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Shewanella decolorationis LDS1 Chromate Resistance

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

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14

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

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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. A 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* LDS1 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* S12, *S. decolorationis* LDS1 possesses the phosphorothioate modification
34 machinery that has been described to participate to survival against various abiotic stresses by
35 protecting DNA. We demonstrate that its heterologous production in *S. oneidensis* allows it to
36 resist higher 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 powerful tool for bioremediation and unveil one of the mechanisms
46 involved in 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 although presenting an optimal growth from 20
116 to 30 °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 ± 0.17 $\mu\text{mol}/\text{min}/\text{mg}$ versus 5.95 ± 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* S12 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* S12,
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 to guaranty a robust growth, and reduction of Cr(VI) to Cr(III)
196 in the growth medium was followed. Strikingly, when the medium was inoculated with cells of
197 *S. decolorationis* LDS1, the Cr reduction appeared when the culture entered into the stationary
198 phase (Figure 4). From that time, the reduction of chromate was almost complete within 15
199 hours. A similar experiment was performed at 40 °C. As for the lower temperature, the
200 reduction of chromate started when the cells reached the stationary phase (Figure 4). From these
201 results, it is clear that *S. decolorationis* LDS1 can reduce chromate at high temperature with the
202 same efficiency as at 28 °C. The mechanism involved is clearly acting from the beginning of
203 the stationary phase as suggested by the trigger of the reduction when the bacteria reach it, as
204 already described under 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* S12, 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 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 *ndnBCDE* 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,

236 respectively. This probably indicates that *S. decolorationis* LDS1 has acquired the genes of the
237 Dnd machinery from another 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 H₂O₂, heat or salinity by protecting DNA from
252 oxidative damage and restriction enzymes (44). In agreement with experiments 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 vector and *S. decolorationis* LDS1

259 were grown aerobically in the presence of arabinose to induce the *dndBCDE* expression from
260 the plasmid, and serial dilutions were spotted on plates containing chromate (0.4 mM) (Figure
261 5). The growth of *S. oneidensis* MR-1 harboring pBAD33 was drastically affected, whereas
262 when 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 higher chromate
265 concentrations. However, since *S. decolorationis* LDS1 resists better and also higher
266 concentrations than the recombinant *S. oneidensis* MR-1 strain, we suggest that the DndBCDE
267 machinery is only one element of the bacterial equipment allowing the high level of resistance
268 of *S. decolorationis* LDS1. To ascertain the effect due to the expression of *dndBCDE* operon in
269 *S. oneidensis* MR1, the reverse experiment was done and increased chromate concentration
270 were spotted on a lawn of bacteria. As shown on figure 5B, the presence of *dndBCDE* genes
271 led to a better resistance of the strain after 24- and 48h incubation. Moreover, we tested whether
272 the DndBCDE machinery led *S. oneidensis* MR-1 to develop at temperatures higher than 28 °C.
273 At both 38 and 40 °C, the growth of *S. oneidensis* MR-1 was not improved by the presence of
274 DndBCDE suggesting that this machinery is not sufficient to allow 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⁻¹ agar. When necessary, TMAO
318 (40 mM) and FeCl₃ (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 OD₆₀₀=0.1 in fresh LB medium and were incubated at
325 28°C. At OD₆₀₀=0.7, arabinose (0.2%, w/vol) was added for 2 hours to induce *dndBCDE*
326 expression. Cells were then diluted to OD₆₀₀=1 and 2 µL drops of 10-fold serial dilutions were
327 spotted on LB-agar plates that contained arabinose (0.2%, w/vol) in the presence or absence of
328 chromate (0.4 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 FeCl₃.

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 % (w/vol) crystal violet (Sigma Aldrich), 0.005
347 % (w/vol) Congo Red (Sigma Aldrich) or 0.005 % (w/vol) Remazol Brilliant Blue (Sigma
348 Aldrich). These media were either inoculated with *S. decolorationis* LDS1 preculture in order
349 to reach an OD₆₀₀ of 0.2 or not inoculated (negative control) and incubation was carried out
350 during ten days. For the inoculated media, cells were removed by centrifugation and remaining
351 medium was transferred in a 24 well transparent plate for imaging.

352

353 **Chromate resistance and reduction**

354 Chromate solutions were made by dilution of potassium chromate K₂CrO₄ (Sigma Aldrich) in
355 deionized water.

356 For chromate resistance assay on agar plate, 150 μ L of bacterial culture at OD₆₀₀ of 0.001 were
357 spread out on LB agar plate. A sterile cotton patch containing 50 μ 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 %, w/vol). 400 μ L of bacterial culture diluted at OD₆₀₀ of 0.01 were spread out. After a 2 h-
364 incubation, three sterile cotton patches containing 25 μ L of potassium chromate (5, 10, 20 mM)
365 were placed on the plates. Diffusion of the solution in the agar forms a chromate gradient.
366 Lengths of the bacteriostatic halo around the patch for the two strains were compared after 24-
367 and 48-hour 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. OD₆₀₀ 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 H₃PO₄ 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

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415

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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-day culture of *S. decolorationis* LDS1, in LB medium in the presence of Crystal violet
597 (CV), Congo red (CR) or Remazol brilliant blue (RBB) from the triarylmethane, azo and
598 anthraquinone dye families, respectively. “-” and “+” correspond to the addition or not of
599 bacteria prior incubation.

600

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

602 **A.** Chromate resistance was tested on solid LB medium. 150 μ l of cells (OD= 0.001) were
603 spread on the plate and 50 μ 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%, w/vol) was added at OD₆₀₀=0.7 during 2 hours. The three
620 strains were diluted to OD₆₀₀=1. 10-fold serial dilutions were spotted on LB plates
621 containing arabinose (0.2%, w/vol) and in the absence or presence of chromate (0.4
622 mM) as indicated. Then, the plates were incubated 24 or 72 hours at 28 °C. Plates are
623 representative of at 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 %, w/vol) induction, cells were spread on
626 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
Enzyme activity		
Nitrate reductase	+	+
TMAO reductase*	+	+
Gelatinase	+	+
Iron reduction	+	+
Assimilation		
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	+	+	-	-	-	-	-
Enzyme activity (28 °C)							
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	-	+	-	-	-	-	-
Assimilation (28°C)							
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* S12 and *S.***
 644 ***oneidensis* MR-1.**

	<i>S. decolorationis</i> LDS1	<i>S. decolorationis</i> S12	<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

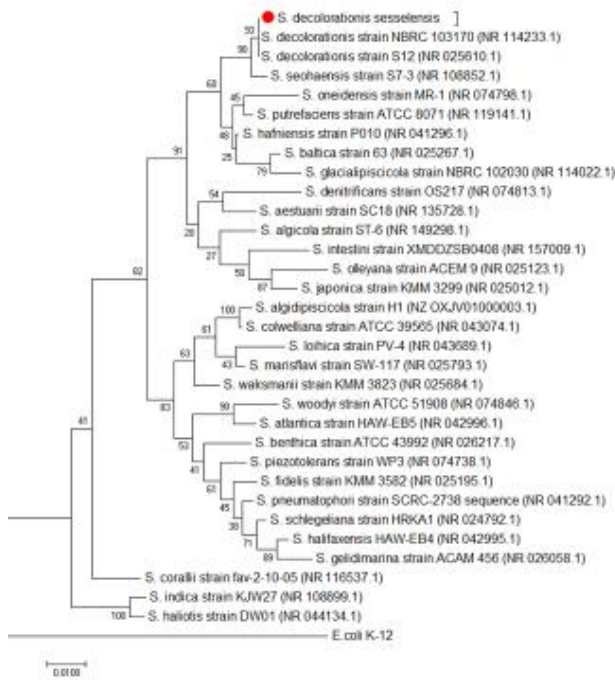
647

648

649

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

Locus	Predicted function
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



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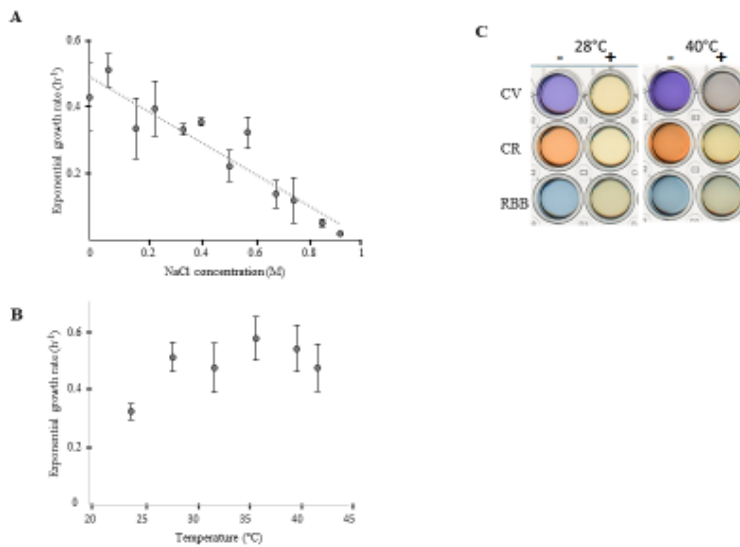


Figure 2

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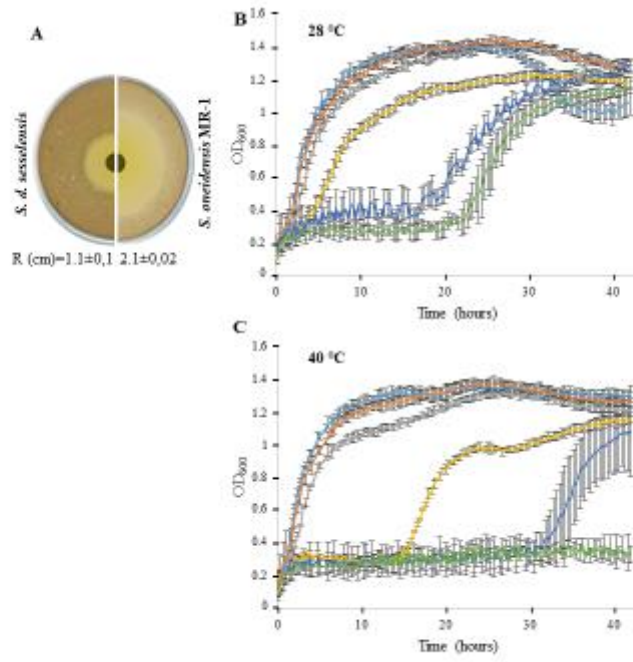


Figure 3

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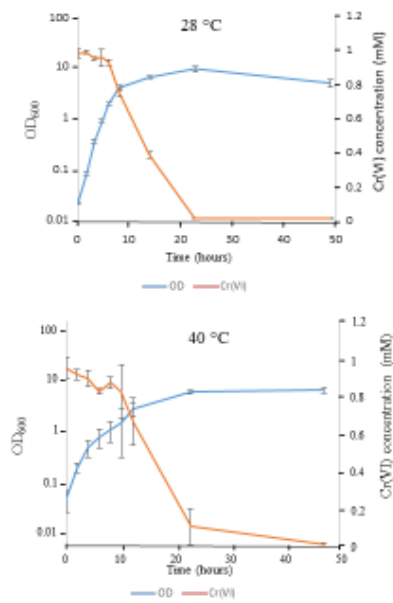


Figure 4

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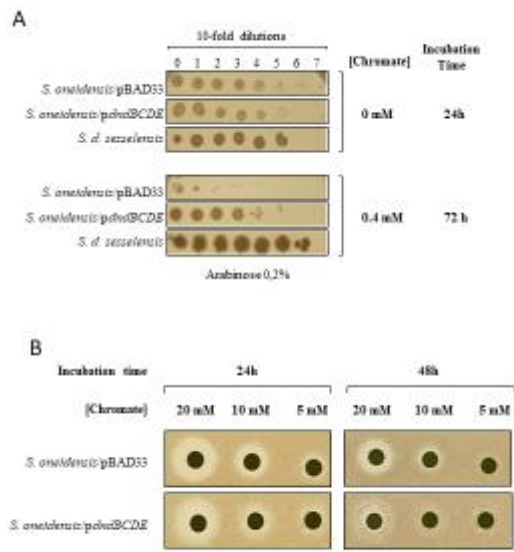


Figure 5.