



**HAL**  
open science

## **Shewanella decolorationis LDS1 Chromate Resistance**

Olivier Lemaire, Flora Honore, Sébastien Tempel, Emma Fortier, Silke Leimkuhler, Vincent Méjean, Chantal Iobbi-Nivol

► **To cite this version:**

Olivier Lemaire, Flora Honore, Sébastien Tempel, Emma Fortier, Silke Leimkuhler, et al.. Shewanella decolorationis LDS1 Chromate Resistance. Applied and Environmental Microbiology, 2019, 85 (18), 10.1128/AEM.00777-19 . hal-02371711

**HAL Id: hal-02371711**

**<https://hal.science/hal-02371711>**

Submitted on 25 Nov 2019

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 ***Shewanella decolorationis* LDS1 chromate resistance**

2

3 Olivier N. Lemaire<sup>a</sup>, Flora A. Honoré<sup>a</sup>, Sébastien Tempel<sup>b</sup>, Emma M. Fortier<sup>a</sup>, Silke  
4 Leimkühler<sup>c</sup>, Vincent Méjean<sup>a</sup> and Chantal Iobbi-Nivol<sup>a</sup>#

5

6

7 <sup>a</sup>Aix-Marseille Université, Institut de Microbiologie de la Méditerranée, Laboratoire de  
8 Bioénergétique et Ingénierie des Protéines, Centre National de la Recherche Scientifique,  
9 Marseille, France.

10 <sup>b</sup>Aix-Marseille Université, Institut de Microbiologie de la Méditerranée, Laboratoire de Chimie  
11 Bactérienne, Centre National de la Recherche Scientifique, Marseille, France.

12 <sup>c</sup>Institute of Biochemistry and Biology, Department of Molecular Enzymology, University of  
13 Potsdam, Potsdam, Germany.

14

15 Running Head: Properties of *S. decolorationis* LDS1

16 # Address correspondence to Chantal Iobbi-Nivol, iobbi@imm.cnrs.fr

17

## 18 Abstract

19 The *Shewanella* genus is well known for its genetic diversity, its outstanding respiratory  
20 capacity and its high potential for bioremediation. Here, a novel strain isolated from sediments  
21 of the Indian Ocean was characterized. 16S rRNA analysis indicated that it belongs to the *S.*  
22 *decolorationis* species. It was named *Shewanella decolorationis* LDS1. This strain presented  
23 an unusual ability to grow efficiently from 24 °C to 40 °C, without apparent modifications of  
24 its metabolism as shown by testing respiratory activities or carbon assimilation and in a wide  
25 range of salt concentrations. Moreover, *S. decolorationis* LDS1 tolerates high chromate  
26 concentrations. Indeed, it was able to grow in the presence of 4 mM chromate at standard  
27 temperature and 3 mM at 40 °C. Interestingly, whatever the temperature, when the culture  
28 reached the stationary phase, the strain reduced the chromate present in the growth medium. In  
29 addition, *S. decolorationis* LSD1 degrades different toxic dyes including anthraquinone-,  
30 triarylmethane- and azo-dye. Thus, compared to *S. oneidensis*, this strain presented better  
31 capacity to cope with various abiotic stresses in particular at high temperatures. The analysis of  
32 genome sequence preliminary data indicated that, in contrast to *S. oneidensis* and *S.*  
33 *decolorationis* ST2, *S. decolorationis* LDS1 possesses the phosphorothioate modification  
34 machinery that has been described to participate against various abiotic stresses by protecting  
35 DNA. We demonstrate that its heterologous production in *S. oneidensis* allows it to resist higher  
36 concentrations of chromate.

## 37 Importance

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

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

## 47 Introduction

48 *Shewanella* species are part of the gammaproteobacteria class of the Proteobacteria phylum.  
49 They have been found all around the world in briny or fresh water and sediments from polar  
50 circles to equatorial biotopes (1, 2). Their trade mark is their tremendous variety of respiratory  
51 capacities allowing their growth with a large panel of exogenous electron acceptors including  
52 solid and soluble metals, explaining why they belong to the restricted family of dissimilatory  
53 metal-reducing bacteria (3). Partly due to their reducing abilities, *Shewanella* species have the  
54 capacity to cope with elevated concentrations of toxic products as for example hydrocarbons,  
55 arsenate and hexahydro-1,3,5-trinitro-1,3,5-triazine (4–9). This ability has made them a useful  
56 tool for bioremediation and clean-up (1–3, 10, 11). Moreover, it appears that the members of  
57 this genus adapt easily to various life conditions. Although they are usually found in aquatic  
58 habitats, some can develop in other biotopes like butter, crude oil or even human corpses (1, 2,  
59 12).

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

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

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

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

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

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

100

## 101 **Results and Discussion**

### 102 **Strain isolation and characterization.**

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

115 most other species, since they survive at 40 °C and present an optimal growth from 20 to 30  
116 °C.

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

139 To better determine the physiological capacities of the strain at high temperature, we decided  
140 to compare its TMAO reductase activity at 28 and 40 °C. The TMAO reductase activity was  
141 first followed through the alkalization of the growth medium indicating that the increase of the  
142 temperature has no effect since the alkalization of the medium was similar (Table 1). For a more  
143 accurate activity, we measured the TMAO reductase activity of crude extracts of cells grown at  
144 28 or 40 °C. The TMAO reductase specific activity of this strain at 40 °C was half that at 28 °C  
145 ( $2.8 \pm 0.17$   $\mu\text{mol}/\text{min}/\text{mg}$  versus  $5.95 \pm 0.40$   $\mu\text{mol}/\text{min}/\text{mg}$ , respectively). Measures of nitrate  
146 reduction and other phenotypic characteristics obtained with API20NE plates (Biomérieux,  
147 France) are recorded in Table 1 and showed no significant difference at 28 and 40 °C, except  
148 for the hydrolysis of capric acid that could be due to the instability of the product rather than  
149 the activity of the enzyme. Altogether these results suggest that the strain has fully adapted from  
150 medium to elevated temperatures by increasing the range of its optimal growth temperature.  
151 The tested respiratory activities (oxygen, TMAO, nitrate) confirmed that they were efficient  
152 from 28 to 40 °C. In contrast to *S. decolorationis* S12T and the other strains presented in Table  
153 2, *S. decolorationis* LDS1 could not grow at very low temperature. The fact that this strain  
154 seemed to have lost the latter ability was not surprising considering that the biotope from where  
155 it was isolated probably does not encounter temperature below 25 °C.

156 Other differences could be pointed out. Indeed, counter to the *S. decolorationis* S12T,  
157 *S. decolorationis* LDS1 as many other *Shewanella* species did not use glucose for fermentation  
158 although it performed its assimilation (Table 2). Moreover, the range of optimal pH for growth  
159 ranged from 6.0 to 11.0, which was slightly wider compared to that of other *Shewanella* strains  
160 (27). This probably results from the sampling of *S. decolorationis* LDS1 from brackish water  
161 that can be more acidic than sea water. Altogether, these results indicate that the  
162 *S. decolorationis* LDS1 shares with other *S. decolorationis* strains the ability to grow at higher  
163 temperatures than most of the other *Shewanella* species. However, only *S. decolorationis* LDS1

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

167

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

169 Recent studies have focused on the ability of *Shewanella* species to reduce chromate (19, 21-  
170 24). For instance, *S. oneidensis* MR-1 can resist 1 mM chromate; whereas, *S. algidipiscicola*  
171 H1 grows in 3 mM of chromate (18, 26). In order to compare the resistance against chromate  
172 of *S. decolorationis* LDS1 with that of the model bacterium *S. oneidensis*, a preliminary test  
173 was done on solid medium containing a filter soaked with a 1 M-chromate solution, leading to  
174 the formation of a gradient of chromate by diffusion in the solid medium. As shown in Figure  
175 3A, *S. decolorationis* LDS1 was more resistant than *S. oneidensis* at 28 °C since it was able to  
176 grow closer to the filter. Moreover, in liquid medium, *S. oneidensis* barely resisted 1 mM  
177 chromate (18) while this concentration did not affect *S. decolorationis* LDS1 (Figure 3B). This  
178 indicates that *S. decolorationis* LDS1 can resist higher chromate concentrations. To confirm  
179 this point, its growth was followed under aerated conditions at 28 °C in the presence of  
180 increasing concentrations of chromate. As shown in Figure 3B, no drastic growth difference  
181 was noticeable in the range of chromium concentration from 0 to 2 mM, although a short lag  
182 phase was observed at 2 mM. In the presence of 3 and 4 mM chromate, the cell growth presented  
183 a pronounced lag phase of about 20 hours, but finally the curves reached the same plateau value  
184 in comparison to the cultures grown at lower concentrations or without the addition of  
185 chromate. This long lag phase could be necessary for the bacteria to adapt to the high chromate  
186 concentrations by producing either Cr(VI) reducing systems or Cr efflux pumps. In addition,  
187 the chromate resistance was followed at 40 °C. The results indicate that the growth is not

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

192 Finally, we wondered whether *S. decolorationis* LDS1 was able to reduce chromate during its  
193 growth. This is of great interest since chromate reduction leads to a less soluble and less toxic  
194 form for the environment (20, 23). Thus, *S. decolorationis* LDS1 was grown at 28 °C, in the  
195 presence of 1 mM of chromate and reduction of Cr(VI) to Cr(III) in the growth medium was  
196 followed. Strikingly, when the medium was inoculated with cells of *S. decolorationis* LDS1,  
197 the Cr reduction appeared when the culture entered into the stationary phase (Figure 4). From  
198 that time, the reduction of chromate was almost complete within 15 hours. A similar experiment  
199 was performed at 40 °C. As for the lower temperature, the reduction of chromate started when  
200 the cells reached the stationary phase (Figure 4). From these results, it is clear that  
201 *S. decolorationis* LDS1 can reduce chromate at high temperature with the same efficiency as at  
202 28 °C. The mechanism involved is clearly acting from the beginning of the stationary phase as  
203 suggested by the trigger of the reduction when the bacteria reach it, as already described under  
204 anaerobic growth of *S. oneidensis* MR-1 (23).

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

206 Since *S. decolorationis* LDS1 was able to cope better with various stresses than does *S.*  
207 *oneidensis* and has a better heat resistance than *S. decolorationis* S12T, we wondered whether  
208 this strain has specific machineries leading to an increase of its resistance to environmental  
209 cues. Indeed, *Shewanella* species are described to present a high level of genetic variability that  
210 confers to each of them peculiar properties (3, 25). The genome of *S. decolorationis* LDS1 was  
211 rsequenced at the Molecular Research LP (MR DNA) Laboratory (USA). A preliminary

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

236 that *S. decolorationis* LDS1 has acquired the genes of the Dnd machinery from another  
237 bacterial genus.

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

247

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

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

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

275

## 276 **Conclusion.**

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

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

290

## 291 **Material and methods**

### 292 **Isolation and genome sequencing**

293

294 The *S. decolorationis* LDS1 strain was isolated from a sample of the uppermost 3 centimeters  
295 of muddy sediment of a brackish pond from the Anse Coco Beach in La Digue island  
296 (Seychelles Republic, 4°21'56.309"S 55°51'4.367"E). The sample was conserved in sterile  
297 plastic tube kept a room temperature during expedition. The sediments were suspended 24 hours  
298 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  
299 7.2 in the presence of 40 mM of TMAO and 0.5 M NaCl in closed plastic tubes and at room  
300 temperature (around 20 °C). To avoid alkalization of the medium due to trimethylamine  
301 production, 50 mM of MOPS pH 7.2 were added. The upper part of the culture, mud-less due  
302 to the sedimentation, was submitted to repeated enrichments in similar medium and growth  
303 conditions. Among the different colony morphotypes of isolates on agar plates, a red-brownish  
304 colony was selected for characterization and conserved at - 80 °C in LB medium with 20 %  
305 (vol/vol) glycerol.

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

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

310

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

312

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

317 When required, media were solidified by adding 17 g.L<sup>-1</sup> agar. When necessary, TMAO  
318 (40 mM) and FeCl<sub>3</sub> (1 mM) were added in the medium as alternative electron acceptors. When  
319 mentioned, in the presence of TMAO, MOPS pH 7.2 (25 mM) was added to buffer the growth  
320 medium and avoid alkalization by trimethylamine production.

321 Bacterial growth on plates: Strains containing either the pBAD33 vector or *pdndBCDE* plasmid  
322 were grown overnight at 28°C in LB medium amended with chloramphenicol (25 µg/mL).  
323 *S. decolorationis* LDS1 was grown under the same conditions except that no chloramphenicol  
324 was added. They were then diluted to OD<sub>600</sub>=0.1 in fresh LB medium and were incubated at  
325 28°C. At OD<sub>600</sub>=0.7, arabinose (0.2%) was added for 2 hours to induce *dndBCDE* expression.  
326 Cells were then diluted to OD<sub>600</sub>=1 and 2 µL drops of 10-fold serial dilutions were spotted on  
327 LB-agar plates that contained arabinose (0.2%) in the presence or absence of chromate (0.4  
328 mM), but no chloramphenicol. The plates were incubated at 28 °C.

329

### 330 **Physiological characterization**

331

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

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

342

#### 343 **Dye decolorization**

344

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

352

#### 353 **Chromate resistance and reduction**

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

356 For chromate resistance assay on agar plate, 150  $\mu$ L of bacterial culture at OD<sub>600</sub> of 0.001 were  
357 spread out on LB agar plate. A sterile cotton patch containing 50  $\mu$ L of potassium chromate  
358 1 M solution was placed at the center of the plate. Diffusion of the solution in the agar forms a  
359 chromate gradient. Lengths of the bacteriostatic halo around the patch for each strain were  
360 compared after incubation at 28 °C for 12 hours.

361 For chromate resistance assay on agar plates, recombinant *S. oneidensis* MR1 were first grown  
362 at 28 °C until late exponential phase and then, induced for 2 h in the presence of arabinose (0.2  
363 %). 400  $\mu$ L of bacterial culture diluted at OD<sub>600</sub> of 0.01 were spread out. After a 2 h-incubation,  
364 three sterile cotton patches containing 25  $\mu$ L of potassium chromate (5, 10, 20 mM) were placed  
365 on the plates. Diffusion of the solution in the agar forms a chromate gradient. Lengths of the  
366 bacteriostatic halo around the patch for the two strains were compared after 24- and 48-hour  
367 incubation at 28 °C.

368

369 Chromate resistance in liquid culture was assayed in LB medium in the presence of 0, 0.5, 1, 2,  
370 3 or 4 mM of potassium chromate. OD<sub>600</sub> was measured in a Tecan Spark 10M microplate  
371 reader in 24 well transparent plates under agitation at 28 or 40 °C and with a humidity cassette  
372 to avoid dehydration.

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

378

### 379 **Phylogenetic tree construction**

380

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

391

392

### 393 **Homologous genes**

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

398

### 399 ***pdndBCDE* construction**

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

406

#### 407 **Data availability**

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

411

412

413

#### 414 **Acknowledgements**

415

416 We would like to thank Olivier Genest, Michel Fons and the whole BIP03 team for interesting  
417 discussions. We are grateful to Scot E. Dowd (Molecular Research LP [MR DNA] Laboratory)  
418 for sequencing and assembly of our genome. The LABGeM (CEA/IG/Genoscope and CNRS  
419 UMR8030) and the France Génomique National infrastructure (funded as part of the  
420 Investissement d'avenir program managed by the Agence Nationale pour la Recherche, contract  
421 ANR-10-INBS-09) are acknowledged for allowing access to the MicroScope platform. This  
422 work was supported by the Centre National de la Recherche Scientifique (CNRS) and Aix-  
423 Marseille Université (AMU). O.N. L. was supported by a MESR fellowship, AMU and CNRS.  
424 We thank the Aix-Marseille Université for supporting S.L. with a visiting professor position.  
425 The funders had no role in study design, data collection and interpretation, or the decision to  
426 submit the work for publication.

#### 427 **References**

- 428 1. Hau HH, Gralnick JA. 2007. Ecology and biotechnology of the genus *Shewanella*. *Annu*  
429 *Rev Microbiol* 61:237–258.

- 430 2. Nealson KH, Scott J. 2006. Ecophysiology of the Genus *Shewanella*, p. 1133–1151. *In*  
431 *The Prokaryotes*. Springer, New York, NY.
- 432 3. Fredrickson JK, Romine MF, Beliaev AS, Auchtung JM, Driscoll ME, Gardner TS,  
433 Nealson KH, Osterman AL, Pinchuk G, Reed JL, Rodionov DA, Rodrigues JLM, Saffarini  
434 DA, Serres MH, Spormann AM, Zhulin IB, Tiedje JM. 2008. Towards environmental  
435 systems biology of *Shewanella*. *Nat Rev Microbiol* 6:592–603.
- 436 4. Gao P, Tian H, Li G, Sun H, Ma T. 2015. Microbial diversity and abundance in the  
437 Xinjiang Luliang long-term water-flooding petroleum reservoir. *MicrobiologyOpen*.
- 438 5. Saltikov CW, Cifuentes A, Venkateswaran K, Newman DK. 2003. The ars detoxification  
439 system is advantageous but not required for As(V) respiration by the genetically tractable  
440 *Shewanella* species strain ANA-3. *Appl Environ Microbiol* 69:2800–2809.
- 441 6. Sibanda T, Selvarajan R, Tekere M. 2017. Synthetic extreme environments: overlooked  
442 sources of potential biotechnologically relevant microorganisms. *Microb Biotechnol*  
443 10:570–585.
- 444 7. Suganthi SH, Murshid S, Sriram S, Ramani K. 2018. Enhanced biodegradation of  
445 hydrocarbons in petroleum tank bottom oil sludge and characterization of biocatalysts and  
446 biosurfactants. *J Environ Manage* 220:87–95.
- 447 8. Sun W, Xiao E, Krumins V, Dong Y, Xiao T, Ning Z, Chen H, Xiao Q. 2016.  
448 Characterization of the microbial community composition and the distribution of Fe-  
449 metabolizing bacteria in a creek contaminated by acid mine drainage. *Appl Microbiol*  
450 *Biotechnol* 100:8523–8535.

- 451 9. Zhao J-S, Manno D, Leggiadro C, O'Neil D, Hawari J. 2006. *Shewanella halifaxensis* sp.  
452 nov., a novel obligately respiratory and denitrifying psychrophile. Int J Syst Evol  
453 Microbiol 56:205–212.
- 454 10. Orellana R, Macaya C, Bravo G, Dorochesi F, Cumsille A, Valencia R, Rojas C, Seeger  
455 M. 2018. Living at the Frontiers of Life: Extremophiles in Chile and Their Potential for  
456 Bioremediation. Front Microbiol 9:2309.
- 457 11. Sekar R, DiChristina TJ. 2017. Degradation of the recalcitrant oil spill components  
458 anthracene and pyrene by a microbially driven Fenton reaction. FEMS Microbiol Lett 364.
- 459 12. Kakizaki E, Kozawa S, Tashiro N, Sakai M, Yukawa N. 2009. Detection of  
460 bacterioplankton in immersed cadavers using selective agar plates. Leg Med Tokyo Jpn  
461 11 Suppl 1: S350-353.
- 462 13. Venkateswaran K, Moser DP, Dollhopf ME, Lies DP, Saffarini DA, MacGregor BJ,  
463 Ringelberg DB, White DC, Nishijima M, Sano H, Burghardt J, Stackebrandt E, Nealson  
464 KH. 1999. Polyphasic taxonomy of the genus *Shewanella* and description of *Shewanella*  
465 *oneidensis* sp. nov. Int J Syst Bacteriol 49 Pt 2:705–724.
- 466 14. Honoré FA, Méjean V, Genest O. 2017. Hsp90 Is Essential under Heat Stress in the  
467 Bacterium *Shewanella oneidensis*. Cell Rep 19:680–687.
- 468 15. Bouillet S, Genest O, Jourlin-Castelli C, Fons M, Méjean V, Iobbi-Nivol C. 2016. The  
469 General Stress Response  $\sigma$ S Is Regulated by a Partner Switch in the Gram-negative  
470 Bacterium *Shewanella oneidensis*. J Biol Chem 291:26151–26163.

- 471 16. Bouillet S, Genest O, Méjean V, Iobbi-Nivol C. 2017. Protection of the general stress  
472 response  $\sigma^S$  factor by the CrsR regulator allows a rapid and efficient adaptation of  
473 *Shewanella oneidensis*. J Biol Chem 292:14921–14928.
- 474 17. Liu Y, Gao W, Wang Y, Wu L, Liu X, Yan T, Alm E, Arkin A, Thompson DK, Fields  
475 MW, Zhou J. 2005. Transcriptome Analysis of *Shewanella oneidensis* MR-1 in Response  
476 to Elevated Salt Conditions. J Bacteriol 187:2501–2507.
- 477 18. Brown SD, Thompson MR, Verberkmoes NC, Chourey K, Shah M, Zhou J, Hettich RL,  
478 Thompson DK. 2006. Molecular dynamics of the *Shewanella oneidensis* response to  
479 chromate stress. Mol Cell Proteomics MCP 5:1054–1071.
- 480 19. Viamajala S, Peyton BM, Apel WA, Petersen JN. 2002. Chromate/nitrite interactions in  
481 *Shewanella oneidensis* MR-1: evidence for multiple hexavalent chromium [Cr(VI)]  
482 reduction mechanisms dependent on physiological growth conditions. Biotechnol Bioeng  
483 78:770–778.
- 484 20. Barnhart J. 1997. Chromium chemistry and implications for environmental fate and  
485 toxicity. J Soil Contam 6:561–568.
- 486 21. Meng Y, Zhao Z, Burgos WD, Li Y, Zhang B, Wang Y, Liu W, Sun L, Lin L, Luan F.  
487 2018. Iron(III) minerals and anthraquinone-2,6-disulfonate (AQDS) synergistically  
488 enhance bioreduction of hexavalent chromium by *Shewanella oneidensis* MR-1. Sci Total  
489 Environ 640–641:591–598.
- 490 22. Myers CR, Carstens BP, Antholine WE, Myers JM. 2000. Chromium (VI) reductase  
491 activity is associated with the cytoplasmic membrane of anaerobically grown *Shewanella*  
492 *putrefaciens* MR-1. J Appl Microbiol 88:98–106.

- 493 23. Viamajala S, Peyton BM, Apel WA, Petersen JN. 2002. Chromate reduction in  
494 *Shewanella oneidensis* MR-1 is an inducible process associated with anaerobic growth.  
495 Biotechnol Prog 18:290–295.
- 496 24. Baaziz H, Gambari C, Boyeldieu A, Ali Chaouche A, Alatou R, Méjean V, Jourlin-Castelli  
497 C, Fons M. 2017. ChrASO, the chromate efflux pump of *Shewanella oneidensis*, improves  
498 chromate survival and reduction. PloS One 12:e0188516.
- 499 25. Zhong C, Han M, Yu S, Yang P, Li H, Ning K. 2018. Pan-genome analyses of 24  
500 *Shewanella* strains re-emphasize the diversification of their functions yet evolutionary  
501 dynamics of metal-reducing pathway. Biotechnol Biofuels 11:193.
- 502 26. Baaziz H, Lemaire ON, Jourlin-Castelli C, Iobbi-Nivol C, Méjean V, Alatou R, Fons M.  
503 2018. Draft Genome Sequence of *Shewanella algidipiscicola* H1, a Highly Chromate-  
504 Resistant Strain Isolated from Mediterranean Marine Sediments. Microbiol Res Announc  
505 7:e00905-18.
- 506 27. Xu M, Guo J, Cen Y, Zhong X, Cao W, Sun G. 2005. *Shewanella decolorationis* sp. nov.,  
507 a dye-decolorizing bacterium isolated from activated sludge of a waste-water treatment  
508 plant. Int J Syst Evol Microbiol 55:363–368.
- 509 28. Dos Santos JP, Iobbi-Nivol C, Couillault C, Giordano G, Méjean V. 1998. Molecular  
510 analysis of the trimethylamine N-oxide (TMAO) reductase respiratory system from a  
511 *Shewanella* species. J Mol Biol 284:421–433.
- 512 29. Lemaire ON, Honoré FA, Jourlin-Castelli C, Méjean V, Fons M, Iobbi-Nivol C. 2016.  
513 Efficient respiration on TMAO requires TorD and TorE auxiliary proteins in *Shewanella*  
514 *oneidensis*. Res Microbiol 167:630–637.

- 515 30. Lemaire ON, Infossi P, Ali Chaouche A, Espinosa L, Leimkühler S, Giudici-Orticoni M-  
516 T, Méjean V, Iobbi-Nivol C. 2018. Small membranous proteins of the TorE/NapE family,  
517 crutches for cognate respiratory systems in Proteobacteria. *Sci Rep* 8:13576.
- 518 31. Yancey PH, Geringer ME, Drazen JC, Rowden AA, Jamieson A. 2014. Marine fish may  
519 be biochemically constrained from inhabiting the deepest ocean depths. *Proc Natl Acad*  
520 *Sci U S A* 111:4461–4465.
- 521 32. Banerjee R, Mukhopadhyay S. 2012. Niche specific amino acid features within the core  
522 genes of the genus *Shewanella*. *Bioinformatics* 8:938–942.
- 523 33. Kato C, Nogi Y. 2001. Correlation between phylogenetic structure and function: examples  
524 from deep-sea *Shewanella*. *FEMS Microbiol Ecol* 35:223–230.
- 525 34. Chen C-H, Chang C-F, Ho C-H, Tsai T-L, Liu S-M. 2008. Biodegradation of crystal violet  
526 by a *Shewanella* sp. NTOU1. *Chemosphere* 72:1712–1720.
- 527 35. Eckstein F. 2007. Phosphorothioation of DNA in bacteria. *Nat Chem Biol* 3:689–690.
- 528 36. Wang L, Chen S, Xu T, Taghizadeh K, Wishnok JS, Zhou X, You D, Deng Z, Dedon PC.  
529 2007. Phosphorothioation of DNA in bacteria by *dnd* genes. *Nat Chem Biol* 3:709–710.
- 530 37. Wang L, Chen S, Vergin KL, Giovannoni SJ, Chan SW, DeMott MS, Taghizadeh K,  
531 Cordero OX, Cutler M, Timberlake S, Alm EJ, Polz MF, Pinhassi J, Deng Z, Dedon PC.  
532 2011. DNA phosphorothioation is widespread and quantized in bacterial genomes. *Proc*  
533 *Natl Acad Sci U S A* 108:2963–2968.
- 534 38. Cao B, Chen C, DeMott MS, Cheng Q, Clark TA, Xiong X, Zheng X, Butty V, Levine  
535 SS, Yuan G, Boitano M, Luong K, Song Y, Zhou X, Deng Z, Turner SW, Korlach J, You

- 536 D, Wang L, Chen S, Dedon PC. 2014. Genomic mapping of phosphorothioates reveals  
537 partial modification of short consensus sequences. *Nat Commun* 5:3951.
- 538 39. Tong T, Chen S, Wang L, Tang Y, Ryu JY, Jiang S, Wu X, Chen C, Luo J, Deng Z, Li Z,  
539 Lee SY, Chen S. 2018. Occurrence, evolution, and functions of DNA phosphorothioate  
540 epigenetics in bacteria. *Proc Natl Acad Sci U S A* 115: E2988–E2996.
- 541 40. Bordi C, Ansaldi M, Gon S, Jourlin-Castelli C, Iobbi-Nivol C, Méjean V. 2004. Genes  
542 regulated by TorR, the trimethylamine oxide response regulator of *Shewanella oneidensis*.  
543 *J Bacteriol* 186:4502–4509.
- 544 41. Barchinger SE, Pirbadian S, Sambles C, Baker CS, Leung KM, Burroughs NJ, El-Naggar  
545 MY, Golbeck JH. 2016. Regulation of Gene Expression in *Shewanella oneidensis* MR-1  
546 during Electron Acceptor Limitation and Bacterial Nanowire Formation. *Appl Environ*  
547 *Microbiol* 82:5428–5443.
- 548 42. Xu T, Liang J, Chen S, Wang L, He X, You D, Wang Z, Li A, Xu Z, Zhou X, Deng Z.  
549 2009. DNA phosphorothioation in *Streptomyces lividans*: mutational analysis of the *dnd*  
550 locus. *BMC Microbiol* 9:41.
- 551 43. Xu T, Yao F, Zhou X, Deng Z, You D. 2010. A novel host-specific restriction system  
552 associated with DNA backbone S-modification in *Salmonella*. *Nucleic Acids Res*  
553 38:7133–7141.
- 554 44. Yang Y, Xu G, Liang J, He Y, Xiong L, Li H, Bartlett D, Deng Z, Wang Z, Xiao X. 2017.  
555 DNA Backbone Sulfur-Modification Expands Microbial Growth Range under Multiple  
556 Stresses by its anti-oxidation function. *Sci Rep* 7: 3516.

- 557 45. Bordi C, Théraulaz L, Méjean V, Jourlin-Castelli C. 2003. Anticipating an alkaline stress  
558 through the Tor phosphorelay system in *Escherichia coli*. Mol Microbiol 48:211–223.
- 559 46. Li J, Wei H, Guo S, Wang E. 2008. Selective, peroxidase substrate based “signal-on”  
560 colorimetric assay for the detection of chromium (VI). Anal Chim Acta 630:181–185.
- 561 47. Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics  
562 Analysis Version 7.0 for Bigger Datasets. Mol Biol Evol 33:1870–1874.
- 563 48. Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high  
564 throughput. Nucleic Acids Res 32:1792–1797.
- 565 49. Saitou N, Nei M. 1987. The neighbor-joining method: a new method for reconstructing  
566 phylogenetic trees. Mol Biol Evol 4:406–425.
- 567 50. Tamura K, Nei M, Kumar S. 2004. Prospects for inferring very large phylogenies by using  
568 the neighbor-joining method. Proc Natl Acad Sci U S A 101:11030–11035.
- 569 51. Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL.  
570 2009. BLAST+: architecture and applications. BMC Bioinformatics 10:421.
- 571 52. Wang M-Q, Sun L. 2016. *Shewanella inventionis* sp. nov., isolated from deep-sea  
572 sediment. Int J Syst Evol Microbiol 66:4947–4953.

573

574

## 575 **Legends**

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

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

587

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

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

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

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

600

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

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

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

610

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

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

615

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

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

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

627 Then, the plates were incubated 24 or 48 hours at 28 °C. Plates are representative of at  
628 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* S12T; 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

641

642

643 **Table 3. Genome overview of *S. decolorationis* LDS1, *S. decolorationis* S12T and *S.***  
 644 ***oneidensis* MR-1.**

	<i>S. decolorationis</i> LDS1	<i>S. decolorationis</i> S12T	<i>S. oneidensis</i> 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

645

646

647

648

649

650 **Table 4. Proteins specifically found in *S. decolorationis* LDS1 from genome sequencing**  
 651 **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