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Transformation of (per)chlorate into chloride by a newly isolated bacterium: reduction and dismutation

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Abstract Bacterial strain GR-1 was isolated from activated sludge for its ability to oxidize acetate with perchlorate as electron acceptor. Sequencing of 16S rDNA revealed the isolate to belong to the β subgroup of *Proteobacteria*. When strain GR-1 was grown on acetate and perchlorate, the release of chloride was proportional to the disappearance of perchlorate, showing that this compound was completely reduced. In addition to perchlorate, strain GR-1 used chlorate, oxygen, nitrate and Mn(IV) as electron acceptor. The oxidation of acetate is coupled to the reduction of perchlorate and chlorate, whereas chlorite reduction is not affected by the addition of acetate. Strain GR-1 disproportionates chlorite into molecular oxygen and chloride. As a consequence, the strain oxidizes acetate by simultaneously reducing perchlorate to chlorite and molecular oxygen to water. Comparison of growth yields with oxygen, chlorate and perchlorate and calculated $\Delta G^{0\prime}$ values confirms this finding.

Introduction

Perchlorate, chlorate, chlorine dioxide and hypochlorite are produced on a large scale by the chemical industry and used in a wide range of applications. In water, chlorine dioxide and hypochlorite decompose into chlorite, chlorate and chloride. Chlorite, in turn, is dismutated into chlorate and chloride. The occurrence of chlorate in surface water has therefore been ascribed to the use of chlorine dioxide and hypochlorite (Malmqvist et al. 1991; Versteegh et al. 1993). Perchlorate occurs in aqueous wastes of the aerospace and defence industries (Ataway and Smith 1993) and naturally in Chile salpeter. As a consequence, the release of chlorooxo acids into the environment occurs by a variety of routes, and knowledge of the microbial transformation of these acids is therefore important.

Both dissimilatory and assimilatory nitrate-reducing microorganisms have the ability to destroy perchlorate and chlorate (Quastel et al. 1925; Hackenthal 1965; Rigano 1970; Tromballa and Broda 1971; Karki and Kaiser 1979). Denitrifying bacteria convert chlorate into chlorite, whereas algae are able to reduce chlorate completely. Although the reduction of (per)chlorate by denitrifying bacteria and algae is probably cometabolic, chlorooxo-acid-reducing reactions may generate energy. Indeed, (per)chlorate reduction with various energy substrates has been demonstrated with a number of enrichment cultures (Bryan and Rohlich 1954; Malmqvist et al. 1991; Ataway and Smith 1993). Anaerobic conditions favour reduction of (per)chlorate. Up to now, a few microorganisms have been isolated that are able to grow through (per)chlorate-reducing reactions. The pure cultures studied are *Vibrio dechloraticans* (Korenkov et al. 1976), *Ideonella dechloratans* (Malmqvist et al. 1994) and an *Acinetobacter* sp. (Stepanyuk et al. 1992). However, the current understanding of microbial reduction of (per)chlorate by pure cultures is limited, because the mechanism of perchlorate, chlorate and chlorite reduction has not been studied by these authors.

In this paper we describe the isolation and characterization of a bacterium capable of anaerobic growth in a defined medium on perchlorate and acetate. Preliminary evidence for the transformation of perchlorate, chlorate and chlorite into chloride by the isolate is presented.

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Materials and methods

Chemicals

Sodium perchlorate and sodium chlorate were purchased from Acros Chimica, Tilburg, The Netherlands, and sodium chlorite was obtained from Fluka, Breda, The Netherlands. Sodium chlorite was impure, the main impurity being chloride (20% v/v). Aqueous sodium chlorite solutions are unstable, and therefore fresh stock solutions were prepared daily. Agar was obtained from Oxoid, Basingstoke, Hampshire, England. All other chemicals used were of reagent grade.

Media and growth conditions

All enrichments and growth of pure cultures were performed in mineral salts medium free of chloride. The mineral salts medium contained per litre of deionized water: 1.0 g $NaClO_4$, 1.55 g K_2HPO_4, 0.85 g $NaH_2PO_4 \cdot H_2O$, 0.5 g $(NH_4)_2HPO_4$, 0.1 g $MgSO_4 \cdot 7H_2O$, 1.7 mg Na_2SeO_3 and 0.1 ml trace elements solution described by Vishniac and Santer (1957). The mineral salts media were added to 1-l flasks with gas-tight screw caps and supplied with 2 g l^{-1} sodium acetate as the sole carbon and energy source. The final pH of the medium was 7.2. The batch cultures were incubated on an orbital incubator (100 rev/min) at 30°C.

Enrichment, isolation and identification

Enrichment cultures were started with activated sludge from a plant treating predominantly domestic waste water. Sludge was incubated anaerobically in a completely filled 300-ml Biological Oxygen Demand flask. For the enrichment culture the flask supplied with mineral salts medium and 1 g l^{-1} sodium acetate was inoculated with 2 mg dry weight l^{-1} activated sludge. This culture was incubated at 30°C. After two subcultures a bacterium in this culture was streaked to purity on agar plates containing mineral salts medium, 2 g l^{-1} sodium acetate and 15 g l^{-1} agar. These plates were incubated at 30°C in an anaerobic jar. The isolate was checked for purity by streaking onto yeast/acetate plates. The biochemical features (API 20NE) of strain GR-1 were examined according to the instructions of the manufacturers (API system, Montalieu-Vercieu, France). The 16S rDNA sequence of strain GR-1 was determined by Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSM, Braunschweig, Germany) as described by Rainey et al. (1992).

Growth

Growth (absorbance) of the isolate on different energy substrates was determined in 100-ml serum bottles with gas-tight caps filled to 75% of their volume with mineral medium and 1.0 g l^{-1} of the appropriate substrate and incubated at 30°C. To evaluate other electron acceptors for growth with acetate, cultures were grown with oxygen, nitrate, nitrite, chlorate, chlorite, bromate, iodate, sulphate, selenate, Mn(IV) and Fe(III) instead of perchlorate. The growth rates on acetate were determined as follows: cells were inoculated in 300-ml side-arm conical flasks containing 30 ml medium. The flasks were incubated in an orbital incubator at 30°C. Growth was followed by measuring turbidity with a nephelometer Ratio XR (Hach, Loveland, Colo., USA). Growth rates were calculated from logarithmically plotted growth curves. For anaerobic growth these cultures were flushed with oxygen-free nitrogen gas.

Growth yields for aerobically and anaerobically growing cultures were obtained from growth experiments in batch cultures in quadruplicate. The yields were calculated from dry weights and acetate determinations in the batch cultures. Temperature and pH were kept constant at 30°C and 7.0 respectively.

Larger-scale cultivations were carried out at 30°C in 1-l serum flasks with gas-tight screw caps containing 0.8 l mineral medium with sodium acetate as sole carbon and energy source. The gas phase contained air.

Washed cell suspension

Cells were harvested by centrifugation at 26 000 *g* for 5 min and washed twice in 15 mM potassium phosphate/sodium phosphate buffer (1.55 g K_2HPO_4 and 0.85 g $NaH_2PO_4 \cdot H_2O$), pH 7.2, being resuspended each time in the same buffer.

Reduction of chlorooxo acids, nitrate and nitrite by washed cell suspensions

To demonstrate the reduction of chlorooxo acids, nitrate and nitrite, washed cell suspensions were anaerobically incubated at 30°C with and without acetate. Portions of the suspension were removed at various times and analysed for chloride, nitrate and nitrite.

Determination of the oxygen formation and consumption

Oxygen consumption and formation by whole cells was measured in a biological oxygen monitor (Yellow Springs Instruments, Yellow Springs, Ohio, USA). The total incubation mixture (5 ml) contained washed cell suspension in 15 mM potassium sodium phosphate buffer, pH 7.2. The oxygen consumption and formation were determined in the absence and presence of sodium acetate (1.0 g l^{-1}). The oxygen formation from chlorite was measured by injecting 0.035 mM chlorite into the vessel.

Analyses

The pH was determined with a microcomputer pH meter Consort P207 (Consort, Turnhout, Belgium). The dry weight was measured by filtering a sample of the microbial suspension over a preweighed 0.2-mm cellulose nitrate filter. The dry weight of the biomass was then determined gravimetrically after the filter had been dried at 105°C for 90 min.

After the cells had been disrupted by sonification using a Vibra cell ultrasonic processor VC-375 (Sonics & Materials Inc., Dansbury, USA) five times for 20 s at 50 W, the protein of these cells was determined by the bicinchoninic acid method (Pierce, Rockford, Wis., USA).

Acetate was analysed by automatic injection of 20 ml into an HPLC system equipped with an LKB pump (Pharmacia, Woerden, The Netherlands), a Plysper OAHY column (Merck, Darmstadt, Germany) and a differential refractometer (Pharmacia, Woerden, The Netherlands). The eluent was 20 mM H_2SO_4. Prior to injection all samples were filtered (0.8 mm, and diluted 1:1 with 200 mM H_2SO_4.

Chloride was measured by means of volumetric precipitation titration with silver nitrate. The equivalence point of the titration was determined potentiometrically using a Metrohm titroprocessor 672 (Metrohm AG, Herisau, Switzerland).

Chlorite was analysed by reacting chlorite with iodide at pH 2, forming iodine. The iodine formed was titrated with a sodium thiosulphate solution (10 mM). The equivalence point was determined visually using starch as indicator.

After the cells had been separated from the culture solutions by centrifugation at 26 000 *g* for 5 min, the chlorate content in the

supernatant was measured. Chlorate was analysed by direct injection of 50 ml into an HPLC system consisting of a M300 precision pump (Separations, H.I. Ambacht, The Netherlands), an Ionospher 5A 100.3-mm column with an anion precolumn (Chrompack, Middelburg, The Netherlands) and a differential refractometer (Waters, Etten Leur, The Netherlands). The mobile phase was 20 mM potassium hydrogen phthalate containing 1 mM ammonium nitrate (pH 4.1) at a flow rate of 0.4 ml min^{-1}.

Perchlorate was determined by precipitation of perchlorate with an excess of nitron. The excess nitron was analysed spectrophotometrically at 490 nm in a Shimadzu spectrophotometer UV/Vis 160A (Shimadzu, Kyoto, Japan).

Nitrite was determined colorimetrically, with Griess-Romijn reagent (Gries Romijn van Eck 1966). Nitrate was measured colorimetrically in solutions containing sulphuric acid with 2,6-dimethylphenol to form 4-nitro-2,6-dimethylphenol (Hartley and Asai 1963).

Results

Enrichment, isolation and characterization

Activated sludge from a plant treating predominantly domestic waste water was placed in a closed 300-ml flask completely filled with mineral medium containing acetate and perchlorate. This primary enrichment was incubated at 30°C and monitored by turbidity for growth. Growth was observed within 1 week with the concomitant appearance of chloride. A 1% transfer was made into a fresh medium and this transfer process was repeated twice. Microscopic examination of the second transfer revealed that the enrichment consisted primarily of rods. The second transfer was plated on a solidified medium and incubated in an anaerobic jar. After about 2 weeks colonies became visible. Colonies of perchlorate-reducing bacteria growing on agar plates were circular and red. White colonies were obtained when the bacterium was growing with oxygen as electron acceptor. The strain obtained was a gram-negative, oxidase-positive, motile rod-shaped organism. The strain was unable to assimilate glucose, arabinose, mannose, mannitol, *N*-acetylglucosamine, maltose, gluconate, adipate and phenyl acetate. Other biochemical features of this isolate are no indole formation from tryptophan, no acidification of glucose, no β-glucosidase, no β-galactosidase and no protease. An examination of 16S rDNA demonstrated that the isolate contained sequences that are specific to members of the β subdivision of the *Proteobacteria.*

Growth experiments

A typical pattern for anaerobic growth of strain GR-1 on acetate under perchlorate-reducing conditions is shown in Fig. 1. The relatively long lag period was reduced to 3 days when the nitrogen gas in the batch culture was replaced by air. Acetate consumption was

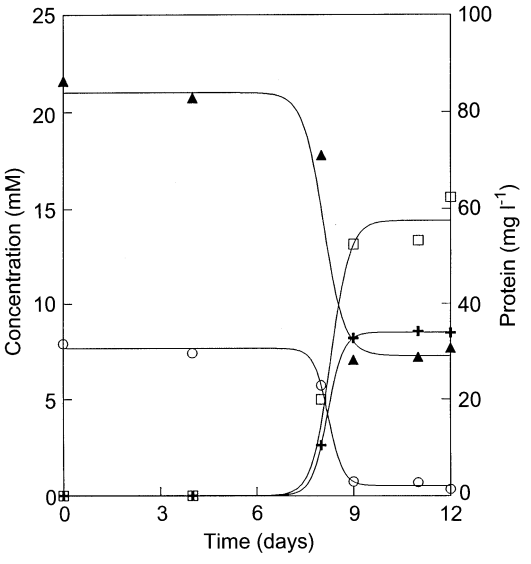

Fig. 1 The stoichiometric conversion of perchlorate (○) into chloride (+) by strain GR-1 growing on acetate (▲) as sole source of carbon and energy in a batch culture. The biomass concentration in the batch culture was determined as protein (□)

paralleled by chloride formation and perchlorate disappearance. Chlorate and chlorite were not detected in the growth medium. Linear regression of a logarithmically plotted growth curve yielded a doubling time of 7 h. A very similar curve was obtained for growth with chlorate.

The isolate grew under aerobic conditions with a doubling time of 3 h. When nitrate was present as sole electron acceptor, the doubling time was decreased to 9 h. Strain GR-1 was also capable to carry out anaerobic respiration of Mn(IV). Strain GR-1 was not able to utilize sulphate, iodate, bromate, chlorite, selenate or Fe(III) as electron acceptors.

Strain GR-1 can utilize many fatty acids and dicarboxylic acids as a sole source of carbon and energy for growth with perchlorate as electron acceptor in a mineral salts medium. Included are acetate, propionate, caprionate, malate, succinate and lactate. Strain GR-1 lacks the ability to catabolize glycine, formate, glycolate and citrate. The organism neither ferments nor oxidatively attacks glucose.

Cell growth yields on acetate from growth by perchlorate, chlorate, nitrate and oxygen reduction were measured in separate batch cultures. After growth cultures were harvested and analysed for acetate and dry weight content. The growth yields were 14.4 ± 2.2 g dry weight mol $acetate^{-1}$ (perchlorate), 16.4 ± 0.3 g dry weight mol $acetate^{-1}$ (chlorate), 15.9 ± 0.6 g dry weight mol $acetate^{-1}$ (oxygen) and 11.9 ± 0.4 g dry weight mol $acetate^{-1}$ (nitrate). These growth yields were statistically analysed using a *t*-test, and only the yield with nitrate differed significantly.

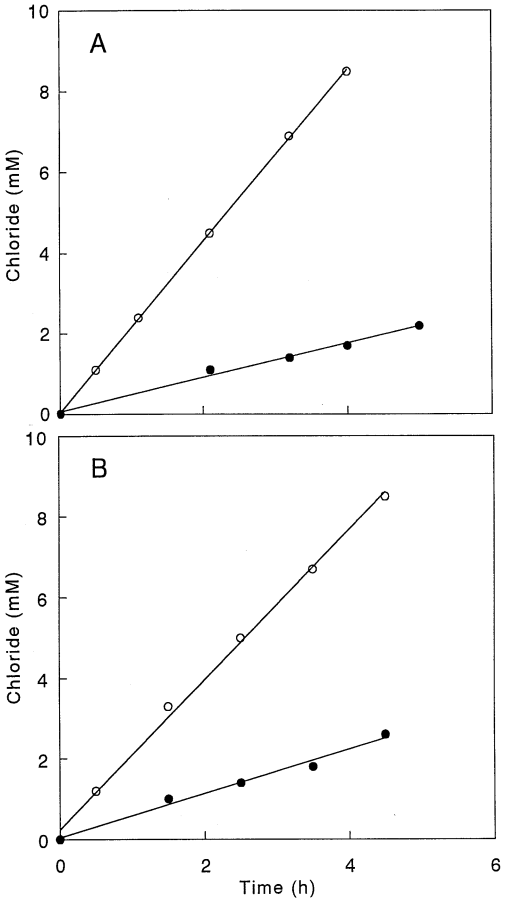

Fig. 2A,B Formation of chloride from perchlorate (**A**) or chlorate (**B**) in the presence of (○) and absence (●) or acetate by a washed cell suspension of strain GR-1. The protein concentrations were 0.83 $g l^{-1}$ and 0.54 $g l^{-1}$ for perchlorate and chlorate respectively

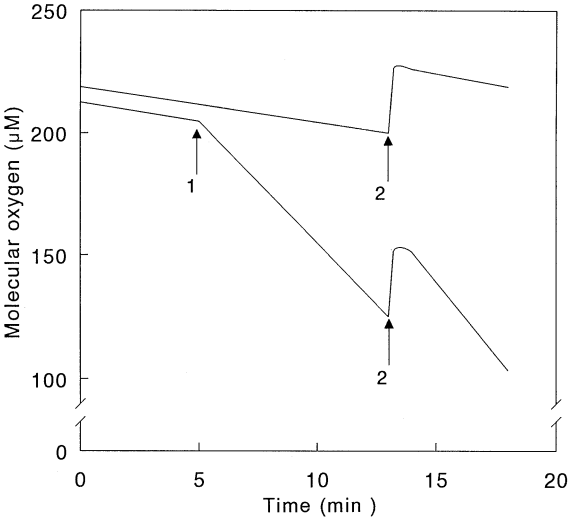

Fig. 3 Endogenous and substrate respiration and the formation of oxygen upon the addition of chlorite by washed cell suspensions of strain GR-1 grown with perchlorate as electron acceptor. *Arrows* addition of acetate (*1*) and chlorite (*2*)

Washed cell suspensions

To elucidate the role of acetate, the formation of chloride from perchlorate, chlorate and chlorite in the presence and absence of acetate was measured. Slow formation of chloride from perchlorate and chlorate by washed cell suspension of perchlorate-grown cells was observed in the absence of acetate. In the presence of acetate, which serves as a reducing agent, complete reduction of perchlorate and chlorate to chloride occurred within a few hours (Fig. 2). The rates (mmol chloride formed mg $protein^{-1}$ h^{-1}) achieved by washed cell suspensions in the presence of acetate incubated with perchlorate and chlorate were 0.043 and 0.057 respectively. Substrate specificity towards oxygen, nitrate and nitrite was also tested with washed cell suspensions of perchlorate-grown cells in the presence of acetate. Oxygen, nitrate and nitrite were reduced at rates of 0.088, 0.001 and 0.003 mmol min^{-1} mg $protein^{-1}$ respectively. Washed cell suspensions of oxygen-grown cells were not able to reduce perchlorate and chlorate. Nitrate-grown cells were not capable of utilizing oxygen.

In contrast to perchlorate and chlorate reduction, the transformation of chlorite was not stimulated by acetate. The activity of washed cell suspensions with chlorite differed significantly from the activities obtained with perchlorate and chlorate. In this case chloride was formed at a rate of 10 mmol min^{-1} mg $protein^{-1}$. Chloride was produced stoichiometrically from chlorite by washed cell suspensions of perchlorate-grown cells. The perchlorate and chlorate reduction only occurred in the absence of molecular oxygen, whereas the conversion of chlorite occurred under both aerobic and anaerobic conditions. These findings led to the hypothesis that chlorite disproportionates to form molecular oxygen and chloride. Evidence in support of this hypothesis was the formation of 0.034 mM oxygen upon the addition of 0.035 mM chlorite to a washed cell suspension of perchlorate-grown cells (Fig. 3). No increase in chloride could be observed in heat-killed controls or in controls without washed cell suspensions (data not provided). These controls were also unable to form oxygen from chlorite. Oxygen-grown cells disproportionate chlorite in the absence and presence of acetate at a rate of 1 mmol min^{-1} mg $protein^{-1}$.

Discussion

To provide information about the mechanism of perchlorate reduction, a bacterium growing on acetate was isolated from an enrichment culture inoculated with activated sludge. Degradation kinetics of this isolate

were followed by measuring the protein content, perchlorate and acetate degradation, and the appearance of chloride (Fig. 1). Reduction of the lag period was observed upon the introduction of oxygen, which is described for denitrifying bacteria (Tiedje 1988). The release of chloride corresponded to perchlorate transformation, confirming that perchlorate is entirely reduced and used as electron acceptor by strain GR-1. Mixed and pure microbial cultures, characterized by their ability to reduce perchlorate or chlorate to chloride, have already been described (Malmqvist et al. 1991; Ataway and Smith 1993).

Analysis of the 16S rDNA demonstrated that the newly isolated bacterium belongs to the β sub-division of the *Proteobacteria*. Another perchlorate-reducing bacterial strain is described in the literature (Korenkov et al. 1976). This strain was assigned to the genus *Vibrio*. 16S rDNA analysis of a chlorate-reducing bacterium, *I. dechloratans*, revealed that this isolate also clusters within the β subgroup of the *Proteobacteria* (Malmqvist et al. 1994). Strain GR-1 also reductively tranformed chlorate into chloride with acetate as electron donor. Oxygen, nitrate and Mn(IV) could replace (per)chlorate as electron acceptor. These metabolic features are similar to those described for *I. dechloratans* (Malmqvist et al. 1994).

Microorganisms have been shown capable of reducing perchlorate and chlorate. The reduction of perchlorate to chlorate is catalysed by cell-free extracts of *Bacillus cereus* (Hackenthal 1965). Chlorate and nitrate reductases present in denitrifying bacteria are involved in the reduction of chlorate (Pichinoty 1969; de Groot and Stouthamer 1969). These nitrate-reducing bacteria reduce chlorate to chlorite (Quastel et al. 1925; Karki and Kaiser 1979). A hypothetical pathway for the reduction of perchlorate was established on the basis of chlorooxo acids and nitrate transformations by denitrifying bacteria. Degradation could proceed first via chlorate and chlorite and then via hypochlorite or dichlorooxide. The disappearance of perchlorate, and the liberation of stoichiometric amounts of chloride in the batch culture inoculated with strain GR-1 suggested that there was no accumulation of intermediates (Fig. 1). Indeed, analysis revealed that there was no production of potential intermediates such as chlorite and hypochlorite by strain GR-1.

The oxidation of acetate is coupled to the reduction of perchlorate and chlorate (Fig. 2). The results presented here show that acetate is the source of reducing equivalents for the transformation of perchlorate into chlorite. The significant amount of (per)chlorate reduced in the absence of acetate demonstrates that intracellular storage material is used as electron donor (Fig. 2). The activities of perchlorate, chlorate, nitrate and nitrite reduction by washed cell suspensions of perchlorate-grown strain GR-1 indicate that specific (per)chlorate reductases are involved. Loss of the capacity to denitrify by *I. dechloratans* after several subcultures with chlorate as electron acceptor proves this assumption (Malmqvist et al. 1994).

The transformation of chlorite by strain GR-1 is not dependent on the presence of acetate. This is consistent with the mechanism in which chlorite is directly converted into molecular oxygen and chloride. To confirm the operation of this mechanism washed cell suspensions were shown to be capable of liberating molecular oxygen from chlorite without the addition of any reductive substrates (Fig. 3). This feature agrees with the inability of strain GR-1 to grow with chlorite because the energy released by the dismutation reaction cannot be used for biosynthesis.

Chlorite dismutation is known from inorganic chemistry but spontaneously occurs only at temperatures above 200°C (Taylor et al. 1940). Enzymes catalysing dismutation reactions such as catalases, peroxidases and superoxide dismutases have been found in many aerobic prokaryotes. Although oxygen production from chlorite by a chloroperoxidase system has been found previously (Shahangian and Hager 1981), stoichiometric conversion of chlorite into oxygen has not been reported. Chlorite and hypochlorite are toxic to microorganisms. The dismutation of chlorite to chloride and oxygen is probably a detoxification which enables strain GR-1 to grow with perchlorate and chlorate. The potential of oxygen-grown cells to decompose chlorite demonstrates that the enzyme required for the dismutation is constitutively expressed.

The $\Delta G^{o\prime}$ values for acetate oxidation with the redox couples perchlorate/chlorite and chlorate/chlorite are only $-801\ kJ\ mol^{-1}$ and $-787\ kJ\ mol^{-1}$ respectively (Table 1). These $\Delta G^{o\prime}$ values explain the preference of (per)chlorate-reducing bacteria for oxygen as electron acceptor. Under standard conditions the $\Delta G^{o\prime}$ of chlorate reduction to chloride with acetate is $-1015\ kJ\ mol^{-1}$ (Table 1). Comparing this with the $\Delta G^{o\prime}$ of respiration with oxygen, Malmqvist et al. (1991) expected the highest growth yield with chlorate as electron acceptor. However, the growth yields on acetate with perchlorate, chlorate and oxygen are almost identical. Comparable growth yields can be explained by the dismutation of chlorite. The $\Delta G^{o\prime}$ values for the overall reactions with oxygen and chlorate or perchlorate are slightly less negative than for the reaction with oxygen alone (Table 1). It is therefore proposed that (per)chlorate-reducing microorganisms utilize these chlorooxo acids and oxygen simultaneously. As expected, the growth yield from nitrate-reducing cultures was the lowest of all.

Oxygen inhibits perchlorate and chlorate reduction by both pure and mixed cultures (Ataway and Smith 1993). Results obtained with strain GR-1 demonstrate that the transformation of chlorite generates oxygen. Therefore, pure cultures of strain GR-1 have to use (per)chlorate and oxygen simultaneously. Washed cell suspensions of perchlorate-grown strain GR-1 reduce oxygen and perchlorate simultaneously, as is shown by

Table 1 Stoichiometric reactions of acetate with different electron acceptors and the calculated Gibbs free-energy changes $\Delta G^{o\prime}$) (Milazzo and Caroli 1978). The $\Delta G^{o\prime}$ values for the oxidation with perchlorate and chlorate are calculated for the reduction to both chloride and chlorite

Reaction	$\Delta G^{o\prime}$ (kJ mol acetate^{-1})
$CH_3COO^- + 2O_2 \rightarrow 2\ HCO_3^- + H^+$	− 844
$CH_3COO^- + 3/5\ NO_3^- + 13/5\ H^+ \rightarrow 2\ HCO_3^- + 4/5\ H_2O + 4/5\ N_2$	− 792
$CH_3COO^- + ClO_4^- \rightarrow 2\ HCO_3^- + H^+ + Cl^-$	− 966
$CH_3COO^- + 4/3\ ClO_3^- \rightarrow 2HCO_3 + H^+ + 4/3\ Cl^-$	− 1015
$1/2\ CH_3COO^- + ClO_4^- \rightarrow HCO_3^- + 1/2\ H^+ + ClO_2^-$	− 801
$ClO_2^- \rightarrow O_2 + Cl^-$	[a]
$1/2\ CH_3COO^- + O_2 \rightarrow HCO_3^- + 1/2H^+$	− 844
$CH_3COO^- + ClO_4^- \rightarrow 2\ HCO_3^- + H^+ + Cl^-$	− 822
$1/3\ CH_3COO^- + 4/3\ ClO_3^- \rightarrow 2/3\ HCO_3^- + 1/3H^+ + 4/3\ ClO_2^-$	− 787
$4/3\ ClO_2^- \rightarrow 4/3\ O_2 + 4/3\ Cl^-$	[a]
$2/3\ CH_3COO^- + 4/3\ O_2 \rightarrow 4/3\ HCO_3^- + 2/3H^+$	− 844
$CH_3COO^- + 4/3\ ClO_3^- \rightarrow 2\ HCO_3^- + H^+ + 4/3\ Cl^-$	− 825

[a] The disproportionation of chlorite does not yield energy for biosynthesis

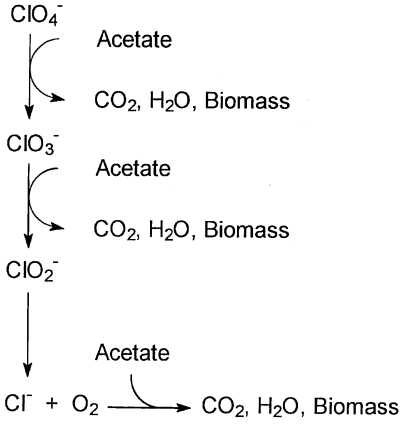

Fig. 4 Proposed reduction pathway of perchlorate catalysed by strain GR-1

activities in the presence of acetate of 0.088 and 0.043 mmol min^{-1} mg protein^{-1} respectively. The higher oxygen respiration rate ensures anaerobic conditions. Washed cell suspensions of nitrate-grown cells do not respire, demonstrating that the enzymes involved in oxygen reduction are induced in perchlorate-grown cells.

From the above we propose a three-step mechanism of perchlorate reduction in which chlorate and chlorite are intermediate products (Fig. 4). In this process, the reduction of perchlorate is rate-limiting. The reduction of perchlorate to chloride involves eight electrons, of which four are provided by acetate. Strain GR-1 dismutates chlorite to form oxygen and chloride. This dismutation involves another four electrons.

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