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Isolation of perchlorate-reducing *Azospira suillum* strain JB524 from tidal flats of the Yellow Sea

Nirmala Bardiya[1, 2*], Jaeho Bae[1]

[1]*Department of Civil and Environmental Engineering, Inha University, Inharo 100, Nam-Gu, Inchon 402-751, South Korea*

[2]*Department of Animal Sciences, The Ohio State University, and Ohio Agricultural Research and Development Center (OARDC), 304 Gerlaugh Hall, 1680 Madison Avenue, Wooster, OH-44691, USA*

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ABSTRACT

Objective: To isolate and identify perchlorate-reducing bacterium from an enriched consortium from tidal flats of the Yellow Sea.
Methods: A perchlorate-enriched consortium from tidal flats of the Yellow Sea was used to isolate *Azospira suillum* (*A. suillum*) strain JB524. The strain was identified based on partial 16S rDNA sequencing. Perchlorate reduction by the strain was tested with acetate as the e^- donor in the presence of NaCl, nitrate and at different growth temperatures using standard anaerobic techniques. The complete enzymatic destruction of perchlorate was confirmed as evolution of O_2 by chlorite dismutase in the absence of acetate.
Results: Strain JB524 shared 100% 16S rDNA sequence similarity with the type strain *A. suillum* PS^T isolated from a swine waste treatment lagoon. Perchlorate reduction coincided with concomitant increase in cell density. Although, acclimatization of the strain PS^T at suboptimal temperature for perchlorate reduction is not reported, the newly isolated strain could rapidly reduce perchlorate at 22 °C after brief acclimatization.
Conclusions: Reduction of perchlorate by *A. suillum* strain JB524 was negatively affected in the presence of NaCl, suboptimal temperature, presence of nitrate, and limiting amount of acetate as the e^- donor.

1. Introduction

Anthropogenic activities such as use of ammunitions, electroplating, organic syntheses, manufacturing of explosives and fireworks have lead to large-scale contamination of surface and groundwater with perchlorate. As an oxyanion of chlorine, perchlorate is highly soluble and mobile in water which enters the human chain through the drinking water supplies. Several studies have linked perchlorate with functional impairment of human thyroid gland[1]. After large-scale contamination with perchlorate came to light in 1997[2] several technological options for it's removal such as ion exchange, carbon adsorption, reverse osmosis, and biological treatment have been tried[3]. While most of the physico-chemical processes merely remove perchlorate and generate highly concentrated perchlorate-laden waste streams which need further treatment for safe disposal, complete destruction of perchlorate into innocuous dioxygen and chloride is possible through biological treatment with the help of ubiquitous perchlorate-reducing bacteria[4-7]. During the biological treatment of perchlorate, removal of several co-contaminants such as nitrate, 1,2-dibromo-3-chloropropane, 1,1,1-trichloroethylene and 1,2,3-trichloropropane in both pump and treat-type *ex situ* and *in situ* approaches using either acetate or H_2 as the electron donor has been demonstrated[3,8-16].

In order to make biological treatment feasible, in addition to optimization of the bioreactor configurations there is an ongoing effort to isolate novel perchlorate reducing bacteria with diverse metabolic and physiological abilities. Based on conventional isolation techniques and immuno-probe specific to dissimilatory perchlorate reducers, *Dechloromonas* (type strain *Dechloromonas agitata* CKB) and *Azospira*[17] formally *Dechlorosoma* (type strain *Dechlorosoma suillum* PS^T) within the β-subclass of the *Proteobacteria* (Rhodocyclaceae family) have been found to be the most predominant perchlorate reducers in the nature[18]. Since most of the perchlorate-reducing bacteria have been isolated from freshwater pristine and contaminated mesophilic habitats, we reasoned that complex ecological niches

*Corresponding author: Nirmala Bardiya, Department of Animal Sciences, The Ohio State University, and Ohio Agricultural Research and Development Center (OARDC), 304 Gerlaugh Hall, 1680 Madison Avenue, Wooster, OH-44691, USA.
Tel: +1 330 263 3803
E-mails: nbardiya2011@hotmail.com; bardiya.1@osu.edu
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such as tidal flats which receive a diverse array of industrial effluents and waste waters may represent novel perchlorate reducing bacteria. In line with these possibilities, through a previous study we reported enrichment and perchlorate reduction by a mixed consortium from tidal flats of the Yellow Sea[19]. As an extension of the previous work, herein we report isolation of perchlorate-reducing *Azospira suillum* (*A. suillum*) strain JB524 from the enriched consortium.

2. Materials and methods

2.1. Medium

For enrichment and growth of the perchlorate-reducing consortium and isolation of the strain an anoxic medium described earlier[20] was used with $MgSO_4$•$7H_2O$ at 10 mg/L. Filter sterilized (0.22 μm pore size) anoxic stock solutions of sodium acetate (molar mass 82.03 g/mol) and sodium perchlorate (molar mass 122.44 g/mol) were used as the electron (e^-) donor and e^- acceptor, respectively. Resazurin was omitted from the medium for analysis by spectrophotometer.

2.2. Enrichment and isolation of the strain

For collection and handling of the samples, standard anaerobic techniques were followed throughout the study[5-7]. Mud samples from tidal flats of the Yellow Sea, Inchon, South Korea were collected in nitrogen-filled and pre-sterilized (121 ˚C for 15 min) serum bottles. Further enrichment of the consortium on perchlorate was carried out as reported earlier[19]. A 2.5% inoculum (by volume) was used twice a week to establish enrichment by sub-culturing into fresh anoxic medium.

For isolation of the strain, an actively growing enriched consortium was serially diluted up to 10^{-5} and spread plate on an agar medium as described earlier[20]. Well-isolated colonies appearing after 4 to 6 days were transferred into freshly sterilized anoxic medium containing acetate and perchlorate (10 mmol/L each). The alternate tube-plate procedure was repeated 3 to 4 times to obtain the pure culture[5]. All procedures were carried out in an anaerobic glove box under the gas phase of N_2:CO_2:H_2 (85:10:5). Acetate as e^- donor and carbon source was added in molar excess to facilitate complete reduction of perchlorate unless otherwise mentioned. For all the experiments, incubation of single replicate samples was carried out at 30-34 ˚C under constant shaking (125–150 r/min) unless otherwise mentioned.

2.3. Morphology

SEM was used to determine the cell shape and size of the isolated strain on a Hitachi S-4200 electron microscope.

2.4. Amplification and partial 16s rDNA sequence determination

DNA isolation was performed using a CloneSaver™ card from Whatman (http://www.whatman.com). The 25 μL reaction mix for PCR and cycle sequence for DYE-ET terminator sequencing contained; 16.4 μL H_2O, 2.5 μL PCR buffer (10x), 5.0 μL dNTP's (1 mmol/L), 0.5 μL primer 16s 500bp F (50 pmol [5'-TGGAGAGTTTGATCCTGGCTCAG-3']), 0.5 μL primer 16s 500 bp R (50 pmol [5'-TACCGCGGCTGCTGGCAC-3']), and 0.1 μL Taq polymerase solution (5 IU/μL). This 25 μL reaction mix was added to the punches (CloneSaver™ card) and PCR was carried out in PTC-100TM programmable controller (MJ Research, Inc, USA). For amplification, initial denaturation was performed at 95 ˚C for 10 s followed by 30 cycles of 95 ˚C for 30 s, 60 ˚C for 30 s, and 72 ˚C for 45 s; and final elongation at 72˚C for 10 min.

For cycle sequencing, 10 μL mixture for forward and reversed reaction contained PCR DNA 3–10 ng (x μL), 0.5 μL forward or reverse primer (10 pmol), 1.0 μL sequencing reagent mix, 3.0 μL dilution buffer, and 5.5-(x) μL H_2O. The sequencing program comprised of 25 cycles of three steps; 95 ˚C for 20 s, 50 ˚C for 15 s, and 60 ˚C for 60 s. The rDNA sequence analysis was performed with the BLAST program[21].

2.5. Growth on perchlorate

Growth of the strain JB524 on perchlorate and acetate was started at an initial cell density of 0.006 (OD_{600} nm). Perchlorate and acetate were added at 1 056 and 1 180 mg/L, respectively. Perchlorate reduction and cell density were measured up to 70 h at 30–34 ˚C.

2.6. Effect of salinity and temperature

Growth and perchlorate reduction by strain JB524 was tested in the presence of varying concentrations of NaCl ranging from 0% to 1.0% (with an increment of 0.5 between the tests). Perchlorate concentrations were 1 021–1 074 mg/L among the treatments with 1 000 mg/L acetate.

For effect of temperature on perchlorate reduction, the strain was grown at fixed temperatures of 22 ˚C, 32 ˚C, and 40 ˚C at a starting cell density of 0.006 in the presence of perchlorate at 1 000–1 232 mg/L and molar excess of acetate (1 000 mg/L).

2.7. Effect of nitrate on perchlorate reduction

Perchlorate-enriched strain at an initial cell density of approximately 0.008 was grown in presence of only perchlorate (1 653 and 1 600 mg/L perchlorate and acetate, respectively), only nitrate (908 and 1 500 mg/L nitrate and acetate, respectively), a mixture of perchlorate and nitrate with acetate in molar excess (603, 337 and 1 000 mg/L perchlorate, nitrate and acetate, respectively) or under an acetate-limiting condition (748, 443 and 500 mg/L perchlorate, nitrate and acetate, respectively) at a temperature of 35–37 ˚C. When either perchlorate or nitrate was used as the sole e^- acceptor, acetate was added in molar excess to achieve complete reduction of the e^- acceptor.

2.8. Chlorite decomposition

An acetate depleted 48 h culture (50 mL) was centrifuged at 10000 r/min for 10 min at 4 ˚C in a Micrc 17 TR centrifuge equipped with a fixed rotor (Hanil Industrial Co., South Korea). The pellet was washed twice in sterile anoxic saline (0.85% NaCl solution) by centrifugation

and finally re-suspended in 50 mL saline in sealed bottles (300 mL capacity) to contain 62.5 mg/L total protein. Evolution of oxygen was monitored by addition of freshly prepared filter sterile anoxic 12.5 mL sodium chlorite solution (20 mmol/L) to the cell suspension in the absence of acetate. Both the cell suspension and the sodium chlorite solution were pre-equilibrated at 26 ℃ for temperature compensation. Evolution of oxygen was considered as positive for the chlorite dismutase enzyme activity which was recorded at 1 min time interval for 20 min at 26 ℃ using a pre-calibrated YSI model 58 dissolved oxygen meter (YSI Incorporation, Yellow Spring, Ohio, 45387, USA). Heat killed (80 ℃; 5 min) cell suspension served as a control and was processed in the same manner.

2.9. Analytical methods

Samples were centrifuged at 7000 r/min for 10 min and diluted to less than 3.0 mg/L final concentration with ultra pure water for analysis by ion chromatography (DX 500 Dionex). Perchlorate was analysed with IonPac AS11 analytical and IonPac AG11 guard columns. A 40-mmol/L NaOH eluent was used at a flow rate of 2 mL/min with a 100 μL sample loop. Similarly, nitrate was measured with IonPac AS14 analytical and IonPac AG14 guard columns. A 3.5 mmol/L Na_2CO_3 and a 1 mmol/L $NaHCO_3$ buffer were used as the eluent at a flow rate of 1.2 mL/min with a 20 μL sample loop. Optimal function of suppressor was ensured by an external water supply. Cell density (optical density at 600 nm) was measured with an Agilent 8453 UV-vis spectrophotometer as per the method described by American Public Health Association[22]. Total protein was estimated by micro-biuret method at 310 nm in a 1-cm silica cell and bovine serum albumin (Sigma Diagnostics, ST. Louis, USA) served as the calibration standard[23].

3. Results

3.1. Morphology

The morphological features of the strain JB524 as observed by SEM are presented in Figure 1. The cells were Gram-negative, curved motile rods of 0.50 × 3.77 μm in size. Occasionally the cells were arranged in chains and gave an appearance of a spirillum (indicated by arrows).

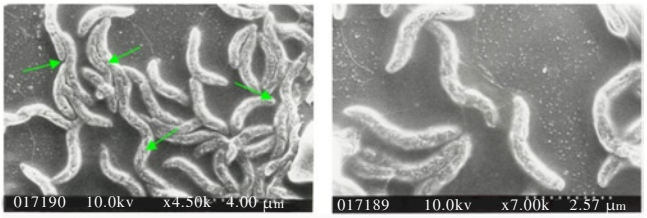

Figure 1. Scanning electron micrograph of the *A. suillum* strain JB524.

3.2. Partial 16S rDNA sequence analysis and strain identification

The 486 nucleotide sequence of 16S rDNA from the strain JB524 used for identification had a base composition of 24.90% (A), 18.31% (T), 33.13% (G), and 23.66% (C) with a purine: pyrimidine ratio of 1.38 and GC content of 56.8%.

Based on the 486 nucleotides sequenced (Figure 2), the strain JB 524 was 100% similar to the type strain *Azopsira suillum* PS^T (formally *Dechlorosoma suillum* PS^T; Figure 2a), *Dechlorosoma* sp. PCC, *Dechlorosoma* sp. Iso1, *Dechlorosoma* sp. KJ, and *Dechlorosoma* sp. PDX. An uncultured bacterium SJA-52 was 99% similar. Other closest relatives with 100% sequence similarity (based on 471 nt) included *Azospira oryzae* and *Azoarcus* sp. BS2-3. *Dechlorosoma* sp. Iso2, *Dechlorosoma* sp. SDGM, and *Dechloromonas* sp. JJ (a non-perchlorate reducer; Figure 2b), respectively shared 99%, 98%, and 93% sequence similarity. Some of the other distant relatives included *Dechloromonas* sp. RCB, *Ferribacterium limneticum* strain cda-1 and *Dechloromonas* sp. SIUL which all shared 92% similarity. Low sequence similarity among the perchlorate reducers compared to a very high sequence similarity with many of the non-perchlorate reducers shows a horizontal gene transfer of this metabolic ability among diverse perchlorate reducers.

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AS_JB: 1    ACGCTGGCGGCATGCCTTACACATGCAAGTCGAACGGCAGCACGGGAGCTTGCTCCTGGT    60
AS_PS: 26   ACGCTGGCGGCATGCCTTACACATGCAAGTCGAACGGCAGCACGGGAGCTTGCTCCTGGT    85
AS_JB: 61   GGCGAGTGGCGAACGGGTGAGTAATACATCGGAACGTACCCAGGAGTGGGGGATAACGTA    120
AS_PS: 86   GGCGAGTGGCGAACGGGTGAGTAATACATCGGAACGTACCCAGGAGTGGGGGATAACGTA    145
AS_JB: 121  GCGAAAGTTACGCTAATACCGCATATTCTGTGAGCAGGAAAGCGGGGGATCGCAAGACCT    180
AS_PS: 146  GCGAAAGTTACGCTAATACCGCATATTCTGTGAGCAGGAAAGCGGGGGATCGCAAGACCT    205
AS_JB: 181  CGCGCTCTTGGAGCGGCCGATGTCGGATTAGCTAGTTGGTGAGGTAAAAGCTCACCAAGG    240
AS_PS: 206  CGCGCTCTTGGAGCGGCCGATGTCGGATTAGCTAGTTGGTGAGGTAAAAGCTCACCAAGG    265
AS_JB: 241  CGACGATCCGTAGCAGGTCTGAGAGGATGATCTGCCACACTGGGACTGAGACACGGCCCA    300
AS_PS: 266  CGACGATCCGTAGCAGGTCTGAGAGGATGATCTGCCACACTGGGACTGAGACACGGCCCA    325
AS_JB: 301  GACTCCTACGGGAGGCAGCAGTGGGGAATTTTGGACAATGGGGGCAACCCTGATCCAGCC    360
AS_PS: 326  GACTCCTACGGGAGGCAGCAGTGGGGAATTTTGGACAATGGGGGCAACCCTGATCCAGCC    385
AS_JB: 361  ATGCCGCGTGAGTGAAGAAGGCCTTCGGGTTGTAAAGCTCTTTCGGCGGGGAAGAAATGG    420
AS_PS: 386  ATGCCGCGTGAGTGAAGAAGGCCTTCGGGTTGTAAAGCTCTTTCGGCGGGGAAGAAATGG    445
AS_JB: 421  CAACGGCTAATATCCGTTGTTGATGACGGTACCCGCATAAGAAGCACCGGCTAACTACGT    480
AS_PS: 446  CAACGGCTAATATCCGTTGTTGATGACGGTACCCGCATAAGAAGCACCGGCTAACTACGT    505
AS_JB: 481  GCCAGC 486
AS_PS: 506  GCCAGC 511
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Figure 2a. Partial 16S rDNA sequence similarity of the *A. suillum* strain JB524 with *A. suillum* PS^T (formerly *Dechlorosoma suillum* -AF170348), Identities = 486/486 (100%)

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AS_JB: 1    ACGCTGGCGGCATGCCTTACACATGCAAGTCGAACGGCAGCACGGGAGCTTGCTCCTGGT   60
DE_JJ: 22   ACGCTGGCGGCATGCCTTACACATGCAAGTCGAACGGCAGCACGGGAGC_ _AATCCTGGT   79
AS_JB: 61   GGCGAGTGGCGAACGGGTGAGTAATACATCGGAACGTACCCAGGAGTGGGGGATAACGTA   120
DE_JJ: 80   GGCGAGTGGCGAACGGGTGAGTAATGTATCGGAACGTACCTTTCAGTGGGGGATAACGTA   139
AS_JB: 121  GCGAAAGTTACGCTAATACCGCATATTCTGTGAGCAGGAAAGCGGGGGATCGCAAGACCT   180
DE_JJ: 140  GCGAAAGTTACGCTAATACCGCATATTCTGTGAGCAGGAAAGCAGGGGATCGCAAGACCT   199
AS_JB: 181  CGCGCTCTTGGAGCGGCCGATGTCGGATTAGCTAGTTGGTGAGGTAAAAGCTCACCAAGG   240
DE_JJ: 200  TGCGCTGATTGAGCGGCCGATATCAGATTAGCTAGTTGGTGAGGTAAAGGCTCACCAAGG   259
AS_JB: 241  CGACGATCCGTAGCAGGTCTGAGAGGATGATCTGCCACACTGGGACTGAGACACGGCCCA   300
DE_JJ: 260  CGACGATCTGTAGCGGGTCTGAGAGGATGATCCGCCACACTGGAACTGAGACACGGTCCA   319
AS_JB: 301  GACTCCTACGGGAGGCAGCAGTGGGGAATTTTGGACAATGGGGGCAACCCTGATCCAGCC   360
DE_JJ: 320  GACTCCTACGGGAGGCAGCAGTGGGGAATTTTGGACAATGGGGGCAACCCTGATCCAGCC   379
AS_JB: 361  ATGCCGCGTGAGTGAAGAAGGCCTTCGGGTTGTAAAGCTCTTTCGGCGGGGAAGAAATGG   420
DE_JJ: 380  ATGCCGCGTGAGTGAAGAAGGCCTTCGGGTTGTAAAGCTCTTTCGGCCGGGAAGAAATCG   439
AS_JB: 421  CAACGGCTAATATCCGTTGTTGATGACGGTACCCGCATAAGAAGCACCGGCTAACTACGT   480
DE_JJ: 440  CATGGGTTAATACCCTGTGTGGATGACGGTACCGGAATAAGAAGCACCGGCTAACTACGT   499
AS_JB: 481  GCCAGC  486
DE_JJ: 500  GCCAGC  505
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Figure 2b. Partial 16S rDNA sequence similarity of the *A. suillum* strain JB524 with *Dechloromonas* sp. JJ (AY032611.1) Identities = 452/486 (93%), Gaps = 2/486 (nucleotides differences are highlighted in red)

3.3. Growth on perchlorate

Growth and perchlorate reduction by *A. suillum* strain JB 524 is illustrated in Figure 3. The strain completely reduced approximately 1056 mg/L of perchlorate in less than 45 h. During the first 30 h of growth approximately 16% of added perchlorate (175 mg/L) was reduced with concomitant increase in cell density from an initial cell density of 0.006 (OD_{600} nm) to 0.074. The remaining 880 mg/L was reduced between 30 h and 45 h of incubation with further increase in cell density to 0.225. Beyond 45 h, once perchlorate reached to an undetectable level growth started to decline gradually, indicating dependence of the strain on perchlorate as the terminal e^- acceptor. During the active perchlorate reduction phase (*i.e.* between 30 h and 45 h), the strain reduced perchlorate at an average rate of 58 mg/L/h resulting in an average increase in cell density by 0.01/h.

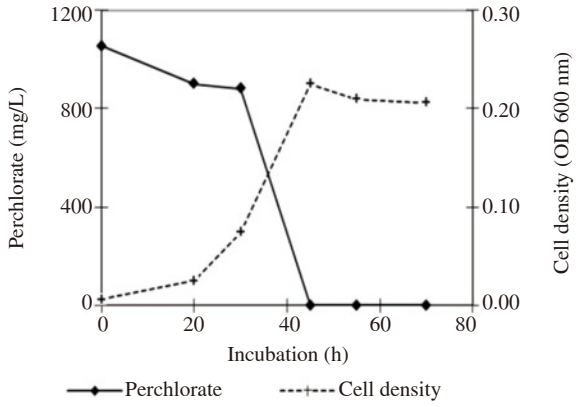

Figure 3. Growth and perchlorate reduction by the *A. suillum* strain JB524

3.4. Effect of salinity on perchlorate reduction

Removal of perchlorate by ion exchange and subsequent regeneration of resins typically generates highly saline (7%–12% salinity) perchlorate-laden brines which require adequate biological treatment with salt tolerant perchlorate reducing bacteria. Since *A. suillum* strain JB524 was enriched from a marine sample, we tested its ability to reduce perchlorate up to 1% salinity level (Figure 4a). Complete reduction of perchlorate was observed only in fresh water medium. At 0% NaCl concentration approximately 1000 mg/L of perchlorate was reduced in less than 48 h. However, at 0.5 % NaCl, perchlorate reduction by the strain was drastically inhibited and only 7% of added perchlorate could be reduced during the same duration.

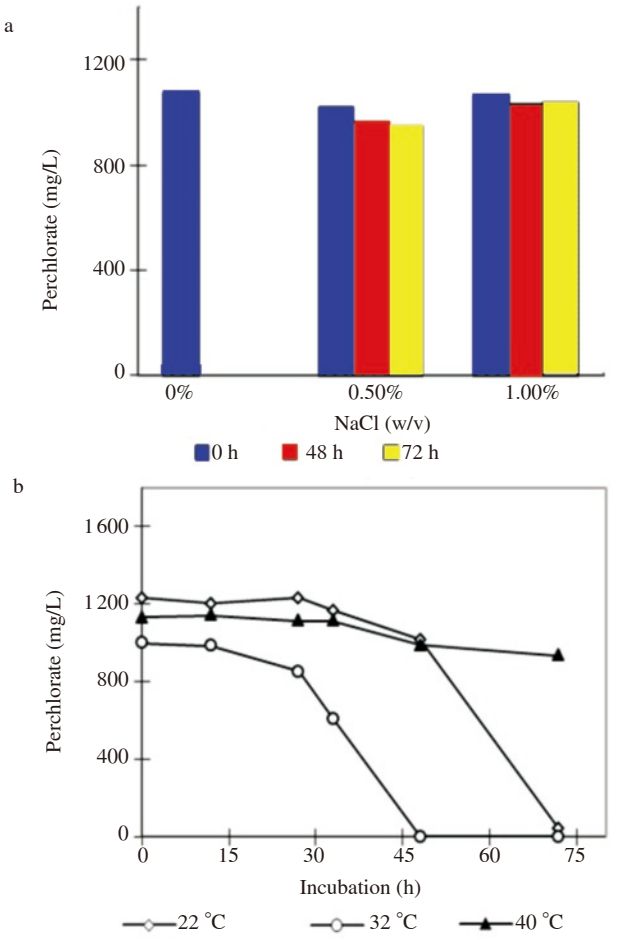

Figure 4. a: Effect of salinity on perchlorate reduction by the *A. suillum* strain JB524; b: Perchlorate reduction at different temperatures by the *A. suillum* strain JB524.

3.5. Effect of temperature on perchlorate reduction

Similar to optimal growth at 37 ˚C of *A. suillum* PS^T which was isolated from a swine waste treatment lagoon[18] the newly isolated *A. suillum* strain JB524 from the tidal flats had optimal growth above 30 ˚C (Figure 4b). Perchlorate reduction by the strain was severely affected both at low (22 ˚C) and high (40 ˚C) temperature, and only 18% and 13% of the reduction could be observed in first 48 h, respectively. However, after this slow reduction at 22 ˚C, the strain could completely reduce over 1 000 mg/L of perchlorate in next 24 h, suggesting towards its ability to acclimatize at low temperature.

3.6. Effect of nitrate on perchlorate reduction

The perchlorate-enriched strain JB524 could reduce not only perchlorate (Figure 5a) but also nitrate (Figure 5b) when the two e^- acceptors were provided separately and the strain was grown at 35–37 ˚C. In fact, reduction of nitrate was faster than perchlorate and no lag phase was observed in either case. Near complete reduction of 900 mg/L nitrate occurred in less than 20 h with corresponding reduction of 800 mg/L perchlorate during the same duration.

The ability of the strain to reduce perchlorate in the presence of nitrate was tested both under acetate-limiting and acetate-sufficient conditions. Stoichiometrically, for the complete reduction of 1 mol of nitrate to N_2 about 5/3 mol of acetate is required whereas for the reduction of 1 mol of perchlorate 1 mol of acetate is needed[24]. Under acetate-limiting condition, nitrate was preferentially utilized and approximately 425 mg/L nitrate (95% of added nitrate) was reduced within 20 h (Figure 5c). However, when acetate was supplied in molar excess, reduction of perchlorate and nitrate could proceed simultaneously (Figure 5d). During the first 20 h approximately 540 mg/L (89% of perchlorate) and 325 mg/L (97 % of nitrate) could be reduced.

3.7. Chlorite decomposition

Presence of chlorite dismutase enzyme activity in strain JB524 was confirmed by evolution of oxygen when whole-cell suspension was exposed to sodium chlorite in the absence of acetate (Figure 6a). During 20 min reaction, approximately 1.93 mg O_2 was evolved which was 24% of the added O_2 (8 mg) from sodium chlorite. Maximum evolution of O_2 occurred during the first 1 min followed by gradual decrease in O_2 evolution rate over the 20 min reaction time (Figure 6b) implying towards hypochlorite induced irreversible inactivation of chlorite dismutase. During the initial 10 min, O_2 evolved at a consistent rate of 0.07–0.08 mg/min which first reduced to 0.06 mg/min at the 11th min and then to 0.04 mg/min at the 15th min of the reaction time. The heat-killed whole-cell suspension (80 ˚C; 5 min) tested negatively for the enzyme activity.

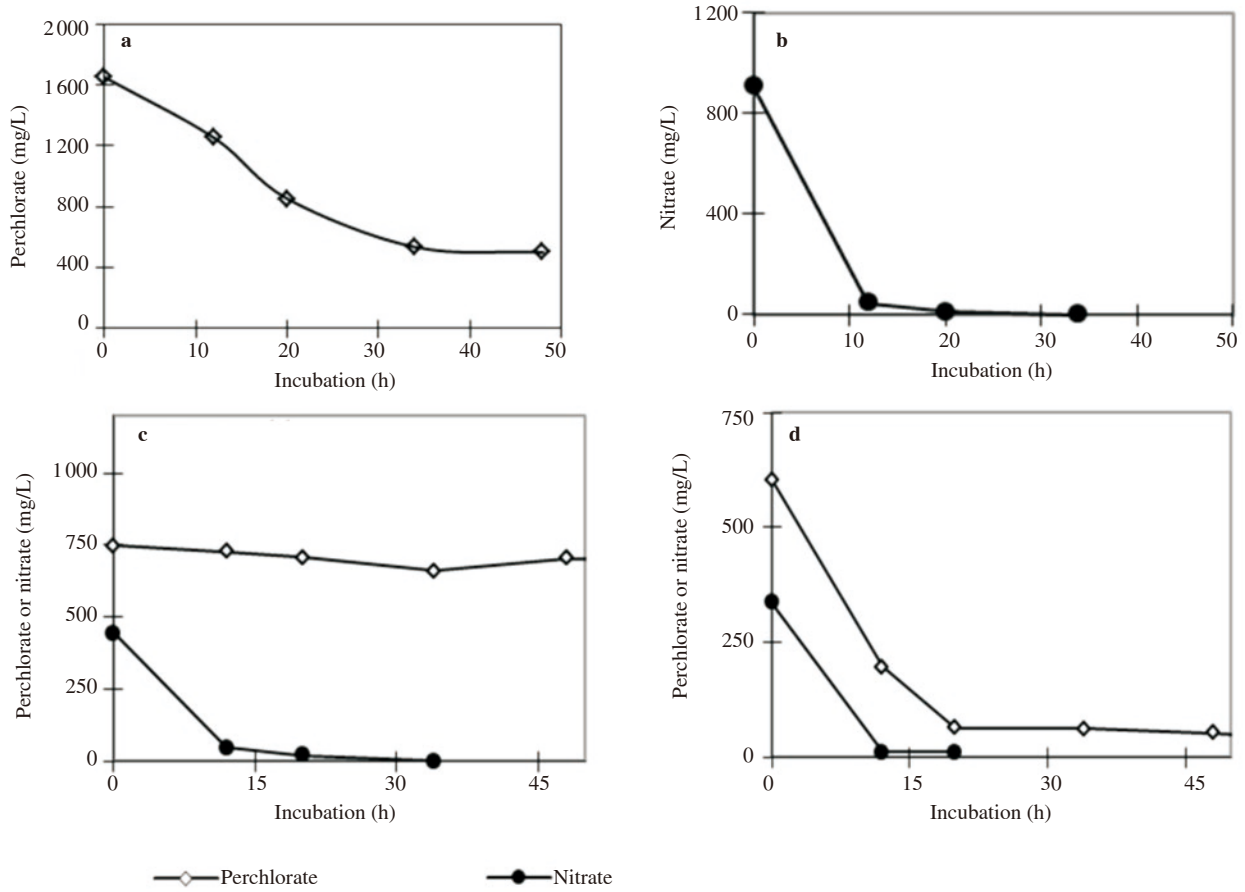

Figure 5. Perchlorate reductions by the *A. suillum* strain JB524 in the presence or absence of nitrate.
a: Only perchlorate; b: Only nitrate; c: Perchlorate and nitrate with limiting acetate; d: Perchlorate and nitrate with sufficient acetate.

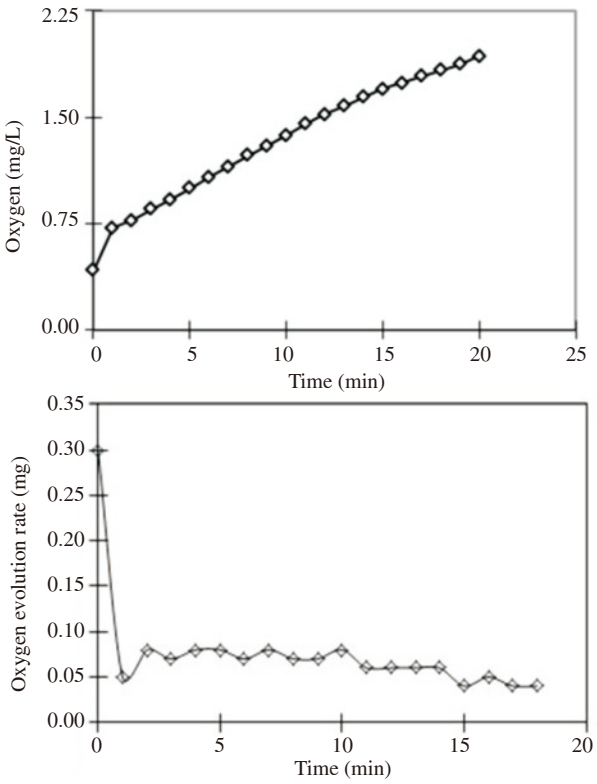

Figure 6. Chlorite decomposition by the *A. suillum* strain JB524.
a: Evolution of O_2; b: Rate of O_2 evolution.

4. Discussion

Though *A. suillum* strain JB524 was isolated from a marine sample, it was genetically and physiologically similar to the type strain *A. suillum* PS^T reported from a swine waste treatment lagoon. The two strains shared 100% sequence similarity in the 16S rDNA region suggesting towards similar % GC content which was however, slightly lower than the average 65.3% GC content reported for the 2.5 Mb genome of *A. suillum* PS^T[25]. During growth on perchlorate average increase in cell density of strain JB524 was also within the range (0.012 to 0.280/h) observed for various perchlorate-reducing consortia and pure cultures under heterotrophic conditions[6,19,24,26-28].

Environmental factors such as presence of NaCl, nitrate and temperature affected perchlorate-reducing ability of the strain JB524 in a manner reported earlier for various dissimilatory perchlorate-reducing bacteria. Inhibition up to 30% and 40%, respectively at 0.5% and 1.0% NaCl has been commonly observed during dissimilatory perchlorate reduction[29]. However, few enriched consortia and isolates obtained from marine environments have been shown to reduce perchlorate up to 3%–6% salinity[30-32]. Improvement in culture stability and sustained perchlorate reduction under high salinity conditions has also been reported with supplementation of Ca^{2+}, Mg^{2+} and K^+ in the growth medium[33]. Perchlorate reduction by the strain JB524 at submesophilic temperature was similar to perchlorate reduction by an acclimatized consortium above 10 °C[34], a *Citrobacter* sp. between 20–35 °C with considerable less reduction at 40 °C[35], by a marine bacterium *Arcobacter* sp. strain CAB at 30 °C[32] and by *Acinetobacter bereziniae* strain GWF at 30 °C[36]. Similarly, simultaneous perchlorate and nitrate reduction has also been observed for few perchlorate reducers[37,38].

In spite of phylogenetic dissimilarities, perchlorate reducers share one common feature *i.e.* chlorite decomposition as a central step during the perchlorate and chlorate respiration, mediated by chlorite dismutase enzyme. Previous studies proposed a non-energy yielding mechanism in which chlorite is dismutated into dioxygen and chloride by the non-respiratory chlorite dismutase enzyme[24,39]. However, emerging evidences from biochemical, biophysical and molecular dynamics simulations revealed transient formation of hypochlorite and compound I intermediates, and hypochlorite-induced irreversible inactivation of the chlorite dismutase enzyme. A gradual decrease in O_2 evolution rate and recovery of only about one fourth of the added O_2 (as sodium chlorite) with whole cell suspension of strain JB524 during chlorite decomposition implies towards a similar inactivation of chlorite dismutase enzyme as contribution of water in O_2 formation has been ruled out[40-46].

From this study, we can concluded that 1) *A. suillum* strain JB524 could reduce perchlorate with acetate as the e^- donor; 2) *A. suillum* strain JB 524 shared 100% rDNA sequence similarity with the type strain *A. suillum* PS^T; 3) the isolated strain also shared 93% sequence similarity with a non-perchlorate reducing strain *Dechloromonas* sp. JJ; 4) perchlorate reduction was inhibited in the presence of NaCl; 5) perchlorate reduction was also affected at suboptimal growth temperatures; 6) nitrate was preferentially utilized when limiting amount of acetate was supplied as the e^- donor; 7) Simultaneous reduction of both perchlorate and nitrate occurred in the presence acetate when supplied in molar excess.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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