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

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

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>

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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. 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* 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* 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 ( $2.8 \pm 0.17$   $\mu\text{mol}/\text{min}/\text{mg}$  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* 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 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 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<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%, w/vol) was added for 2 hours to induce *dndBCDE*  
326 expression. Cells were then diluted to OD<sub>600</sub>=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 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 % (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 OD<sub>600</sub> 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 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 %, w/vol). 400  $\mu$ L of bacterial culture diluted at OD<sub>600</sub> of 0.01 were spread out. After a 2 h-  
364 incubation, three sterile cotton patches containing 25  $\mu$ 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. 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

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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  $\mu$ 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%, w/vol) was added at OD<sub>600</sub>=0.7 during 2 hours. The three  
620 strains were diluted to OD<sub>600</sub>=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
<b>Enzyme activity</b>		
Nitrate reductase	+	+
TMAO reductase*	+	+
Gelatinase	+	+
Iron reduction	+	+
<b>Assimilation</b>		
D-glucose	+	+
N-acetylglucosamine	+	+
D-maltose	+	+
Caprate	+	-
Malate	+	+

633

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

635

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

640

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

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

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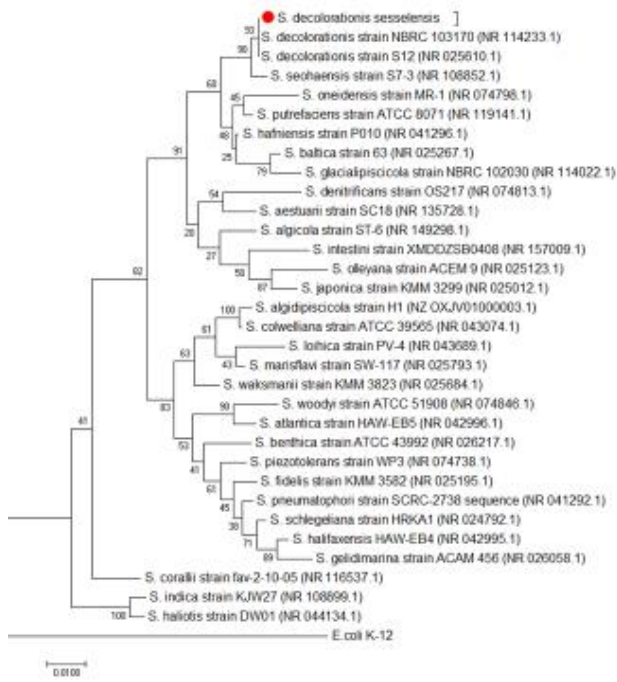
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650 **Table 4. Proteins specifically found in *S. decolorationis* LDS1 from genome sequencing**  
 651 **and annotation.**

<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



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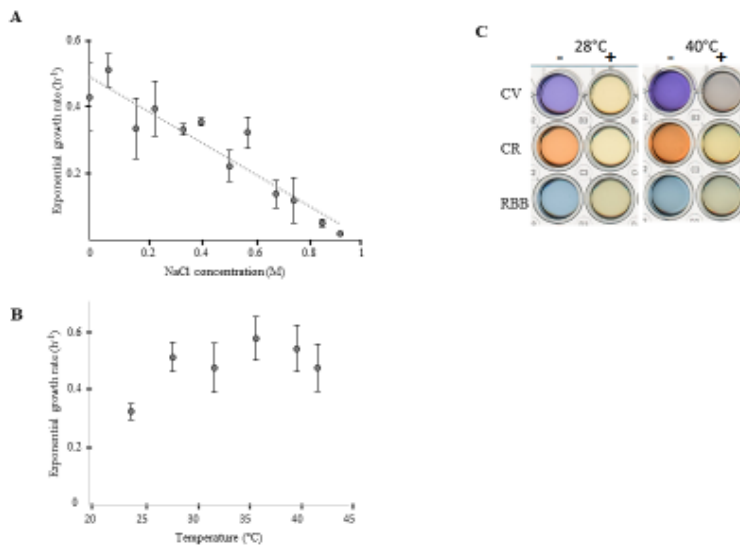


Figure 2

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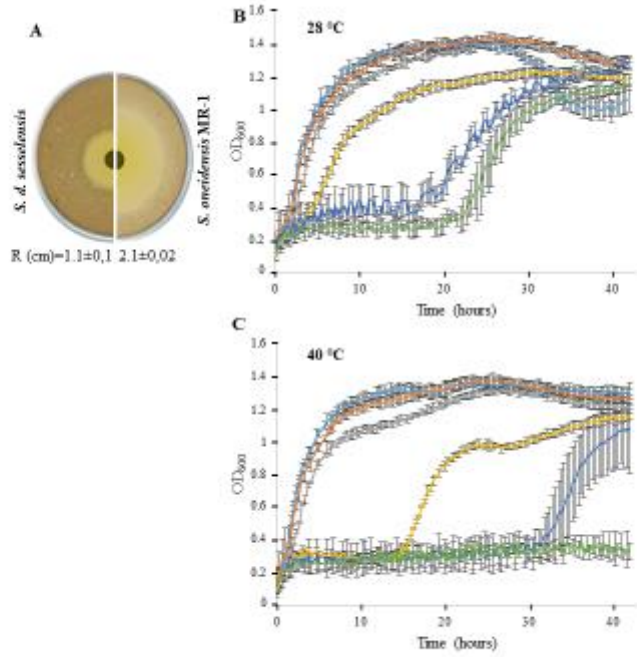


Figure 3

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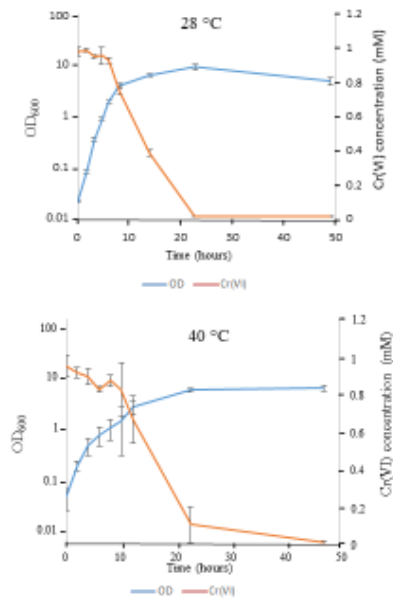


Figure 4

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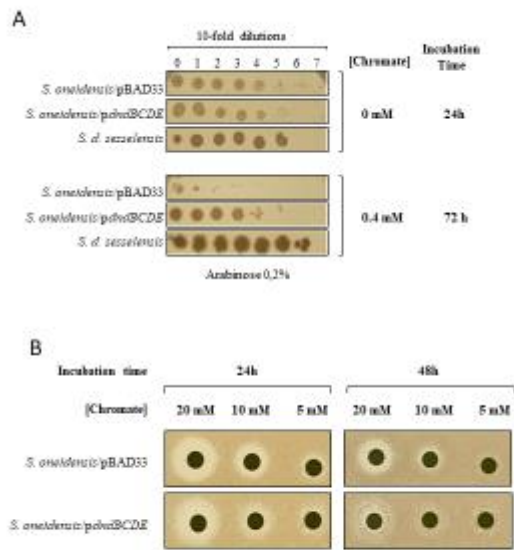


Figure 5.