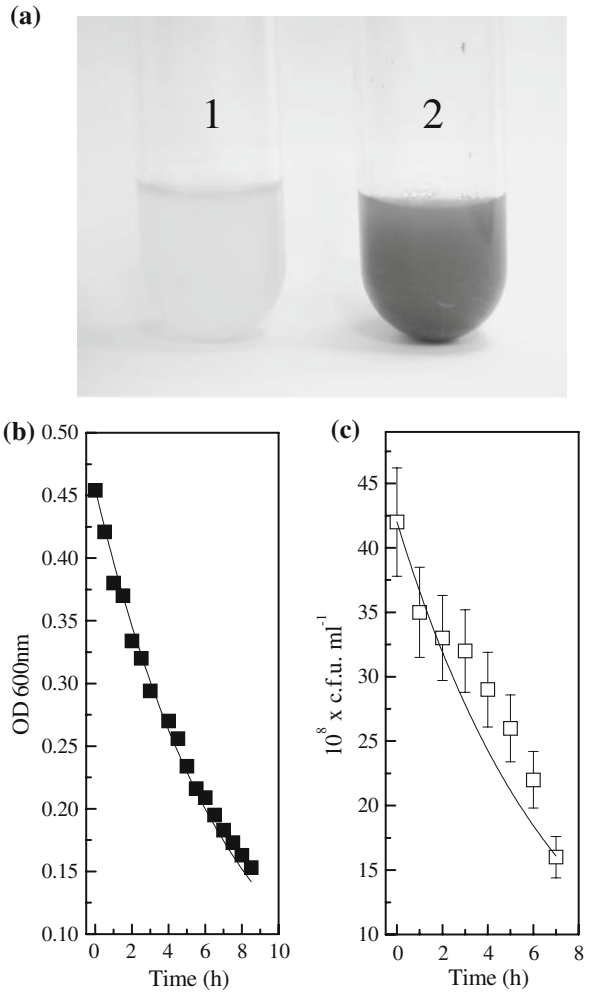

Fig. 4 Growth of wild-type *E. cloacae* (sub sp. *cloacae*) under selenate-rich conditions. **a** *E. cloacae* (sub sp. *cloacae*) (*tube 1*) is unable to reduce selenate (7 mM) to elemental selenium which is observed as a *dark precipitate* (red in colour) in the culture medium of *E. cloacae* SLD1a-1 (*tube 2*). **b**, **c** Response of the growth profile of wild-type *E. cloacae* (sub sp. *cloacae*) to selenate wash-in conditions in continuous culture. **b** The decrease in culture turbidity (OD_{600nm}) and **c** the decrease in cell viability (c.f.u. ml^{-1}) over time following the switch to BSM depleted in nitrate, but containing 7 mM selenate. The *solid lines* in each case represent the simulated predicted wash-out profile for a non-growing culture. The cultures were maintained at a dilution rate of $D = 0.14\ h^{-1}$

main function of the selenate reductase is primarily related to selenium detoxification [22, 27]. However, the location of the selenate reductase within the cytoplasmic membrane does suggest that the Q-pool forms the source of electrons [22] and that under certain selenate-rich conditions may act to draw electrons via a primary dehydrogenase through the respiratory electron transfer chain and generate enough Δp ($\Delta pH + \Delta\psi$) to support a low level of ATP production. In an attempt to address this hypothesis the present paper reports the study of selenate respiration by *E. cloacae* SLD1a-1 in continuous cultures, switching from nitrate-rich to selenate-rich conditions. The evidence presented indicates that the membrane-bound selenate reductase supports slow growth that retards the wash-out of the culture when switching to nitrate-depleted selenate-rich media, and provides a proton-motive-force for sustained cell maintenance. In contrast, a strain of *E. cloacae* (sub sp. *cloacae*) that does not readily reduce selenate, cannot sustain cell maintenance when switching to a selenate-rich medium. These results support the hypothesis that the novel membrane-bound selenate reductase, purified recently by Ridley et al. [22], may have an important respiratory function when colonizing selenate-contaminated environments and that under nitrate-depleted conditions selenate can be utilised as an alternative electron acceptor. The function of the selenate reductase would thus be to conserve energy by the utilization of an electrogenic primary dehydrogenase, such as a formate dehydrogenase (Fig. 5).

The observation that the non-selenate reducing wild-type strain gains no advantage over a non-growing culture when switching from nitrate to a selenate-rich environment shows convincingly that selenate respiration is supported solely by the unique selenate reductase system of strain SLD1a-1 and is not a consequence of alternative substrate utilization by the respiratory nitrate reductase, a side reaction that has been reported by others [1, 23]. This is also consistent with our previous observations that show that the purified respiratory membrane bound nitrate reductase from *E. cloacae* SLD1a-1, which is typical of the NAR type, displays no selenate reductase activity in vitro [22].

Conclusions

This work has demonstrated for the first time that selenate reduction by *E. cloacae* SLD1a-1 can energize the

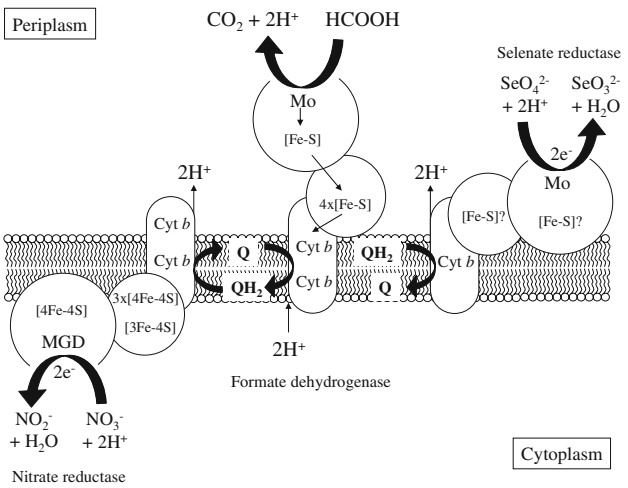

Fig. 5 Schematic diagram showing redox loops for both nitrate and selenate respiration

cytoplasmic membrane and generate enough ATP to support slow growth in continuous culture, which retards wash-out when switching from nitrate rich to nitrate-depleted selenate-rich habitats. These data suggest that the evolution of a selenate-reducing pathway has given *E. cloacae* strain SLD1a-1 a distinct selective advantage over non-selenate reducing bacteria when colonizing selenate-rich anaerobic environments.

Acknowledgments This work was supported by BBSRC research grants (13/P17219 and BBS/B/10110) to Clive S. Butler and David J. Richardson. James T. Leaver acknowledges receipt of a BBSRC quota Ph.D studentship. We thank Dr. Helen Ridley, Dr. Carys Watts and Professor Jeremy H. Lakey (University of Newcastle) for helpful discussions. We also kindly acknowledge Dr. Elizabeth Dridge (University of Exeter) and Juliane Klein (University of Newcastle) for help with experiments.

References

1. Avazeri C, Turner RJ, Pommier J, Weiner JH, Giordano G, Verméglio A (1997) Tellurite reductase activity of the nitrate reductase is responsible for the basal resistance of *Escherichia coli* to tellurite. Microbiology 143:1181–1189
2. Craske A, Ferguson SJ (1986) The respiratory nitrate reductase from *Paracoccus denitrificans*. Molecular characterisation and kinetic properties. Eur J Biochem 158:429–436
3. DeMoll-Decker H, Macy JM (1993) The periplasmic nitrite reductase of *Thauera selenatis* may catalyse the reduction of selenite to elemental selenium. Arch Microbiol 160:241–247
4. Dungan RS, Frankenberger WT (2001) Biotransformation of selenium by *Enterobacter cloacae* SLD1a-1: formation of dimethylselenide. Biogeochemistry 55:73–86
5. Dridge EJ, Watts CA, Jepson BJN, Line K, Santini JM, Richardson DJ, Butler CS (2007) Investigation of the redox centres of selenate reductase from *Thauera selenatis* by electron paramagnetic resonance spectroscopy. Biochem J 408:19–28
6. Ellington MJ, Richardson DJ, Ferguson SJ (2003) *Rhodobacter capsulatus* gains a competitive advantage from respiratory nitrate reduction during light–dark transitions. Microbiology 149:941–948
7. Haygarth PM (1994) Global importance and global cycling of selenium. In: Frankenberger WT Jr, Benson S (eds) Selenium in the environment. Marcel Dekker, New York, pp 1–28
8. Knight V, Blakemore R (1998) Reduction of diverse electron acceptors by *Aeromonas hydrophila*. Arch Microbiol 169:239–248
9. Krafft T, Bowen A, Theis F, Macy JM (2000) Cloning and sequencing of the genes encoding the periplasmic-cytochrome *b*-containing selenate reductase from *Thauera selenatis*. DNA Seq 10:365–377
10. Lane DJ (1991) 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M (eds) Nucleic acid techniques in bacterial systematics, chap 6. Wiley, New York, pp 115–175
11. Losi ME, Frankenberger WT (1997) Reduction of selenium oxyanions by *Enterobacter cloacae* SLD1a-1: isolation and growth of the bacterium and its expulsion of selenium particles. Appl Environ Microbiol 63:3079–3084
12. Macy JM, Rech S, Auling G, Dorsch M, Stackenbrandt E, Sly LI (1993) *Thaurea selenatis* gen-nov, sp-nov, a member of the beta-subclass of proteobacteria with a novel type of anaerobic respiration. Int J Syst Bacteriol 43:135–142
13. Maher MJ, Santini J, Pickering IJ, Prince RC, Macy JM, George GN (2004) X-ray absorption spectroscopy of selenate reductase. Inorg Chem 43:402–404
14. Ohlendorf HM, Hoffman DJ, Saiki MK, Aldrich TW (1986) Embryonic mortality and abnormalities of aquatic birds: apparent impacts of selenium from irrigation drainwater. Sci Total Environ 52:49–63
15. Ohlendorf HM, Hothem RL, Aldrich TW, Krynitsky AJ (1987) Selenium contamination of the Grasslands, a major California waterfowl area. Sci Total Environ 66:169–183
16. Oremland RS, Hollibaugh JT, Maest AS, Presser TS, Miller LG, Culbertson CW (1989) Selenate reduction to elemental selenium by anaerobic bacteria in sediments and culture: biogeochemical significance of a novel, sulfate-independent respiration. Appl Environ Microbiol 55:2333–2343
17. Oremland RS, Steinberg NA, Presser TS, Miller LG (1991) In situ bacterial selenate reduction in the agricultural drainage systems of western Nevada. Appl Environ Microbiol 57:615–617
18. Oremland RS, Switzer Blum J, Burns Bindi A, Dowdle PR, Herbel M, Stolz JF (1999) Simultaneous reduction of nitrate and selenate by cell suspensions of selenium-respiring bacteria. Appl Environ Microbiol 65:4385–4392
19. Rayman M (2002) Se brought to Earth. Chem Br 38:28–31
20. Rech SA, Macy JM (1992) The terminal reductases for selenate and nitrate respiration in *Thauera selenatis* are two distinct enzymes. J Bacteriol 174:7316–7320
21. Richardson DJ (2000) Bacterial respiration: a flexible process for a changing environment. Microbiology 146:551–571
22. Ridley H, Watts CA, Richardson DJ, Butler CS (2006) Resolution of distinct membrane-bound enzymes from *Enterobacter cloacae* SLD1a-1 that are responsible for selective reduction of nitrate and selenate oxyanions. Appl Environ Microbiol 72:5173–5180
23. Sabaty M, Avazeri C, Pignol D, Verméglio A (2001) Characterization of the reduction of selenate and tellurite by nitrate reductases. Appl Environ Microbiol 67:5122–5126
24. Schröder I, Rech S, Krafft T, Macy JM (1997) Purification and characterization of the selenate reductase from *Thauera selenatis*. J Biol Chem 272:23765–23768
25. Stolz JF, Basu P, Santini JM, Oremland RS (2006) Arsenic and selenium in microbial metabolism. Annu Rev Microbiol 60:107–130
26. Stolz JF, Oremland RS (1999) Bacterial respiration of arsenic and selenium. FEMS Microbiol Rev 23:615–627
27. Watts CA, Ridley H, Condie KL, Leaver JT, Richardson DJ, Butler CS (2003) Selenate reduction by *Enterobacter cloacae* SLD1a-1 is catalysed by a molybdenum-dependent membrane-bound enzyme that is distinct from the membrane-bound nitrate reductase. FEMS Microbiol Lett 228:273–279
28. Watts CA, Ridley H, Dridge EJ, Leaver JT, Reilly AJ, Richardson DJ, Butler CS (2005) Microbial reduction of selenate and nitrate: common themes and variations. Biochem Soc Trans 33:173–175
29. Wolin EA, Wolin MJ, Wolfe RS (1963) Formation of methane by bacterial extracts. J Biol Chem 238:2882–2886
30. Yee N, Ma J, Dalia A, Boonfueng T, Kobayashi DY (2007) Se(VI) reduction and the precipitation of Se(0) by the facultative bacterium *Enterobacter cloacae* SLD1a-1 are regulated by FNR. Appl Environ Microbiol 73:1914–1920