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**Insights into chromate resistance of *Shewanella decolorationis* LDS1**

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Running Head: Properties of *S. decolorationis* LDS1

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## Abstract

The *Shewanella* genus is well known for its genetic diversity, its outstanding respiratory capacity and its high potential for bioremediation. Here, a novel strain isolated from sediments of the Indian Ocean was characterized. A 16S rRNA analysis indicated that it belongs to the *S. decolorationis* species. It was named *Shewanella decolorationis* LDS1. This strain presented an unusual ability to grow efficiently from 24 °C to 40 °C, without apparent modifications of its metabolism as shown by testing respiratory activities or carbon assimilation and in a wide range of salt concentrations. Moreover, *S. decolorationis* LDS1 tolerates high chromate concentrations. Indeed, it was able to grow in the presence of 4 mM chromate at standard temperature and 3 mM at 40 °C. Interestingly, whatever the temperature, when the culture reached the stationary phase, the strain reduced the chromate present in the growth medium. In addition, *S. decolorationis* LDS1 degrades different toxic dyes including anthraquinone-, triarylmethane- and azo-dye. Thus, compared to *S. oneidensis*, this strain presented better capacity to cope with various abiotic stresses in particular at high temperatures. The analysis of genome sequence preliminary data indicated that, in contrast to *S. oneidensis* and *S. decolorationis* S12, *S. decolorationis* LDS1 possesses the phosphorothioate modification machinery that has been described to participate to survival against various abiotic stresses by protecting DNA. We demonstrate that its heterologous production in *S. oneidensis* allows it to resist higher concentrations of chromate.

## Importance

*Shewanella* species have long been described as interesting microorganisms in regards to their ability to reduce many organic and inorganic compounds including metals. However, members of the *Shewanella* genus are often depicted as cold water microorganisms although usually their optimal growth temperature ranges around 25-28 °C under laboratory growth conditions. *S.*

*decolorationis* LDS1 is highly attractive since its metabolism allows it to develop efficiently from 24 to 40 °C conserving its ability to respire alternative substrates and to reduce toxic compounds such as chromate or toxic dyes. Our results clearly indicate that this novel strain has the potential to be a powerful tool for bioremediation and unveil one of the mechanisms involved in the chromate resistance.

## Introduction

*Shewanella* species are part of the gammaproteobacteria class of the Proteobacteria phylum. They have been found all around the world in briny or fresh water and sediments from polar circles to equatorial biotopes (1, 2). Their trade mark is their tremendous variety of respiratory capacities allowing their growth with a large panel of exogenous electron acceptors including solid and soluble metals, explaining why they belong to the restricted family of dissimilatory metal-reducing bacteria (3). Partly due to their reducing abilities, *Shewanella* species have the capacity to cope with elevated concentrations of toxic products as for example hydrocarbons, arsenate and hexahydro-1,3,5-trinitro-1,3,5-triazine (4–9). This ability has made them a useful tool for bioremediation and clean-up (1–3, 10, 11). Moreover, it appears that the members of this genus adapt easily to various life conditions. Although they are usually found in aquatic habitats, some can develop in other biotopes like butter, crude oil or even human corpses (1, 2, 12).

Within the genus, *S. oneidensis* MR-1 has become the strain of reference in many laboratories and it is clearly the best described. As most of the other species, its optimal growth temperature is around 25-28 °C (13) and it requires the HtpG general chaperone to survive at 35 °C (14). It can grow in either brackish or fresh water and it possesses an efficient chemosensory system that allows it to adapt rapidly under nutrient deprivation or stressful conditions (15–17). It was shown that *S. oneidensis* MR-1 survives to elevated concentrations of chromate by developing

several resistance strategies like oxidative stress protection, detoxification, SOS-controlled DNA repair mechanisms and different pathways for Cr(VI) reduction (18, 19). Indeed, in addition to enzymatic reduction of chromate (hexavalent chromium Cr(VI)) into a less mobile form (Cr(III)) that presents a decreased toxicity (20–23), *S. oneidensis*, as other members of the genus, possesses a chromate efflux pump able to extrude chromate from the cell increasing its resistance against the metal (24).

Although, *S. oneidensis* is the model bacterium of the genus, many other species are studied because, beside their common characteristics, they present peculiar aspects conferring useful properties especially for biotechnology (1–3, 25). Thus, these bacteria often have been isolated from polluted areas, waste water or sewage plants. Recently, the genome of *Shewanella algidipiscicola* H1, isolated from the sediment of the industrial Algerian Skikda harbor, was sequenced (26). This strain is proposed to be the most resistant *Shewanella* in chromium-containing medium. A second strain highly resistant to toxic metals, *Shewanella* ANA-3 comes from water contaminated with arsenate that the bacterium uses as a respiratory substrate (5). *Shewanella halifaxensis*, isolated from an immersed munition-dumping zone, can degrade explosive compounds to generate respiratory substrates (9). As a last example, *Shewanella decolorationis* S12 capable of efficient dye-decolorization activity was isolated from sludges of a textile-printing waste-water treatment plant in China (27). Thus, in the *Shewanella* genus, the various species present distinctive properties that make them genuine tools to cope with a large range of human environmental misconducts.

This large panel of enzymatic activities is correlated to the genetic variability found in the *Shewanella* genus and the latter was difficult to define correctly because of the diversity of the species it contains (1–3, 25). This point is illustrated by the increasing number of *Shewanella* species found in databases and corresponding to almost 70 species and 30 sequenced genomes. The high diversity of *Shewanella* genomes is really astonishing. Recently, pan-genome analyses

of 24 *Shewanella* strains established that the core genome corresponds to only 12 % of the total genes (25). This small number of shared genes is consistent with a genus that is genetically versatile and has had frequent lateral gene transfer events.

The purpose of the current study is to characterize a novel strain, *S. decolorationis* LDS1 to gain greater insight into the *Shewanella* genus behaviors in bioremediation and depollution. Since *S. decolorationis* LDS1 reduced chromate into a less soluble and thus less toxic compound, decolorized various dyes, both in a large range of temperatures, and possessed a DNA modification system that is suspected to protect bacteria against environmental stresses, this novel strain presents an interesting biotechnological potential.

## Results and Discussion

### Strain isolation and characterization.

*S. decolorationis* strain LDS1 (for La Digue Seychelle and also named *S. decolorationis sesselensis*, see accession number) was isolated from a sample of sediments collected in a pond at the Anse Coco beach (La Digue Island, Republic of Seychelles, Indian Ocean) on the basis of its capability to grow on rich medium containing trimethylamine oxide (TMAO). Indeed, the latter is a compound widespread in marine environments, and with a few exceptions, it is used as a major respiratory substrate by *Shewanella* species (28–31). Based on a comparison of the conserved 16S rRNA genes of over thirty *Shewanella* species, a phylogenetic tree was constructed (Figure 1). The *Shewanella* genus can be divided into two types, the mesophilic and the piezotolerant species (32, 33). *S. decolorationis* LDS1 was among mesophilic subgroup in the clade containing the two other defined strains of *S. decolorationis* species with which it shared 100 % 16S rRNA gene identity (27, 34). These species known for their decolorization activities are described to grow at higher temperatures than *S. oneidensis* and

most other species, since they survive at 40 °C although presenting an optimal growth from 20 to 30 °C.

Since *Shewanella* species are originally marine bacteria (2), *S. decolorationis* LDS1 was cultivated at various salt concentrations. It was able to grow from very low to high concentrations of salt, even higher than that of the ocean (about 0.5 M, Figure 2A) (17). *S. decolorationis* LDS1 was then phenotypically characterized in order to test its ability to grow at high temperature and to decolorize dyes, and thus to explore if it exhibits different features compared to the already known strains of the clade. The strain was cultivated at various temperatures from 24 to 42 °C and the exponential growth rates are presented in Figure 2B. As the other *S. decolorationis* strains (27), *S. decolorationis* LDS1 could grow at more elevated temperatures than most of the other *Shewanella* species and, although less efficient, growth still occurred at 42 °C. Interestingly, in contrast to the other *Shewanella* species described, *S. decolorationis* LDS1 showed a uniquely wide optimal growth temperature since it presented a plateau ranging from 24 to 40 °C. *S. decolorationis* LDS1 was then challenged for its ability to decolorize Remazol Brilliant Blue, Crystal violet and Congo red, which are members of the three families of dyes: anthraquinone, triarylmethane and azo dye, respectively. As expected for the strains of this species, the dyes were at least partially converted by *S. decolorationis* LDS1 at 28 °C (Figure 2C). We used this property to check the efficiency of the strain at 40 °C, and as shown in Figure 2C, *S. decolorationis* LDS1 was still able to decolorize the three families of dyes. At both temperatures, the color of the media without bacteria remained identical for more than ten days (Figure 2C). This strain seems to be able to decolorize dyes including anthraquinone-, triarylmethane- and azo-dye from 28 to 40 °C. This characterization suggests that *S. decolorationis* LDS1 presents an adapted metabolism that allows it to cope efficiently from medium to elevated temperatures.

To better determine the physiological capacities of the strain at high temperature, we decided to compare its TMAO reductase activity at 28 and 40 °C. The TMAO reductase activity was first followed through the alkalization of the growth medium indicating that the increase of the temperature has no effect since the alkalization of the medium was similar (Table 1). For a more accurate activity, we measured the TMAO reductase activity of crude extracts of cells grown at 28 or 40 °C. The TMAO reductase specific activity of this strain at 40 °C was half that at 28 °C ( $2.8 \pm 0.17$   $\mu\text{mol/min/mg}$  versus  $5.95 \pm 0.40$   $\mu\text{mol/min/mg}$ , respectively). Measures of nitrate reduction and other phenotypic characteristics obtained with API20NE plates (Biomérieux, France) are recorded in Table 1 and showed no significant difference at 28 and 40 °C, except for the hydrolysis of capric acid that could be due to the instability of the product rather than the activity of the enzyme. Altogether these results suggest that the strain has fully adapted from medium to elevated temperatures by increasing the range of its optimal growth temperature. The tested respiratory activities (oxygen, TMAO, nitrate) confirmed that they were efficient from 28 to 40 °C. In contrast to *S. decolorationis* S12 and the other strains presented in Table 2, *S. decolorationis* LDS1 could not grow at very low temperature. The fact that this strain seemed to have lost the latter ability was not surprising considering that the biotope from where it was isolated probably does not encounter temperature below 25 °C.

Other differences could be pointed out. Indeed, counter to the *S. decolorationis* S12, *S. decolorationis* LDS1 as many other *Shewanella* species did not use glucose for fermentation although it performed its assimilation (Table 2). Moreover, the range of optimal pH for growth ranged from 6.0 to 11.0, which was slightly wider compared to that of other *Shewanella* strains (27). This probably results from the sampling of *S. decolorationis* LDS1 from brackish water that can be more acidic than sea water. Altogether, these results indicate that the *S. decolorationis* LDS1 shares with other *S. decolorationis* strains the ability to grow at higher temperatures than most of the other *Shewanella* species. However, only *S. decolorationis* LDS1



presents the same growth rate from 24 to 40 °C. Moreover, it appears that its metabolism is not drastically affected by high temperature since most of the tested activities were similar or still measurable whatever the growth temperature.

### **Chromate resistance and reduction.**

Recent studies have focused on the ability of *Shewanella* species to reduce chromate (19, 21-24). For instance, *S. oneidensis* MR-1 can resist 1 mM chromate; whereas, *S. algidipiscicola* H1 grows in 3 mM of chromate (18, 26). In order to compare the resistance against chromate of *S. decolorationis* LDS1 with that of the model bacterium *S. oneidensis*, a preliminary test was done on solid medium containing a filter soaked with a 1 M-chromate solution, leading to the formation of a gradient of chromate by diffusion in the solid medium. As shown in Figure 3A, *S. decolorationis* LDS1 was more resistant than *S. oneidensis* at 28 °C since it was able to grow closer to the filter. Moreover, in liquid medium, *S. oneidensis* barely resisted 1 mM chromate (18) while this concentration did not affect *S. decolorationis* LDS1 (Figure 3B). This indicates that *S. decolorationis* LDS1 can resist higher chromate concentrations. To confirm this point, its growth was followed under aerated conditions at 28 °C in the presence of increasing concentrations of chromate. As shown in Figure 3B, no drastic growth difference was noticeable in the range of chromium concentration from 0 to 2 mM, although a short lag phase was observed at 2 mM. In the presence of 3 and 4 mM chromate, the cell growth presented a pronounced lag phase of about 20 hours, but finally the curves reached the same plateau value in comparison to the cultures grown at lower concentrations or without the addition of chromate. This long lag phase could be necessary for the bacteria to adapt to the high chromate concentrations by producing either Cr(VI) reducing systems or Cr efflux pumps. In addition, the chromate resistance was followed at 40 °C. The results indicate that the growth is not

modified until 1 mM of chromate in the medium. At higher concentrations, the lag phases were increased (15 and 30 hours for 2 and 3 mM, respectively) while at 4 mM no growth was observed after 40 hours. Altogether these data establish that *S. decolorationis* LDS1 resists chromate even at elevated temperatures.

Finally, we wondered whether *S. decolorationis* LDS1 was able to reduce chromate during its growth. This is of great interest since chromate reduction leads to a less soluble and less toxic form for the environment (20, 23). Thus, *S. decolorationis* LDS1 was grown at 28 °C, in the presence of 1 mM of chromate to guaranty a robust growth, and reduction of Cr(VI) to Cr(III) in the growth medium was followed. Strikingly, when the medium was inoculated with cells of *S. decolorationis* LDS1, the Cr reduction appeared when the culture entered into the stationary phase (Figure 4). From that time, the reduction of chromate was almost complete within 15 hours. A similar experiment was performed at 40 °C. As for the lower temperature, the reduction of chromate started when the cells reached the stationary phase (Figure 4). From these results, it is clear that *S. decolorationis* LDS1 can reduce chromate at high temperature with the same efficiency as at 28 °C. The mechanism involved is clearly acting from the beginning of the stationary phase as suggested by the trigger of the reduction when the bacteria reach it, as already described under anaerobic growth of *S. oneidensis* MR-1 (23).

#### **Data from genome sequence.**

Since *S. decolorationis* LDS1 was able to cope better with various stresses than does *S. oneidensis* and has a better heat resistance than *S. decolorationis* S12, we wondered whether this strain has specific machineries leading to an increase of its resistance to environmental cues. Indeed, *Shewanella* species are described to present a high level of genetic variability that confers to each of them peculiar properties (3, 25). The genome of *S. decolorationis* LDS1 was sequenced at the Molecular Research LP (MR DNA) Laboratory (USA). A preliminary analysis

212 indicated that the draft genome sequence contained 4223 potential protein-coding genes. A  
213 genome comparison was undertaken between the two sequenced *S. decolorationis* strains (S12  
214 and LDS1) and *S. oneidensis* MR-1 (Table 3). Among genes shared by the two other species,  
215 more than 40 code for *c*-type cytochromes, which are a trade mark of bacteria of *Shewanella*  
216 genus. However, the results indicated that 12 % of *S. decolorationis* LDS1 genome was not  
217 shared by the two other strains. Among the 259 potential genes present only in  
218 *S. decolorationis* LDS1 genome, 153 have been annotated but 99 of them have an unknown  
219 function (Table 4). From the other 54 genes, several of them drew our attention (Table 4). For  
220 instance, we noticed two operons that could play a central role in the adaptation of the strain to  
221 environmental stresses. First, the *dndBCDE* operon that encodes the phosphorothioate (PT)  
222 modification machinery that replaces a nonbridging oxygen by a sulfur in the DNA sugar  
223 phosphate backbone (35–37) (Table 4). The sequences recognized by the modification  
224 machinery are not fully established and studies have shown that only 12 % of the potential sites  
225 recognized by the PT system are effectively modified and from one cell to another, the  
226 modifications are not conserved (38, 39). Moreover, it seems that when the potential target site  
227 is present in the binding motif of an important regulatory protein, no modification occurs. This  
228 was demonstrated for TorR and RpoE binding sites (38). In *Shewanella* species the Tor system,  
229 which is regulated by TorR, is a major respiratory system (40) and RpoE controls the expression  
230 of stress response genes (41). Generally, the modification machinery includes an additional  
231 protein, DndA, a cysteine desulfurase (42). The *dndA* gene is absent in *S. decolorationis* LDS1  
232 and, as described in literature, it is probably replaced in the machinery by IscS  
233 (SDECO\_v1\_40139) (39). A bioinformatics analysis using DndC sequence as a probe indicated  
234 that only three unrelated species of *Shewanella*, *S. baltica*, *S. pealeana* ATCC 700345 and  
235 *S. violacea* DSS12 possess this machinery with 92.8 %, 67.8 % and 66.5 % of identity,

respectively. This probably indicates that *S. decolorationis* LDS1 has acquired the genes of the Dnd machinery from another bacterial genus.

*pthFGH*, the other interesting operon (43), is found in the same locus as the *dndBCDE* operon. It is related to the *dnd* gene products and it encodes DNA restriction enzymes, and they form together the modification-restriction (M-R) system. In many bacteria producing the Dnd machinery, the restriction system is absent (38). The inability of *S. decolorationis* LDS1 to grow at low temperature is noticeable. Indeed, it was established in *E. coli* B7A and in *Salmonella enterica* serovar Cerro 87 that the restriction system is temperature dependent and is activated at 15 °C (44). The temperature dependence of the restriction system in *S. decolorationis* LDS1 is yet to be determined as well as the role of that restriction system once activated.

#### **Protection by the DndBCDE machinery.**

The function of this M-R system is not well understood and an additional role in the epigenetic control of gene expression is suspected (39). Indeed, the products of the *dnd* operon were shown to sustain bacterial growth under stresses like H<sub>2</sub>O<sub>2</sub>, heat or salinity by protecting DNA from oxidative damage and restriction enzymes (44). In agreement with experiments done in *Escherichia coli* and *S. piezotolerans*, our hypothesis is that the production of DndB-E proteins improves the growth of *S. decolorationis* LDS1 under oxidative conditions. The presence of chromate leads to oxidative damages in bacteria. Thus, to investigate whether the *dnd* operon plays a role in the robustness of *S. decolorationis* LDS1 towards chromate, *dndBCDE* genes carried by the pBAD33 vector (*pdndBCDE*) were introduced into *S. oneidensis* MR-1. *S. oneidensis* MR-1 harboring either *pdndBCDE* or pBAD33 vector and *S. decolorationis* LDS1

were grown aerobically in the presence of arabinose to induce the *dndBCDE* expression from the plasmid, and serial dilutions were spotted on plates containing chromate (0.4 mM) (Figure 5). The growth of *S. oneidensis* MR-1 harboring pBAD33 was drastically affected, whereas when carrying *pdndBCDE*, the strain was able to grow. Growth of *S. decolorationis* LDS1 was similar in the presence or absence of chromate. This result indicates that the presence and expression of the *dndBCDE* operon allow *S. oneidensis* to resist higher chromate concentrations. However, since *S. decolorationis* LDS1 resists better and also higher concentrations than the recombinant *S. oneidensis* MR-1 strain, we suggest that the DndBCDE machinery is only one element of the bacterial equipment allowing the high level of resistance of *S. decolorationis* LDS1. To ascertain the effect due to the expression of *dndBCDE* operon in *S. oneidensis* MR1, the reverse experiment was done and increased chromate concentration were spotted on a lawn of bacteria. As shown on figure 5B, the presence of *dndBCDE* genes led to a better resistance of the strain after 24- and 48h incubation. Moreover, we tested whether the DndBCDE machinery led *S. oneidensis* MR-1 to develop at temperatures higher than 28 °C. At both 38 and 40 °C, the growth of *S. oneidensis* MR-1 was not improved by the presence of DndBCDE suggesting that this machinery is not sufficient to allow temperature resistance.

## Conclusion.

In conclusion, the novel *S. decolorationis* LDS1 strain is an intriguing organism since it is adapted to higher temperature than most of the other members of this genus. Moreover, it keeps its enzymatic capacity in a large range of elevated temperatures and under these conditions, its metabolism seems not perturbed. Indeed, its ability to degrade the three families of dyes tested in this study is conserved even at high temperature. We can hypothesize that *S. decolorationis* LDS1 probably degrades other pollutants. Its capacity to tolerate high

concentrations of chromate in the culture medium indicates that *S. decolorationis* LDS1 has developed strategies to cope with chromate toxicity. Indeed, from the results obtained here, it appears that during exponential growth, the strain resists the toxic effect of chromate until stationary phase is reached, thus at high cell concentration, when the reduction of chromate occurs. The features of *S. decolorationis* LDS1 that it grows efficiently from 24 to 40 °C and it reduces both toxic dyes and chromate likely makes it an efficient tool for bioremediation of many pollutants.

## **Material and methods**

### **Isolation and genome sequencing**

The *S. decolorationis* LDS1 strain was isolated from a sample of the uppermost 3 centimeters of muddy sediment of a brackish pond from the Anse Coco Beach in La Digue island (Seychelles Republic, 4°21'56.309"S 55°51'4.367"E). The sample was conserved in sterile plastic tube kept a room temperature during expedition. The sediments were suspended 24 hours in 15 mL of a 1:4 dilution of LB (1.25 g yeast extract, 1.25 g NaCl, 2.5 g tryptone per liter) pH 7.2 in the presence of 40 mM of TMAO and 0.5 M NaCl in closed plastic tubes and at room temperature (around 20 °C). To avoid alkalization of the medium due to trimethylamine production, 50 mM of MOPS pH 7.2 were added. The upper part of the culture, mud-less due to the sedimentation, was submitted to repeated enrichments in similar medium and growth conditions. Among the different colony morphotypes of isolates on agar plates, a red-brownish colony was selected for characterization and conserved at - 80 °C in LB medium with 20 % (vol/vol) glycerol.

After DNA purification, the genome sequencing of *S. decolorationis* LDS1 was carried out at the “Molecular Research LP (MR DNA) Laboratory” (USA). The library was prepared with

Nextera DNA Sample Preparation Kit (Illumina) following the manufacturer's user guide and sequenced with the HiSeq 2500 system (Illumina) as previously described (26).

### **Bacterial strains and growth conditions.**

The *S. oneidensis* strain used in this study correspond to the rifampicin resistant MR-1-R (45) and was routinely grown at 28 °C in LB medium under agitation. When necessary, chloramphenicol (25 µg/mL) was added. The *S. decolorationis* LDS1 strain was routinely grown at 28 °C in the same medium.

When required, media were solidified by adding 17 g.L<sup>-1</sup> agar. When necessary, TMAO (40 mM) and FeCl<sub>3</sub> (1 mM) were added in the medium as alternative electron acceptors. When mentioned, in the presence of TMAO, MOPS pH 7.2 (25 mM) was added to buffer the growth medium and avoid alkalization by trimethylamine production.

Bacterial growth on plates: Strains containing either the pBAD33 vector or *pdndBCDE* plasmid were grown overnight at 28°C in LB medium amended with chloramphenicol (25 µg/mL). *S. decolorationis* LDS1 was grown under the same conditions except that no chloramphenicol was added. They were then diluted to OD<sub>600</sub>=0.1 in fresh LB medium and were incubated at 28°C. At OD<sub>600</sub>=0.7, arabinose (0.2%, w/vol) was added for 2 hours to induce *dndBCDE* expression. Cells were then diluted to OD<sub>600</sub>=1 and 2 µL drops of 10-fold serial dilutions were spotted on LB-agar plates that contained arabinose (0.2%, w/vol) in the presence or absence of chromate (0.4 mM), but no chloramphenicol. The plates were incubated at 28 °C.

### **Physiological characterization**

The growth temperature and salinity ranges were assessed in a Tecan Spark 10M microplate reader in 24 well transparent plates under agitation with a humidity cassette to avoid dehydration. For salinity range determination, growths were carried out at 28 °C. During exponential phase, the exponential growth rates were calculated. Growth at 4 °C was tested on agar plates, no growth was detected after two months.

Phenotypic characteristics were determined with API 20NE test strips (Biomérieux), incubated at 28 or 40 °C. TMAO reduction was assessed by the measure of pH during growth in unbuffered LB medium in the presence of TMAO and spectroscopically at 30 °C on crude extracts of the strain grown at 28 or 40 °C as described in 29. Iron reduction was evaluated by black precipitates formation during growth in the presence of FeCl<sub>3</sub>.

### **Dye decolorization**

Decolorization assays were performed in static aerated plastic tubes incubated at 28 or 40 °C. Sterile LB medium was amended with 0.001 % (w/vol) crystal violet (Sigma Aldrich), 0.005 % (w/vol) Congo Red (Sigma Aldrich) or 0.005 % (w/vol) Remazol Brilliant Blue (Sigma Aldrich). These media were either inoculated with *S. decolorationis* LDS1 preculture in order to reach an OD<sub>600</sub> of 0.2 or not inoculated (negative control) and incubation was carried out during ten days. For the inoculated media, cells were removed by centrifugation and remaining medium was transferred in a 24 well transparent plate for imaging.

### **Chromate resistance and reduction**

Chromate solutions were made by dilution of potassium chromate K<sub>2</sub>CrO<sub>4</sub> (Sigma Aldrich) in deionized water.



For chromate resistance assay on agar plate, 150  $\mu$ L of bacterial culture at OD<sub>600</sub> of 0.001 were spread out on LB agar plate. A sterile cotton patch containing 50  $\mu$ L of potassium chromate 1 M solution was placed at the center of the plate. Diffusion of the solution in the agar forms a chromate gradient. Lengths of the bacteriostatic halo around the patch for each strain were compared after incubation at 28 °C for 12 hours.

For chromate resistance assay on agar plates, recombinant *S. oneidensis* MR1 were first grown at 28 °C until late exponential phase and then, induced for 2 h in the presence of arabinose (0.2 %, w/vol). 400  $\mu$ L of bacterial culture diluted at OD<sub>600</sub> of 0.01 were spread out. After a 2 h-incubation, three sterile cotton patches containing 25  $\mu$ L of potassium chromate (5, 10, 20 mM) were placed on the plates. Diffusion of the solution in the agar forms a chromate gradient. Lengths of the bacteriostatic halo around the patch for the two strains were compared after 24- and 48-hour incubation at 28 °C.

Chromate resistance in liquid culture was assayed in LB medium in the presence of 0, 0.5, 1, 2, 3 or 4 mM of potassium chromate. OD<sub>600</sub> was measured in a Tecan Spark 10M microplate reader in 24 well transparent plates under agitation at 28 or 40 °C and with a humidity cassette to avoid dehydration.

Chromate concentration was measured by the 1,5-diphenyl carbazide (DPC, Sigma Aldrich) method slightly modified (24, 46). Briefly, hexavalent chromate was detected in culture supernatant by the colorimetric reaction of a solution of 0.025 % (vol/vol) DPC, 0.5 % (vol/vol) H<sub>3</sub>PO<sub>4</sub> in deionized water. After 5 to 10-minute reaction, optical density at 540 nm was measured. Chromate concentration was calculated by comparison to a standard range.

### **Phylogenetic tree construction**

A classical phylogeny pipeline was used from the phylogeny tool MEGA7 (47). The sequences were aligned with MUSCLE (48). The evolutionary history was inferred from the Neighbor-Joining method (49). The tree with the highest log likelihood (-4930.1497) is shown (200 replicates). The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (50) and are in the units of the number of base substitutions per site. The analysis involved 33 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 1128 positions in the final dataset.

### **Homologous genes**

BLAST version 2.6.0 (51) was used to determine if genes from genome A are homologous to genes from genome B. Two genes were considered homologous if the e-value hit was lower or equal to  $1e^{-5}$ . The BLAST results and a R script shows the genes present in one, two or the three *Shewanella* genomes.

### ***pdndBCDE* construction**

To construct the *pdndBCDE* plasmid, the *dnd* operon from *S. decolorationis* LDS1 chromosome with its Shine Dalgarno sequence was PCR-amplified with forward and reverse primers containing the *Xma*I and *Sph*I restriction sites, respectively. After digestion with *Xma*I and *Sph*I, the DNA fragment was inserted into the pBAD33 vector digested with the same restriction enzymes. The resulting plasmid was checked by DNA sequencing using several internal primers and then introduced into MR1-R by conjugation.

## **Data availability**

The results obtained from the whole-genome shotgun project have been deposited at the National Center for Biotechnology Information (NCBI) under accession number SHEWANELLA\_DECOLORATIONIS\_SESSELENSISCP037898.

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## Legends

**Figure 1. Evolutionary relationships of taxa.** Phylogenetic tree reconstructed by the Maximum Composite Likelihood method (50) based on the nucleotide sequence of 16S rRNA

of 33 species of *Shewanella*. The tree with the highest log likelihood (-4930.1497) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 33 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 1128 positions in the final dataset.

## **Figure 2. *S. decolorationis* LDS1 behaviours**

**A.** Exponential growth rate of *S. decolorationis* LDS1 as a function of NaCl concentration in the medium. The strain was grown aerobically in LB medium. Each point corresponds to the average of at least three independent replicates.

**B.** Exponential growth rate of *S. decolorationis* LDS1 as a function of incubation temperature. The strain was grown aerobically in LB medium. Each point corresponds to the average of at least three independent replicates.

**C.** Dye decolorizing by *S. decolorationis* LDS1 at 28 and 40 °C. Cell free supernatant of a 10-day culture of *S. decolorationis* LDS1, in LB medium in the presence of Crystal violet (CV), Congo red (CR) or Remazol brilliant blue (RBB) from the triarylmethane, azo and anthraquinone dye families, respectively. “-” and “+” correspond to the addition or not of bacteria prior incubation.

## **Figure 3. Chromate resistance of *S. decolorationis* LDS1 and *S. oneidensis* MR-1**

**A.** Chromate resistance was tested on solid LB medium. 150 µl of cells (OD= 0.001) were spread on the plate and 50 µl of chromate (1 M) was loaded on the filter. Plates were incubated 12 hours at 28 °C. The radius (R) of each halo is indicated in cm.

**B. and C.** Chromate resistance of *S. decolorationis* LDS1 was tested on liquid LB medium at 28 and 40 °C with increasing concentration of chromate: 0 mM (light blue), 0.5 mM (orange), 1 mM (grey), 2 mM (yellow), 3 mM (dark blue) and 4 mM (green). Each point corresponds to the average of at least three independent replicates. Averages and standard deviation from at least three independent experiments are shown.

**Figure 4. Chromate reduction by *S. decolorationis* LDS1 at 28 and 40 °C**

Cells were grown in LB medium containing 1 mM chromate. The growth was followed spectrophotometrically (OD= 600 nm) and Cr(VI) reduction was measured by DPC method. Averages and standard deviation from at least three independent experiments are shown.

**Figure 5. Effect of the presence of *dndBCDE* genes in *S. oneidensis***

**A.** *S. oneidensis* harboring either the pBAD33 vector or the *pdndBCDE* plasmid and *S. decolorationis* LDS1 were grown at 28 °C. For the two *S. oneidensis* recombinant strains, arabinose (0.2%, w/vol) was added at OD<sub>600</sub>=0.7 during 2 hours. The three strains were diluted to OD<sub>600</sub>=1. 10-fold serial dilutions were spotted on LB plates containing arabinose (0.2%, w/vol) and in the absence or presence of chromate (0.4 mM) as indicated. Then, the plates were incubated 24 or 72 hours at 28 °C. Plates are representative of at least three experiments.

**B.** *S. oneidensis* harboring either the pBAD33 vector or the *pdndBCDE* plasmid were grown at 28 °C. After a 2-hour arabinose (0.2 %, w/vol) induction, cells were spread on plates and filters containing the indicated concentration of chromate were dropped on

627 the top. Then, the plates were incubated 24 or 48 hours at 28 °C. Plates are  
628 representative of at least three experiments.

629

630

631 **Table 1. Enzymatic activities and carbon source assimilation of *S. decolorationis* LDS1 at**632 **28 and 40 °C.**

|                        | Temperature |       |
|------------------------|-------------|-------|
|                        | 28 °C       | 40 °C |
| <b>Enzyme activity</b> |             |       |
| Nitrate reductase      | +           | +     |
| TMAO reductase*        | +           | +     |
| Gelatinase             | +           | +     |
| Iron reduction         | +           | +     |
| <b>Assimilation</b>    |             |       |
| D-glucose              | +           | +     |
| N-acetylglucosamine    | +           | +     |
| D-maltose              | +           | +     |
| Caprate                | +           | -     |
| Malate                 | +           | +     |

633

634 \*activity estimated by the alkalization of the growth medium (29).

635

636 **Table 2. Phenotypic characteristics of various *Shewanella* species.** Species (27, 52): 1,  
637 *S. decolorationis* LDS1; 2, *S. decolorationis* S12; 3, *S. baltica* DSM 9439; 4,  
638 *S. glacialipiscicola* NBRC 102030T; 5, *S. hafniensis*; 6, *S. oneidensis* ATCC 700550T; 7,  
639 *S. putrefaciens* ATCC 8071. ND, not determined.

640

|                                | 1 | 2  | 3  | 4  | 5  | 6 | 7  |
|--------------------------------|---|----|----|----|----|---|----|
| Growth at 4°C                  | - | +  | +  | +  | +  | + | +  |
| Growth at 40°C                 | + | +  | -  | -  | -  | - | -  |
| <b>Enzyme activity (28 °C)</b> |   |    |    |    |    |   |    |
| Nitrate reductase              | + | ND | ND | ND | ND | + | ND |
| Tryptophanase                  | - | ND | ND | ND | ND | - | ND |
| Arginine dehydrolase           | - | ND | ND | ND | ND | - | ND |
| Urease                         | - | ND | ND | ND | ND | - | ND |
| Esculinase                     | - | ND | ND | ND | ND | - | ND |
| Gelatinase                     | + | +  | +  | +  | +  | + | -  |
| B-galactosidase                | - | ND | ND | ND | ND | - | ND |
| Fermentation of Glucose        | - | +  | -  | -  | -  | - | -  |
| <b>Assimilation (28°C)</b>     |   |    |    |    |    |   |    |
| D-glucose                      | + | +  | +  | -  | +  | - | -  |
| L-arabinose                    | - | -  | -  | -  | -  | - | -  |
| D-mannose                      | - | ND | ND | ND | ND | - | ND |
| D-manitol                      | - | -  | -  | -  | -  | - | -  |
| N-acetylglucosamine            | + | +  | +  | +  | +  | + | +  |
| D-maltose                      | + | +  | -  | -  | +  | - | -  |
| Gluconate                      | - | -  | +  | +  | +  | - | -  |
| Caprate                        | + | +  | -  | -  | +  | - | -  |
| Adipate                        | - | ND | ND | ND | ND | - | ND |
| Malate                         | + | +  | +  | +  | +  | - | +  |
| Citrate                        | - | -  | +  | -  | +  | - | -  |
| Phenylacetate                  | - | ND | ND | ND | ND | - | ND |

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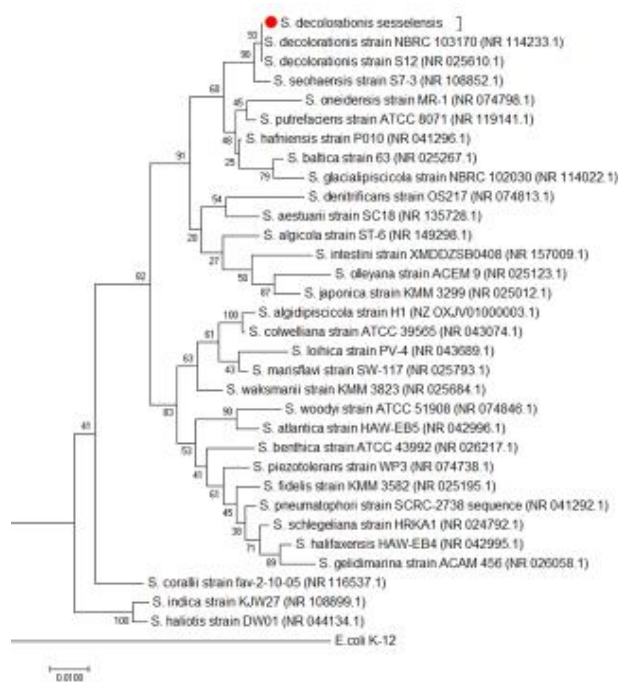


**Table 3. Genome overview of *S. decolorationis* LDS1, *S. decolorationis* S12 and *S. oneidensis* MR-1.**

|                       | <i>S. decolorationis</i><br>LDS1 | <i>S. decolorationis</i><br>S12 | <i>S. oneidensis</i><br>MR-1 |
|-----------------------|----------------------------------|---------------------------------|------------------------------|
| GC content            | 47.12                            | 47.09                           | 45.96                        |
| Size                  | 4719362                          | 4851566                         | 4969811                      |
| DNA scaffolds         | 16                               | 77                              | 0                            |
| Total genes           | 4430                             | 4451                            | 5031                         |
| Coding sequences      | 4223                             | 4309                            | 4444                         |
| Common genes          | 3535                             |                                 |                              |
| Strain-specific genes | 259                              | 345                             | 375                          |

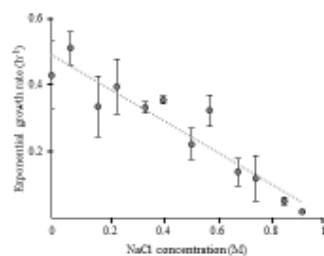
**Table 4. Proteins specifically found in *S. decolorationis* LDS1 from genome sequencing and annotation.**

| <b>Locus</b>   | <b>Predicted function</b>                                 |
|----------------|---|
| SDECO_v1_10304 | transcriptional regulator                                 |
| SDECO_v1_10308 | IcIR family transcriptional regulator                     |
| SDECO_v1_10310 | transposase   |
| SDECO_v1_10317 | phage tail tape measure protein                           |
| SDECO_v1_10321 | DUF1983 domain-containing protein                         |
| SDECO_v1_10405 | DUF262 domain-containing protein                          |
| SDECO_v1_11406 | Arc family DNA-binding protein                            |
| SDECO_v1_10464 | DUF4868 domain-containing protein                         |
| SDECO_v1_10506 | DUF5071 domain-containing protein                         |
| SDECO_v1_10701 | DUF4265 domain-containing protein                         |
| SDECO_v1_10782 | restriction endonuclease                                  |
| SDECO_v1_10884 | DUF3916 domain-containing protein                         |
| SDECO_v1_11229 | SMP-30/gluconolactonase/LRE family protein                |
| SDECO_v1_11230 | glycosyl transferase family 2                             |
| SDECO_v1_11231 | acylneuraminate cytidyltransferase family protein         |
| SDECO_v1_11234 | DUF115 domain-containing protein                          |
| SDECO_v1_11235 | polysaccharide biosynthesis protein                       |
| SDECO_v1_11238 | DUF115 domain-containing protein                          |
| SDECO_v1_11243 | DUF1972 domain-containing protein                         |
| SDECO_v1_11404 | transcriptional regulator                                 |
| SDECO_v1_11405 | DNA-binding protein                                       |
| SDECO_v1_11468 | DGQHR domain-containing protein                           |
| SDECO_v1_20119 | DUF3578 domain-containing protein                         |
| SDECO_v1_20120 | DUF2357 domain-containing protein                         |
| SDECO_v1_20196 | DUF4065 domain-containing protein                         |
| SDECO_v1_20376 | DUF4297 domain-containing protein                         |
| SDECO_v1_20377 | NYN domain-containing protein                             |
| SDECO_v1_20445 | helicase  |
| SDECO_v1_20479 | esterase  |
| SDECO_v1_20492 | DUF4263 domain-containing protein                         |
| SDECO_v1_20493 | DUF4062 domain-containing protein                         |
| SDECO_v1_20559 | zinc chelation protein SecC                               |
| SDECO_v1_20672 | polysaccharide lyase                                      |
| SDECO_v1_30058 | serine protease   |
| SDECO_v1_30517 | type I-F CRISPR-associated endoribonuclease Cas6/Csy4     |
| SDECO_v1_30520 | CRISPR-associated endonuclease Cas3"                      |
| SDECO_v1_30521 | type I-F CRISPR-associated endonuclease Cas1              |
| SDECO_v1_30534 | DNA sulfur modification protein DndB                      |
| SDECO_v1_30535 | DNA phosphorothioation system sulfurtransferase DndC      |
| SDECO_v1_30536 | DNA sulfur modification protein DndD                      |
| SDECO_v1_30537 | DNA sulfur modification protein DndE                      |
| SDECO_v1_30540 | DNA phosphorothioation-dependent restriction protein DptH |
| SDECO_v1_30541 | DNA phosphorothioation-dependent restriction protein DptG |
| SDECO_v1_30542 | DNA phosphorothioation-dependent restriction protein DptF |
| SDECO_v1_40174 | DUF4062 domain-containing protein                         |
| SDECO_v1_40306 | MBL fold hydrolase  |
| SDECO_v1_50042 | HNH endonuclease  |
| SDECO_v1_50046 | ATP-binding protein                                       |
| SDECO_v1_50214 | glycosyl transferase                                      |
| SDECO_v1_50216 | glycosyltransferase                                       |
| SDECO_v1_60106 | ATP-binding protein                                       |
| SDECO_v1_80044 | host-nuclease inhibitor protein Gam                       |
| SDECO_v1_80074 | phage tail tape measure protein                           |
| SDECO_v1_80075 | tape measure domain-containing protein                    |

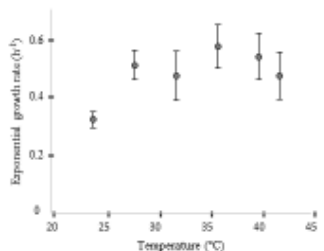


0.0100

A



B



C

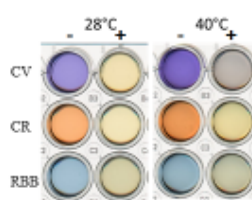


Figure 2

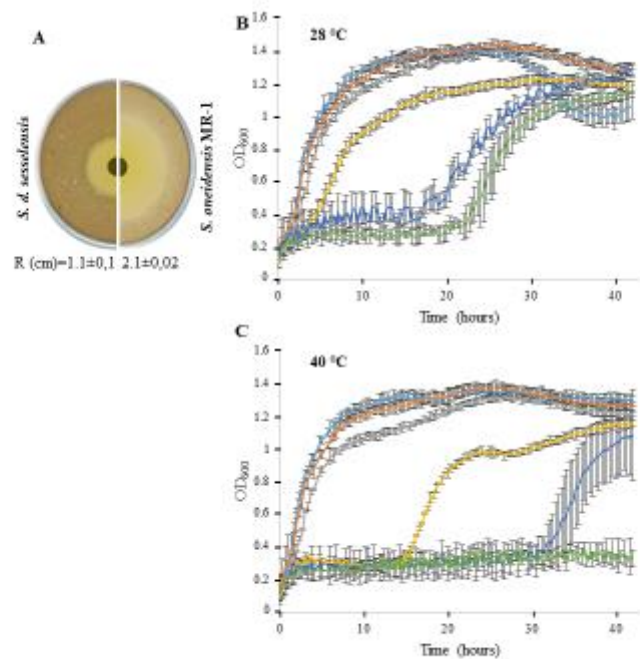


Figure 3

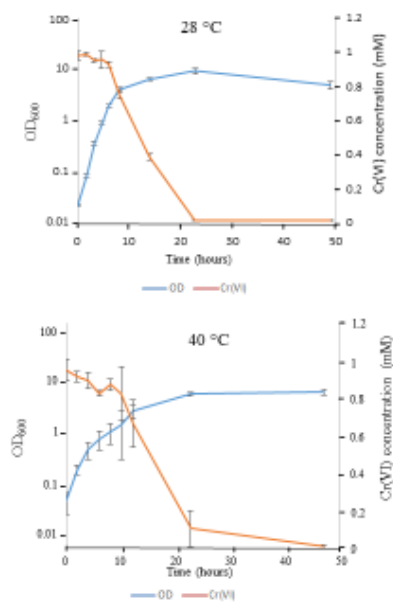


Figure 4

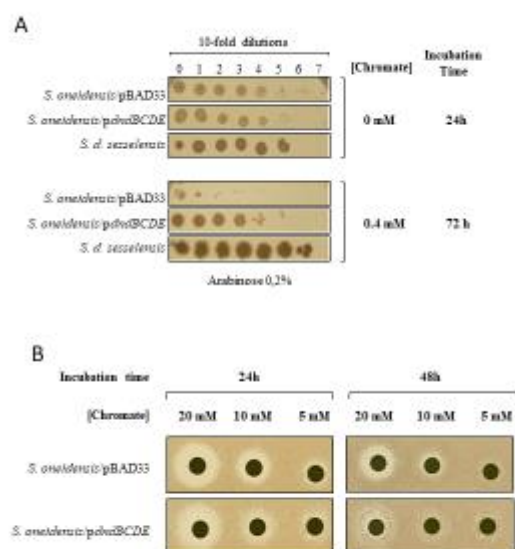


Figure 5.