

**The effect of iron-chelating agents on *Magnetospirillum magneticum* strain AMB-1: stimulated growth and magnetosome production and improved magnetosome heating properties**

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1 The effect of iron chelating agents on *Magnetospirillum*  
2 *magneticum* strain AMB-1: stimulation of growth,  
3 magnetosome production and improvement of  
4 magnetosome heating properties.

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16 **ABSTRACT**

17 The introduction of various iron chelating agents to the AMB-1 bacterial growth medium stimulates the  
18 growth of AMB-1 magnetotactic bacteria and enhances the production of magnetosomes. After seven  
19 days of growth, the number of bacteria and the production of magnetosomes are increased in the  
20 presence of iron chelating agents by factors of up to  $\sim 2$  and  $\sim 6$ , respectively. The presence of iron  
21 chelating agents also produces an increase of the magnetosome sizes and magnetosome chain lengths  
22 and yields an improvement of the magnetosome heating properties. The specific absorption rate (SAR)  
23 of suspensions of chains of magnetosomes isolated from AMB-1 magnetotactic bacteria, measured  
24 under the application of an alternating magnetic field of average field strength  $\sim 20$  mT and frequency  
25 198 kHz, increases from  $\sim 222$  W/g<sub>Fe</sub> in the absence of iron chelating agent up to  $\sim 444$  W/g<sub>Fe</sub> in the  
26 presence of 4  $\mu$ M rhodamine B and  $\sim 723$  W/g<sub>Fe</sub> in the presence of 4  $\mu$ M EDTA. These behaviors are  
27 observed for an iron concentration of 20  $\mu$ M and iron chelating agent concentration lying below 40  $\mu$ M.

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37 **KEYWORDS**

38 Magnetosomes, magnetotactic bacteria, iron chelating agents, siderophore, magnetic hyperthermia,  
39 alternating magnetic field.

## 40 INTRODUCTION

41 For a number of applications, it is advantageous to use large, single magnetic domain and well-  
42 crystallized magnetic nanoparticles, which possess a ferrimagnetic behavior at room temperature and are  
43 not prone to aggregation (Alphandéry et al 2011). Indeed, these types of nanoparticles possess a  
44 magnetic moment, which is stronger and more stable, than that of the smaller superparamagnetic  
45 magnetic nanoparticles, usually tested in biotechnological applications. Whereas monodomain  
46 nanoparticles, which are ferrimagnetic at room temperature, can hardly be produced chemically  
47 (Borderon et al 2011), certain species of bacteria, called magnetotactic bacteria (MTB), synthesize them.  
48 The organelles made of vesicles embedding magnetic crystals are called magnetosomes. They are  
49 usually composed of magnetite in the bacteria but they can oxidize into maghemite after their extraction  
50 from the bacteria due to their contact with oxygen (Alphandéry et al 2008). In addition, the  
51 magnetosomes isolated from the bacteria are not prone to aggregation due to their chain arrangement  
52 and the lipidic bi-layered membrane surrounding the magnetic crystal (Alphandéry et al 2011, Komeili  
53 2006). MTB use the magnetosomes as a compass to navigate in the direction of the Earth magnetic field  
54 and to presumably find with increased efficiency the optimum environment for their growth and survival  
55 (Bazylinski et al 2004). The magnetosomes have already been shown to be useful for a number of  
56 applications in the commercial, scientific or medical fields. For example, they can be used to extract  
57 DNA, to magnetically detect biomolecular interactions or to separate cells (Arakaki et al 2005). The  
58 anti-tumoral activity of a complex formed by bacterial magnetosomes and doxorubicin has also been  
59 demonstrated experimentally (Sun et al 2007). Chains of magnetosomes extracted from AMB-1  
60 magnetotactic bacteria have also been shown to be efficient to eradicate tumors. For that, they have been  
61 administered within tumors xeno-grafted under the skin of mice and heated under the application of an  
62 alternating magnetic field. In several mice, this treatment produced the disappearance of the tumor one  
63 month following the treatment (Alphandéry et al 2011). To consider a commercial application of the  
64 magnetosomes, the production yield of the MTB still needs to be increased. It has recently been  
65 improved in several species of magnetotactic bacteria including *Magnetospirillum magneticum* strain

66 AMB-1 (Matsunaga et al 1990, Matsunaga et al 1996a, Matsunaga et al 1996b, Matsunaga et al 2000,  
67 Yang et al 2001a, Yang et al 2001b, Yang et al 2001b), *Magnetospirillum magnetotacticum* strain MS-1  
68 (Kundu et al 2010) and *Magnetospirillum gryphiswaldense* strain MSR-1 (Guo et al 2011, Heyen et al  
69 2003, Lang et al 2006, Liu et al, 2010, Sun et al, 2008). To date, the highest production yield, which has  
70 been achieved, is 55 mg of magnetosomes synthesized per liter of growth medium and per day (Liu et al,  
71 2010).

72 In this article, we present a method, which can be used to stimulate the growth of MTB and to improve  
73 their production yield. This method uses iron chelating agents, which are introduced to the bacterial  
74 growth medium of AMB-1 magnetotactic bacteria. A series of different iron chelating agents are tested.  
75 In addition, we also show that the introduction of iron chelating agents to the bacterial growth medium  
76 yields magnetosomes with increased magnetosome sizes and magnetosome chain lengths and results in  
77 improved magnetosome heating properties under the application of an alternating magnetic field.

## 78 **MATERIALS AND METHODS**

79 MTB belonging to the species *Magnetospirillum magneticum*, strain AMB-1, available at the ATCC  
80 under the reference 700264, have been cultivated in a volume of either 10 ml or 500 ml. A suspension  
81 containing either 100  $\mu$ l of  $\sim 5 \cdot 10^8$  bacteria or 5 ml of  $\sim 2 \cdot 10^9$  bacteria was first inoculated in 10 ml or  
82 500 ml of ATCC bacterial growth medium 1653 respectively. The cells were cultivated in micro-  
83 anaerobic conditions, *i. e.* in a culture medium that had not been degased, but that was closed and thus  
84 not in contact with air. The culture of MTB was carried out in an incubator at 26 °C using a slightly  
85 modified ATCC medium 1653, whose composition is given by the ATCC. The pH of the culture  
86 medium was adjusted to 6.85 by using a 5 M solution of sodium hydroxide. The suspensions containing  
87 the various chelating agents (EDTA, ethylenediaminetetracetic acid, rhodamine B, ascorbic acid,  
88 erythrosine, anthranilic acid, citric acid, 3-(N-morpholino)propanesulfonic acid, 3-(cyclohexylamino)-1-  
89 propanesulfonic acid, calcein, dextran, alendronic acid, neridronic acid, nicotinamide) mixed in water

90 were then inserted within the bacterial growth medium. The concentration of iron chelating agents  
91 contained within the bacterial growth medium was varied between 0.4  $\mu\text{M}$  and 400  $\mu\text{M}$ .

92 After incubation, two different types of suspensions containing either whole inactive MTB or extracted  
93 chains of magnetosomes mixed in water were prepared. To prepare the suspensions containing the  
94 whole MTB, the cells were harvested by centrifugation at 4 000 g during 20 minutes at room  
95 temperature. The supernate was removed and the cells were resuspended in milli-Q water. To extract the  
96 chains of magnetosomes, 2 ml of cell suspension obtained as previously described was centrifugated  
97 again and resuspended in a 10 ml tris-HCl buffer of pH 7.4. The cellular suspension was then sonicated  
98 during 120 minutes at 30 W in order to lyse the cells and collect the chains of magnetosomes. After  
99 sonication, the suspension containing the chains of magnetosomes was separated from the cellular debris  
100 by positioning a strong neodymium magnet (0.1-1 T) next to the tube, and the magnetic material was  
101 then harvested. The supernate containing the cellular debris and other organic molecules was eliminated.  
102 The chains of magnetosomes were washed 10 to 20 times in deionized water at pH 7.4 and were then  
103 resuspended in 500  $\mu\text{L}$  of milli-Q water.

104 In order to obtain a sufficiently large amount of chains of magnetosomes, the MTB were first cultivated  
105 in a volume of 500 ml either in the absence or in the presence of various chelating agents. They were  
106 centrifugated (4000 g, 20 min) after 7 days of growth. The supernate was then removed and the bacteria  
107 were resuspended in 2 ml of mili-Q-water. For the various suspensions of MTB, the absorption was  
108 measured at 565 nm (Heyen et al 2003) and the number of bacteria was estimated using a Beckman  
109 Coulter Z1 DT. The concentrations in maghemite of the various suspensions of extracted chains of  
110 magnetosomes were measured by absorption at 480 nm (Alphandéry et al 2011).

111 The growth curves of the MTB grown either in the absence or in the presence of the various iron  
112 chelating agents were measured for the MTB cultivated in 10 ml elongated tubes allowing the presence  
113 of an oxygen gradient similar to that encountered in natural environments. The bacteria were  
114 centrifugated 1 day, 2 days, 3 days, 4 days, 7 days, 8 days and 9 days following the inoculation of the

115 MTB. The supernate was removed and the bacteria were resuspended in 1 ml of mili-Q-water. The  
116 growth curves of the various suspensions of whole MTB were then measured during the ten days  
117 following the inoculation by measuring both the absorption at 565 nm (Heyen 2003) and the number of  
118 bacteria using a Beckman Coulter Z1 DT. In order to evaluate the presence (or not) of the  
119 magnetosomes within the bacterial growth medium, the magnetic moments of the different suspensions  
120 of magnetotactic bacteria were measured. For that, 600  $\mu$ l of the various suspensions of MTB were  
121 deposited on top of non magnetic absorbing paper, which was then inserted within a capsule of gelatin.  
122 This capsule was positioned in a SQUID. The magnetic moment of the MTB was estimated under the  
123 application of a magnetic field of 1000 Oe.

124 Transmission electron microscopy (TEM) studies were carried out using a JEOL 2100F with field  
125 emission gun. For the TEM measurements, 5  $\mu$ l of a suspension of whole MTB was deposited on top of  
126 a copper grid covered with a thin amorphous carbon film. TEM measurements were used to measure the  
127 sizes of the magnetosomes and to estimate the lengths of the chains of magnetosomes in bacteria  
128 cultivated either in the absence or in the presence of iron chelating agents.

129 The maghemite composition of the magnetosomes was determined by measuring the saturation  
130 isothermal remanent magnetization curves of the magnetotactic bacteria cultivated in various conditions  
131 following the same method as that described previously (Alphandéry et al 2008). The absence of the  
132 Verwey transition indicates that the magnetosomes are oxidized into maghemite.

133 The heating curves of suspensions containing 300  $\mu$ l of extracted chains of magnetosomes mixed in  
134 water with a concentration in maghemite of 478  $\mu$ g/ml were measured. The maghemite composition of  
135 the chains of magnetosomes extracted from AMB-1 magnetotactic bacteria was estimated following a  
136 method previously described (Alphandéry et al 2008). The concentration in maghemite of the different  
137 suspensions of extracted chains of magnetosomes was measured at 480 nm. To generate heat, the  
138 suspensions of extracted chains of magnetosomes were exposed to an alternating magnetic field of

139 frequency 198 kHz and average field amplitude of 20 mT using a 10 kW EasyHeat power supply from  
140 Ambrell, Soultz, France. The average field strength within the coil was measured using a 2 D probe  
141 (Suppl. Fig. 1). The temperature of the suspensions was measured using a thermocouple microprobe (IT-  
142 18, Physitemp, Clifton, USA).

## 143 **RESULTS**

144 Figure 1 shows the optical absorption, measured at 565 nm, of suspensions of MTB cultivated either in  
145 the absence or in the presence of various iron chelating agents and harvested after 7 days of growth.  
146 About 70 % of the iron chelating agents tested produces an increase of the absorption of the suspension  
147 of MTB. This indicates that in most cases the presence of iron chelating agents within the bacterial  
148 growth medium stimulates the growth of the MTB. The growth stimulation is observed at low  
149 concentrations of iron chelating agents, which lie below 40  $\mu\text{M}$  (Figure 1). The most pronounced growth  
150 stimulations are observed for hemoglobin at concentration 40  $\mu\text{M}$ , citric acid at concentration 4  $\mu\text{M}$ , and  
151 hemoglobin at concentration 4  $\mu\text{M}$  for which the optical absorptions of the suspension of MTB is 1.5 to  
152 2.5 times larger than that of the bacteria cultivated in the absence of iron chelating agents. Figure 2  
153 shows the concentration in maghemite of the suspensions containing chains of magnetosomes extracted  
154 from whole MTB harvested after seven days of growth and cultivated in the same conditions as those  
155 presented in Figure 1. Since the magnetosomes studied here are oxidized into maghemite (Alphandéry  
156 2008), the concentration in maghemite, which is measured, is equal to the concentration in  
157 magnetosomes. We therefore deduce from Figure 2 that the production of magnetosomes is increased  
158 for a majority of iron chelating agents tested ( $\sim 65\%$ ) and for concentrations in iron chelating agents,  
159 which lie below 40  $\mu\text{M}$ . The strongest enhancement of the magnetosome production is observed when  
160 0.4  $\mu\text{M}$  or 4  $\mu\text{M}$  of hemoglobin is introduced within the bacterial growth medium. In this case,  
161 enhancements of the magnetosome production by factors of 6 are achieved. It is also worth mentioning  
162 that the concentration in iron chelating agent, which produces the largest growth stimulation, for



163 example 40  $\mu\text{M}$  for hemoglobin (Figure 1), is not always the same as that, which yields the largest  
164 magnetosome production (for example 4  $\mu\text{M}$  for hemoglobin).

165 The influence of introducing iron chelating agents to the bacteria growth medium on several MTB and  
166 magnetosome properties is studied next. We describe the results obtained with an iron chelating agent,  
167 rhodamine B, which shows a typical behavior, *i. e.* which stimulates well both the growth of the MTB  
168 and the production of the magnetosomes. Figure 3(a) shows the growth curves of MTB cultivated either  
169 in the absence of rhodamine B or in the presence of different concentrations of rhodamine B. In the  
170 presence of 0.4  $\mu\text{M}$ , 4  $\mu\text{M}$  or 40  $\mu\text{M}$  of rhodamine B introduced to the bacterial growth medium, the  
171 growth of MTB is more rapid than in the absence of rhodamine B. The concentration of 400  $\mu\text{M}$  of  
172 rhodamine B does not stimulate the growth of MTB. The growth stimulation induced by the presence of  
173 rhodamine B is significant during the 3 or 4 first days of growth, *i. e.* during the exponential phase. After  
174 that, the bacteria reach the stationary phase and then the absorption of the different suspensions of  
175 bacteria decreases most probably due to the death of the bacteria (Suppl. Fig. 2). In order to determine  
176 the rate at which the magnetosomes grow, the magnetic moments of the suspensions of MTB cultivated  
177 in the absence or in the presence of various concentrations of rhodamine B were measured for an applied  
178 magnetic field of 1000 Oe. Before the fourth day of growth, the magnetic moments of the various  
179 suspensions of bacteria were not detected indicating the absence or undetectable concentrations of  
180 magnetosomes in these suspensions. At days 4, 7 and 8, the magnetic moments of the suspensions  
181 containing MTB cultivated in the presence of 0.4  $\mu\text{M}$ , 4  $\mu\text{M}$  or 40  $\mu\text{M}$  of rhodamine B were detected.  
182 The magnetic moments of the suspensions containing MTB cultivated in the absence of rhodamine B  
183 were detected at a later stage during the ninth day of growth, indicating that the production of the  
184 magnetosomes is more rapid for the MTB cultivated in the presence of rhodamine B than for those  
185 cultivated in the absence of rhodamine B. The stimulations of the growth of the bacteria and of the  
186 production of the magnetosomes were observed for other iron chelating agents introduced within the  
187 bacterial growth medium, such as ascorbic acid (Suppl. Fig. 3).

188 The magnetosome sizes and magnetosome chain lengths of the extracted chains of magnetosomes were  
189 measured for the MTB cultivated in the absence of rhodamine B and for those cultivated in the presence  
190 of 4  $\mu\text{M}$  of rhodamine B (Figure 4). The distributions in magnetosome sizes and magnetosome chain  
191 lengths are bimodal. For the magnetosomes of small sizes, the magnetosome sizes and magnetosome  
192 chain lengths increase from an average of  $\sim 27.5$  nm and  $\sim 150$  nm in the absence of iron chelating agent  
193 up to an average of  $\sim 35$  nm and  $\sim 200$  nm in the presence of 4  $\mu\text{M}$  rhodamine B, respectively (Figures  
194 4(a)-(d)). The largest magnetosomes of the longest chains increase from an average of  $\sim 42.5$  nm and  $\sim$   
195 750 nm in the absence of rhodamine B up to an average of  $\sim 65$  nm and  $\sim 800$  in the presence of  
196 rhodamine B, respectively (Figures 4(a)-(d)). The increase of the magnetosome sizes and magnetosome  
197 chain lengths was also observed in the presence of other iron chelating agents such as EDTA (Suppl.  
198 Fig. 4).

199 Three suspensions containing 300  $\mu\text{l}$  of extracted chains of magnetosomes with a concentration in  
200 maghemite of 478  $\mu\text{g/ml}$  were then heated under the application of an alternating magnetic field of  
201 frequency 198 kHz and average magnetic field strength of 20 mT. These suspensions contain chains of  
202 magnetosomes synthesized either in the absence or in the presence of 4  $\mu\text{M}$  Rhodamine B or 4  $\mu\text{M}$   
203 EDTA. Figure 5(a) shows that the absorption curves of the three suspensions are the same, indicating  
204 that their concentration is identical. The variations of temperature as a function of time of the three  
205 suspensions are shown in Figure 5(b). The increase in temperature is more important in the presence of  
206 4  $\mu\text{M}$  rhodamine B or 4  $\mu\text{M}$  EDTA than in the absence of iron chelating agent. The slopes, measured at  
207 25  $^{\circ}\text{C}$ , of the variations of temperature with time shown in Figure 5(b), designated as  $\Delta T/\delta t$ , increase  
208 from  $\Delta T/\delta t \sim 0.005$   $^{\circ}\text{C}/\text{sec}$ . in the absence of iron chelating agent up to  $\Delta T/\delta t \sim 0.011$   $^{\circ}\text{C}/\text{sec}$  in the  
209 presence of 4  $\mu\text{M}$  Rhodamine and 0.017  $^{\circ}\text{C}/\text{sec}$  in the presence of 4  $\mu\text{M}$  EDTA. Using the relation  
210 between the specific absorption rate (SAR) and  $(\Delta T/\delta t)$ , which is given by  $\text{SAR} = C_v(\Delta T/\delta t)$ , where  $C_v =$   
211 4.2 J/(g.K) is the specific heat capacity of water, we find that the SAR increases from  $\sim 222$  W/g<sub>Fe</sub> in the

212 absence of iron chelating agent up to  $\sim 444$  W/g<sub>Fe</sub> in the presence of 4  $\mu$ M rhodamine B and  $\sim 723$   
213 W/g<sub>Fe</sub> in the presence of 4  $\mu$ M EDTA.

## 214 **DISCUSSION**

215 We have shown that the presence of iron chelating agents within the bacterial growth medium stimulates  
216 the production of the magnetosomes. The function of the iron chelating agents is to chelate Fe<sup>3+</sup> iron  
217 ions and to enhance the bioavailability of iron by forming Fe<sup>3+</sup>-ligand complexes. The iron chelating  
218 agents could be involved in one or several of the following steps of the magnetosome formation, (i) the  
219 formation of the vesicle in which the magnetosomes are formed, (ii) the iron uptake by the cells, (iii) the  
220 iron transport within the cells and (iv) the control of the biomineralization of the magnetosome  
221 formation in Fe<sub>3</sub>O<sub>4</sub> or  $\gamma$ -Fe<sub>2</sub>O<sub>3</sub> (Bazylinski 2004). They could therefore favor the magnetosome formation  
222 by making each or several of these steps more efficient.

223 We examine why the presence of iron chelating agents stimulates the growth of the bacteria. The  
224 mechanisms, which enhance the production of the magnetosomes and those, which stimulate the growth  
225 of the bacteria, are expected to be different. The MTB are believed to synthesize siderophores, which  
226 help to incorporate iron within the bacteria (Calugay 2006). In the absence of such complexing agents,  
227 Fe<sup>3+</sup> iron ions precipitate mostly as ferrihydrite and their bioavailability is likely strongly decreased.  
228 When iron chelating agents are introduced to the bacterial growth medium, the MTB have less need to  
229 synthesize siderophores. It is thus likely that the energy allocated for siderophore synthesis can then be  
230 used for bacterial growth. This could explain the more efficient growth observed in the presence than in  
231 the absence of iron chelating agents.

232 We also observe that the highest stimulation of the bacteria is observed for large iron chelating agents,  
233 such as hemoglobin. This result suggests that iron is chelated in a more efficient way using high  
234 molecular weight than low molecular weight iron chelating agents. This is in agreement with previous  
235 study (Reichard *et al*, 2007). There are several possible explanations for this behavior. The stability

236 formed by a large iron complex could be higher than that formed by a smaller iron complex. A large iron  
237 complex may also more easily enter within the periplasm of the cells. It is also possible that a large iron  
238 complex would more easily release iron within the bacterial cells or that it would favor oxidoreduction  
239 reactions involved in the magnetosome production or crystallization process.

240 We have shown that above a concentration in iron chelating agent lying between 40  $\mu\text{M}$  and 400  $\mu\text{M}$ ,  
241 the stimulation of the growth of the MTB and of the production of the magnetosomes is not observed.  
242 This result agrees with previous reports, which showed that for a ratio of 100 to 500 between the iron  
243 chelating agent and iron concentrations, no growth stimulation was observed for the species MSR-1  
244 (Schüler 1996). This indicates that the iron chelating agents are toxic above concentrations lying  
245 between 40  $\mu\text{M}$  and 400  $\mu\text{M}$ . We propose two hypotheses to explain this result. Either a high iron  
246 chelating agent concentration yields a high iron concentration within the bacterial growth medium,  
247 which is toxic for the magnetotactic bacteria. Or the reactive oxygen species, which are formed because  
248 of the alcohol functional groups getting unbound from iron, for example in rhodamine B, damage the  
249 membrane of the bacteria.

250 The kinetics of the magnetosome formation is reported for the AMB-1 species. For this species, the  
251 magnetosomes are probably mainly formed after the magnetotactic bacteria have reached the stationary  
252 phase (Yang 2001). We have shown that the introduction of iron chelating agents within the bacterial  
253 growth medium of AMB-1 magnetotactic bacteria yields faster growth of magnetosomes. This should  
254 therefore enable a significant increase in the rate of production of the magnetosomes by the AMB-1  
255 magnetotactic bacteria. In strain MSR-1, the magnetosomes are produced earlier, before the bacteria  
256 have reached the stationary phase (Heyen and Schüler 2003). This behavior could be explained by a  
257 different mechanism of iron uptake or of magnetosome formation between these two species of  
258 magnetotactic bacteria. Therefore, the influence of introducing iron chelating agents within the bacterial  
259 growth medium on growth rate of magnetosome production may be less pronounced for the MSR-1 than  
260 for the AMB-1 species.

261 The introduction of iron chelating agents to the bacterial growth medium also increases the  
262 magnetosome sizes and magnetosome chain lengths, which results in an improvement of the  
263 magnetosome heating properties. The specific absorption rate of suspensions of extracted chains of  
264 magnetosomes increases by a factor of 3 between the MTB cultivated in the absence of iron chelating  
265 agent and those cultivated in the presence of 4  $\mu\text{M}$  EDTA. High values of SAR are advantageous to  
266 carry out magnetic hyperthermia treatments of cancers since they enable the use of a small quantity of  
267 magnetosomes and/or the application of an alternating magnetic field of low strength, hence decreasing  
268 the risks of toxicity.

269 Further studies should explore separately the effect of iron on either the bacterial growth or the  
270 production of magnetosomes.

271 In conclusion, the introduction of iron chelating agents within the bacterial growth medium enables the  
272 stimulation of the growth of the MTB, the enhancement of the production yield of the magnetosomes,  
273 the increase of the magnetosome sizes and magnetosome chain lengths as well as the enhancement of  
274 the magnetosome heating properties under the application of an alternating magnetic field.

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276 F. Guyot and M. Amor do not claim any inventive contribution in this work. They only analyzed  
277 experimental results.

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347 **FIGURES**

348 Figure 1: Absorption measured at 565 nm and number of magnetotactic bacteria per ml of suspensions  
349 of magnetotactic bacteria cultivated either in the absence of iron chelating agents or in the presence of  
350 0.4  $\mu\text{M}$ , 4  $\mu\text{M}$ , 40  $\mu\text{M}$  or 400  $\mu\text{M}$  of various iron chelating agents. These chelating agents are EDTA,  
351 rhodamine B, ascorbic acid, erythrosine, anthranilic acid, citric acid, 3-(N-morpholino)propanesulfonic  
352 acid, 3-(cyclohexylamino)-1-propanesulfonic acid, calcein, dextran, alendronic acid, neridronic acid and  
353 nicotinamide. Dotted lines represent control values.

354 Figure 2: The concentration in maghemite of suspensions containing chains of magnetosomes extracted  
355 from whole magnetotactic bacteria. The magnetotactic bacteria have been cultivated either in the  
356 absence or in the presence of the same iron chelating agents as in Figure 1.

357 Figure 3: (a) The growth curves of magnetotactic bacteria cultivated either in the absence of rhodamine  
358 B or in the presence of 0.4  $\mu\text{M}$ , 4  $\mu\text{M}$ , 40  $\mu\text{M}$  or 400  $\mu\text{M}$  of rhodamine B. The growth curves represent  
359 the absorption of the different suspensions of magnetotactic bacteria at 565 nm (left y scale) or the  
360 number of MTB per ml (right y scale) measured at the different days of growth (day 0, day 1, day 3, day  
361 4). (b) The Magnetic moments of the suspensions of magnetotactic bacteria cultivated either in the  
362 absence or in the presence of 0.4  $\mu\text{M}$ , 4  $\mu\text{M}$ , 40  $\mu\text{M}$  or 400  $\mu\text{M}$  of rhodamine B, measured under the  
363 application of a magnetic field of 1000 Oe during the fourth day, the seventh day, the eighth day or the  
364 ninth day of bacterial growth.

365 Figure 4: (a), (b) The magnetosome size distribution of the magnetosomes contained within the  
366 magnetotactic bacteria for the bacteria cultivated either in the absence, (a), or in the presence of 4  $\mu\text{M}$   
367 rhodamine B, (b). (c), (d) The magnetosome chain length distribution of the chains of magnetosomes  
368 contained within the magnetotactic bacteria for the bacteria cultivated either in the absence, (c), or in the  
369 presence of 4  $\mu\text{M}$  rhodamine B, (d).

370 Figure 5: (a) The absorption curves of three suspensions of extracted chains of magnetosomes. The  
371 MTB were cultivated either in the absence of iron chelating agent, in the presence of 4  $\mu$ M rhodamine B  
372 or in presence of 4  $\mu$ M EDTA. (b) The variations of temperature as a function of time of the same  
373 suspensions as in (a) exposed to an alternating magnetic field of frequency 198 kHz and average field  
374 strength of 20 mT. Ch-Std, Ch-EDTA (4 $\mu$ M) and Ch-Rhodamine B (4 $\mu$ M) designate the chains of  
375 magnetosomes isolated from the bacteria obtained by cultivating the bacteria in the absence of iron  
376 chelating agent, in the presence of 4  $\mu$ M EDTA or in the presence of 4  $\mu$ M rhodamine B, respectively.

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**Whole magnetotactic bacteria AMB-1 harvested after 7 days of growth**

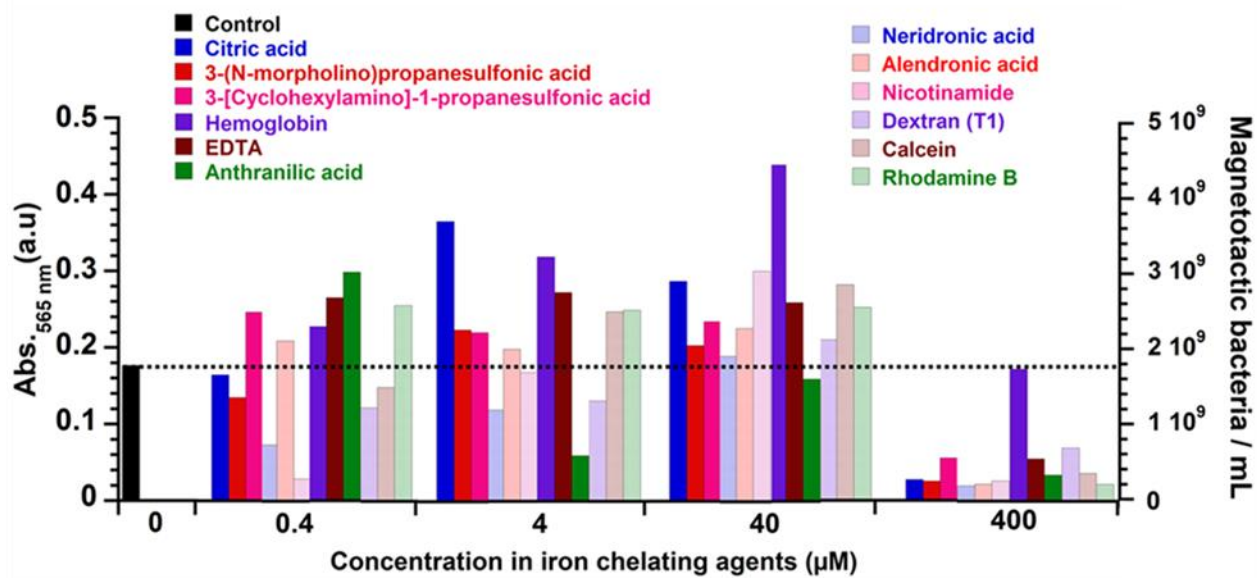


FIG. 1

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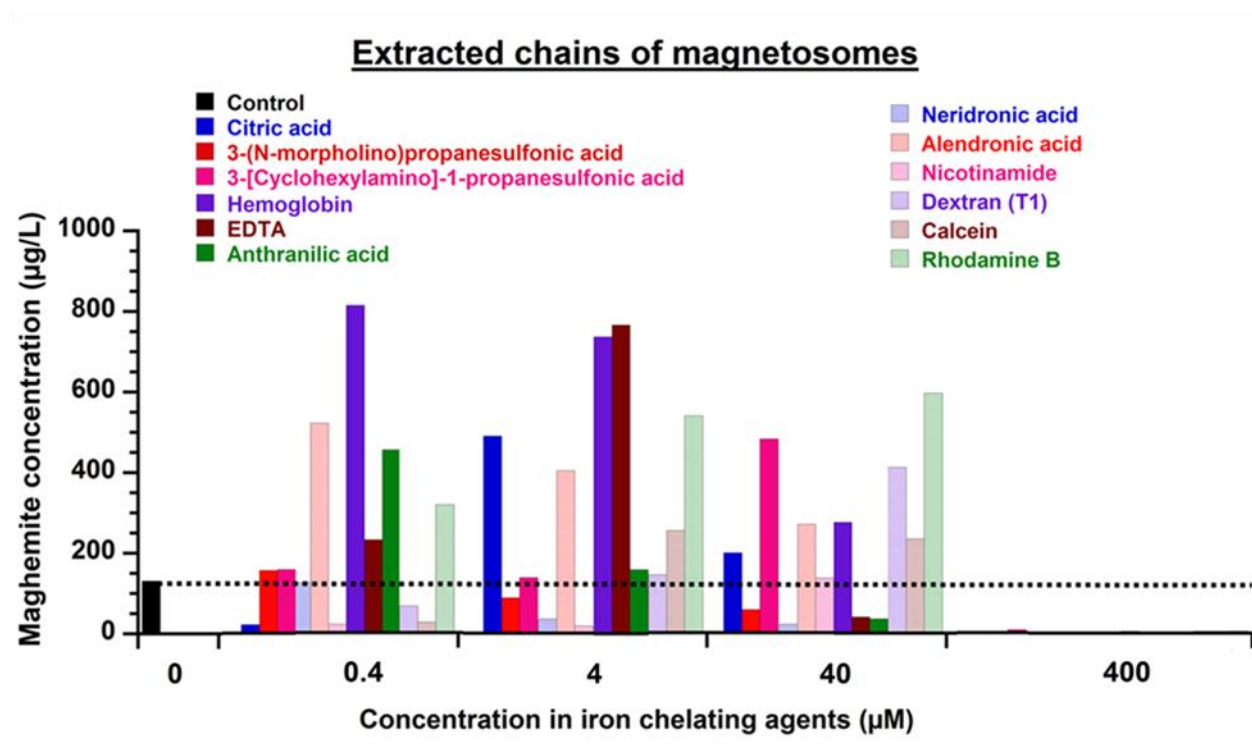


FIG. 2

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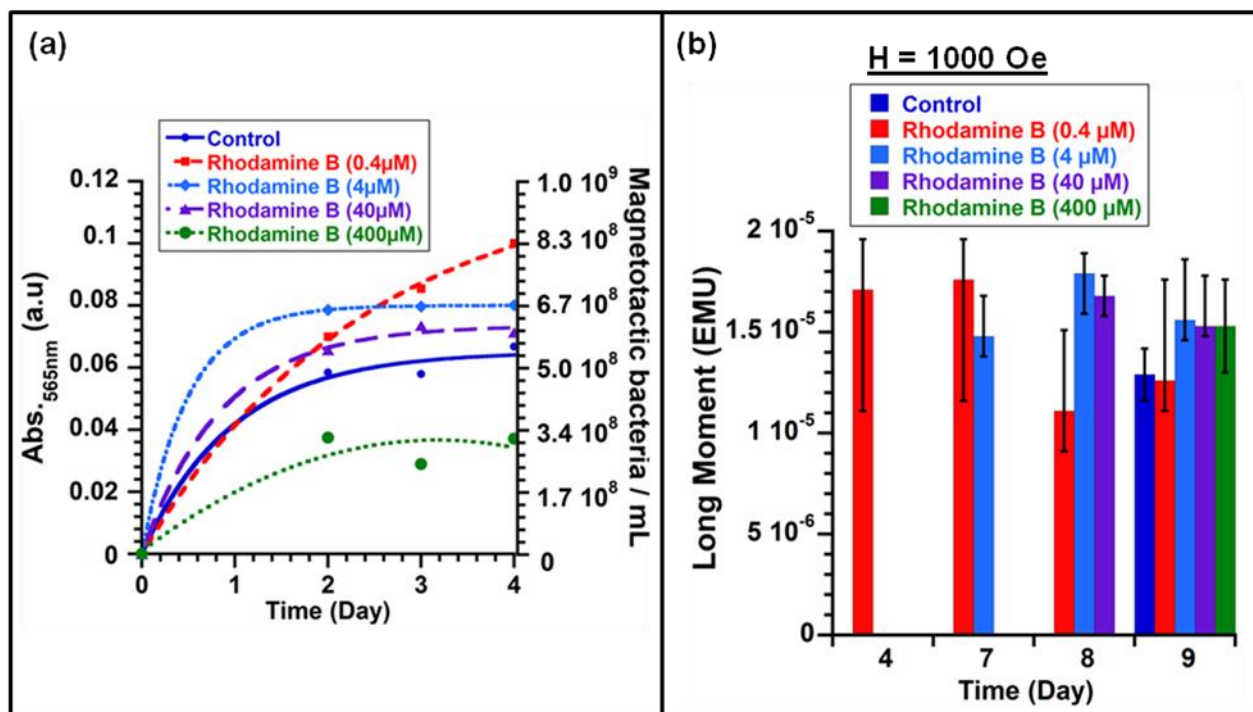


FIG. 3

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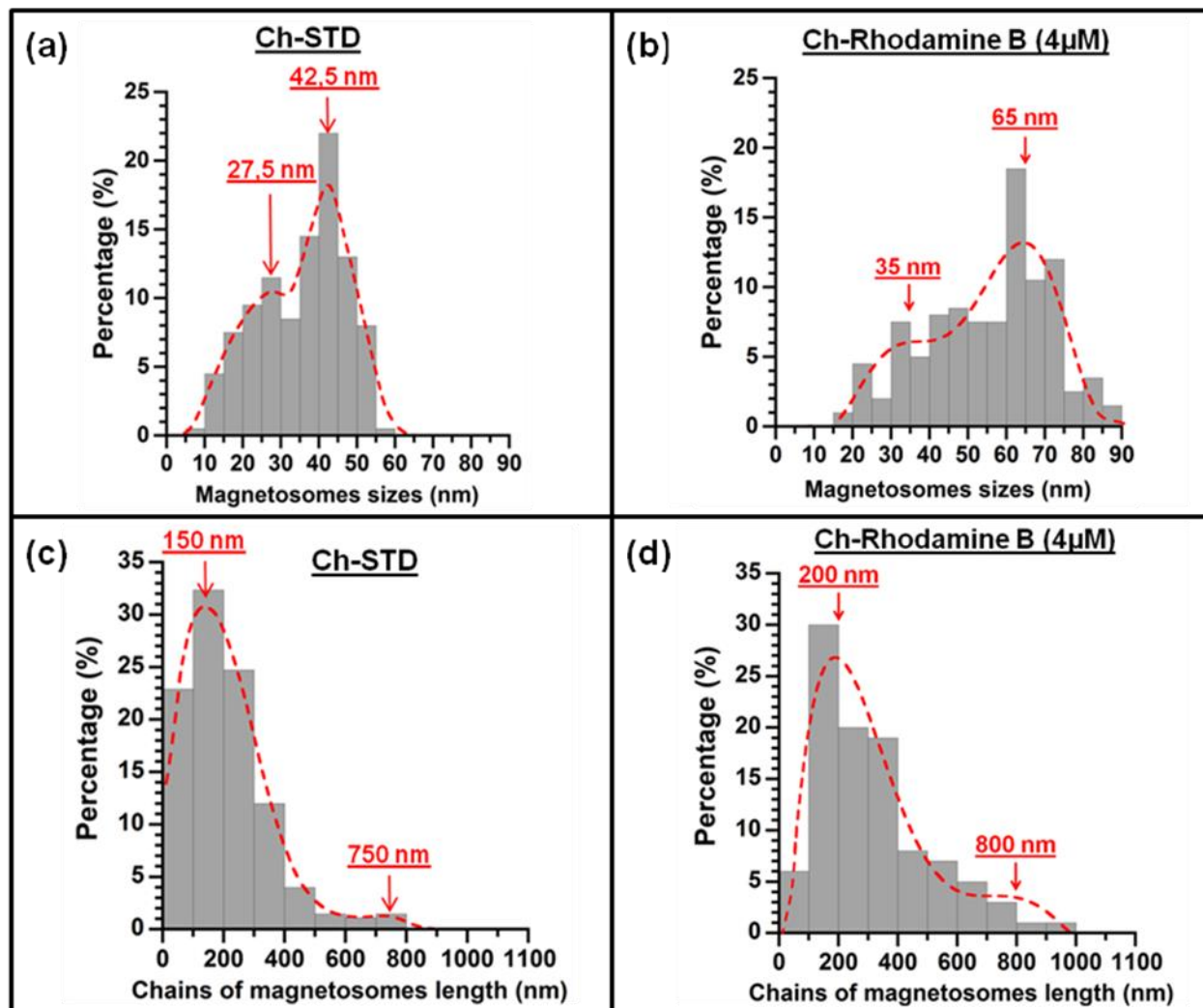


FIG. 4

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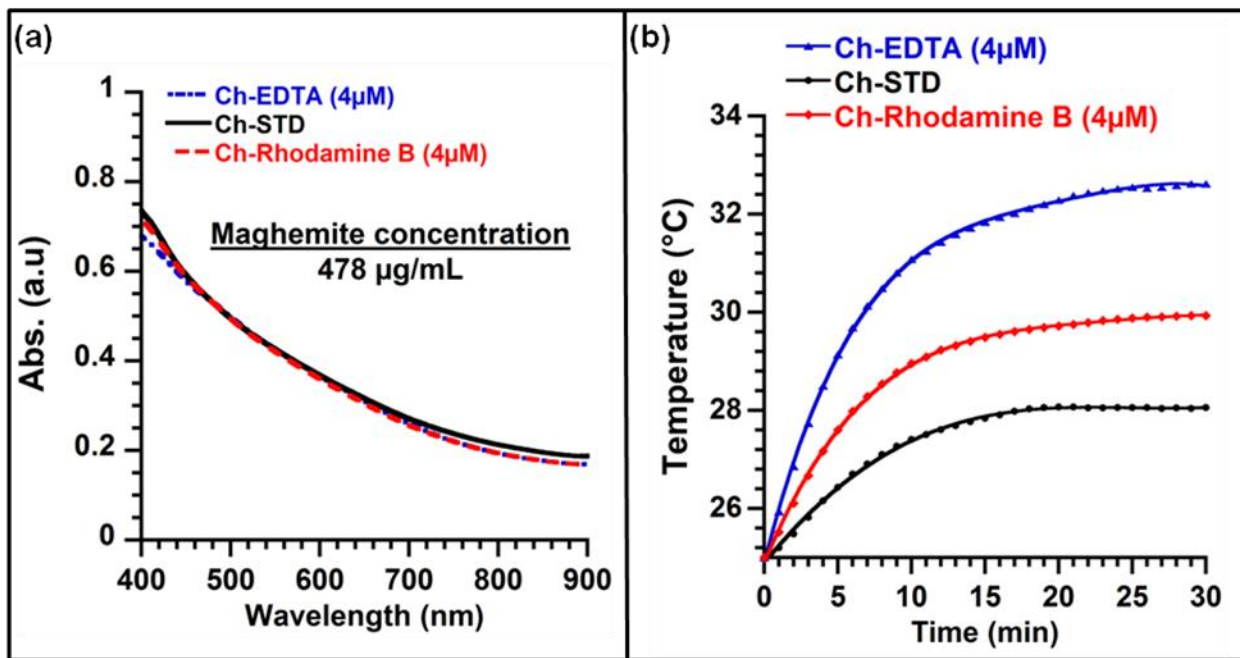


FIG. 5

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