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

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ABSTRACT

The introduction of various iron chelating agents to the AMB-1 bacterial growth medium stimulates the growth of AMB-1 magnetotactic bacteria and enhances the production of magnetosomes. After seven days of growth, the number of bacteria and the production of magnetosomes are increased in the presence of iron chelating agents by factors of up to ~ 2 and ~ 6, respectively. The presence of iron chelating agents also produces an increase of the magnetosome sizes and magnetosome chain lengths and yields an improvement of the magnetosome heating properties. The specific absorption rate (SAR) of suspensions of chains of magnetosomes isolated from AMB-1 magnetotactic bacteria, measured under the application of an alternating magnetic field of average field strength ~ 20 mT and frequency 198 kHz, increases from ~ 222 W/g_{Fe} in the absence of iron chelating agent up to ~ 444 W/g_{Fe} in the presence of 4 μM rhodamine B and ~ 723 W/g_{Fe} in the presence of 4 μM EDTA. These behaviors are observed for an iron concentration of 20 μM and iron chelating agent concentration lying below 40 μM.

KEYWORDS

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

INTRODUCTION

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

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

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

MATERIALS AND METHODS

MTB belonging to the species *Magnetospirillum magneticum*, strain AMB-1, available at the ATCC under the reference 700264, have been cultivated in a volume of either 10 ml or 500 ml. A suspension containing either 100 µl of ~ 5.10^8 bacteria or 5 ml of ~ 2.10^9 bacteria was first inoculated in 10 ml or 500 ml of ATCC bacterial growth medium 1653 respectively. The cells were cultivated in micro-anaerobic conditions, *i. e.* in a culture medium that had not been degased, but that was closed and thus not in contact with air. The culture of MTB was carried out in an incubator at 26 °C using a slightly modified ATCC medium 1653, whose composition is given by the ATCC. The pH of the culture medium was adjusted to 6.85 by using a 5 M solution of sodium hydroxide. The suspensions containing the various chelating agents (EDTA, ethylenediaminetetracetic acid, rhodamine B, ascorbic acid, erythrosine, anthranilic acid, citric acid, 3-(N-morpholino)propanesulfonic acid, 3-(cyclohexylamino)-1-propanesulfonic acid, calcein, dextran, alendronic acid, neridronic acid, nicotinamide) mixed in water

were then inserted within the bacterial growth medium. The concentration of iron chelating agents contained within the bacterial growth medium was varied between 0.4 μM and 400 μM.

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

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

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

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

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

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

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

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

RESULTS

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

example 40 μM for hemoglobin (Figure 1), is not always the same as that, which yields the largest magnetosome production (for example 4 μM for hemoglobin).

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

The magnetosome sizes and magnetosome chain lengths of the extracted chains of magnetosomes were measured for the MTB cultivated in the absence of rhodamine B and for those cultivated in the presence of 4 μM of rhodamine B (Figure 4). The distributions in magnetosome sizes and magnetosome chain lengths are bimodal. For the magnetosomes of small sizes, the magnetosome sizes and magnetosome chain lengths increase from an average of ~ 27.5 nm and ~ 150 nm in the absence of iron chelating agent up to an average of ~ 35 nm and ~ 200 nm in the presence of 4 μM rhodamine B, respectively (Figures 4(a)-(d)). The largest magnetosomes of the longest chains increase from an average of ~ 42.5 nm and ~ 750 nm in the absence of rhodamine B up to an average of ~ 65 nm and ~ 800 in the presence of rhodamine B, respectively (Figures 4(a)-(d)). The increase of the magnetosome sizes and magnetosome chain lengths was also observed in the presence of other iron chelating agents such as EDTA (Suppl. Fig. 4).

Three suspensions containing 300 μl of extracted chains of magnetosomes with a concentration in maghemite of 478 μg/ml were then heated under the application of an alternating magnetic field of frequency 198 kHz and average magnetic field strength of 20 mT. These suspensions contain chains of magnetosomes synthesized either in the absence or in the presence of 4 μM Rhodamine B or 4 μM EDTA. Figure 5(a) shows that the absorption curves of the three suspensions are the same, indicating that their concentration is identical. The variations of temperature as a function of time of the three suspensions are shown in Figure 5(b). The increase in temperature is more important in the presence of 4 μM rhodamine B or 4 μM EDTA than in the absence of iron chelating agent. The slopes, measured at 25 °C, of the variations of temperature with time shown in Figure 5(b), designated as $\Delta T/\delta t$, increase from $\Delta T/\delta t$ ~ 0. 005 °C/sec. in the absence of iron chelating agent up to $\Delta T/\delta t$ ~ 0.011 °C/sec in the presence of 4 μM Rhodamine and 0.017 °C/sec in the presence of 4 μM EDTA. Using the relation between the specific absorption rate (SAR) and $(\Delta T/\delta t)$, which is given by $SAR = C_v(\Delta T/\delta t)$, where C_v = 4.2 J/(g.K) is the specific heat capacity of water, we find that the SAR increases from ~ 222 W/g_{Fe} in the

absence of iron chelating agent up to ~ 444 W/g_{Fe} in the presence of 4 μM rhodamine B and ~ 723 W/gFe in the presence of 4 μM EDTA.

DISCUSSION

We have shown that the presence of iron chelating agents within the bacterial growth medium stimulates the production of the magnetosomes. The function of the iron chelating agents is to chelate Fe^{3+} iron ions and to enhance the bioavailability of iron by forming Fe^{3+}-ligand complexes. The iron chelating agents could be involved in one or several of the following steps of the magnetosome formation, (i) the formation of the vesicle in which the magnetosomes are formed, (ii) the iron uptake by the cells, (iii) the iron transport within the cells and (iv) the control of the biomineralization of the magnetosome formation in Fe_3O_4 or γFe_2O_3 (Bazylinski 2004). They could therefore favor the magnetosome formation by making each or several of these steps more efficient.

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

We also observe that the highest stimulation of the bacteria is observed for large iron chelating agents, such as hemoglobin. This result suggests that iron is chelated in a more efficient way using high molecular weight than low molecular weight iron chelating agents. This is in agreement with previous 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 μM and 400 μM,
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 μM and 400 μM. 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.

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

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

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

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

REFERENCES

Alphandéry E, Faure S, Seksek O, Guyot F, Chebbi I (2011) Chains of magnetosomes extracted from *AMB-1* magnetotactic bacteria for application in alternative magnetic field cancer therapy 5:6279-6296.

Alphandéry E, Ngo A T, Lefèvre C, Lisiecki I, Wu L F, Pileni M P (2008) Difference between the Magnetic Properties of the Magnetotactic Bacteria and Those of the Extracted Magnetosomes: Influence of the Distance between the Chains of Magnetosomes J. Phys. Chem. 112:12304-12309.

284 Arakaki A, Nakazawa H, Nemoto M, Mori T, Matsunaga T (2008) Formation of magnetite by bacteria
285 and its application J. R. Soc. Interface 5:977-999.

286 Bazylinski DA, Frankel RB (2004) Magnetosome formation in prokaryotes Nature Reviews
287 Microbiology 2:217-230.

288 Bordelon DE, Cornejo C, Grüttner C, Westphal F, DeWeese TL, Ivkov R (2011) Magnetic nanoparticle
289 heating efficiency reveals magneto-structural differences when characterized with wide ranging and high
290 amplitude alternating magnetic fields J. Appl. Phys. 109:124904-124904-8.

291 Calugay RJ, Miyashita H, Okamura Y, Matsunaga T (2008) Siderophore production by the magnetic
292 bacterium Magnetospirillum magneticum *AMB-1* FEMS Micribiology Letters 218:371-375.

293 Guo F, Liu Y, Chen Y, Tang T, Jiang W, Li Y, Li J (2011) A novel rapid and continuous procedure for
294 large-scale purification of magnetosomes from *Magnetospirillum gryphiswaldense* Appl. Microbiol.
295 Biotechnol. 90:1277-1283.

296 Heyen U, Schüler D (2003) Growth and magnetosome formation by microaerophilic *Magnetospirillum*
297 strains in an oxygen-controlled fermentor (2003) Appl. Microbiol. Biotechnol. 61:536-544.

298 Komeili A (2006) *Cell biology of magnetosome formation* Microbiol. Monogr. 3:163-174.

299 Kundu S, Kulkarni GR (2010) Enhancement of magnetotactic bacterial yield in a modified MSGM
300 medium without alteration of magnetosomes properties Indian J. Exp. Bio. 48:518-523.

301 Lang C, Schüler D (2006) Biogenic nanoparticles: production, characterization, and application of
302 bacterial magnetosomes J. Phys. Condens. Matter 18:S2815-S2828.

303 Liu Y, Li GR, Guo F, Jiang W, Li Y, Li LJ (2010*)* Large-scale production of magnetosomes by
304 chemostat culture of *Magnetospirillum gryphiswaldense* at high cell density Microbial Cell Factories
305 9:99.

306 Matsunaga T, Tadokoro F, Nakamura N (1990) Mass culture of magnetic bacteria and their application
307 to flow type immunoassays IEE Trans. Mag. 26:1557-1559.

308 Matsunaga T, Kawasaki M, Yu X, Tsujimura N, Nakamura N (1996) Chemiluminescence Enzyme
309 Immunoassay Using Bacterial Magnetic Particles Anal. Chem. 68:3551-3554.

310 Matsunaga T, Tsujimura N, Kamiya S (1996) Enhancement of magnetic particle production by nitrate
311 and succinate fed-batch culture of *Magnetospirillum sp. AMB-1* Biotechnology Techniques 10:495-500.

312 Matsunaga T, Togo H, Tanaka T (2000) Production of Luciferase-Magnetic Particle Complex by
313 Recombinant *Magnetospirillum sp. AMB-1* Biotechnology and bioengineering 70:704-709.

314 Reichard P U, Kretzschmar R, Kraemer S M (2007) Dissolution mechanisms of goethite in the presence
315 of siderophores and organic acids Geochimica et Cosmochimica Acta. 71:5635-5650.

316 Schüler D, Bauerlein E (1996) Iron-limited growth and kinetics of iron uptake in *Magnetospirillum*
317 *gryphiswaldense* Arch. Microbiol. 166:301-307

318 Sun JB, Zhao F, Tang T, Jiang W, Tian JS, Li Y, Li JL (2008) High-yield growth and magnetosome
319 formation by *Magnetospirillum gryphiswaldense MSR-1* in an oxygen-controlled fermentor supplied
320 solely with air Appl. Microbiol. Biotechnol. 79:389-397.

321 Sun JB, Duan JH, Dai SL, Ren J, Zhang YD Tim JS Li Y (2007) In vitro and in vivo antitumor effects
322 of doxorubicin loaded with bacterial magnetosomes (DBMS) on H22 cells: The magnetic bio-
323 nanoparticles as drug carriers Cancer Letters 258:109-117.

324 Yang CD, Takeyama H, Tanaka T, Hasegawa A, Matsunaga T (2001) Synthesis of Bacterial Magnetic
325 Particles During Cell Cycle of *Magnetospirillum magneticum AMB-1* Appl. Biochem. Biotech. 91:155-
326 160.

Yang CD, Takeyama H, Tanaka T, Matsunaga T (2001a*)* Effects of growth medium composition, iron sources and atmospheric oxygen concentrations on production of luciferase-bacterial magnetic particle complex by a recombinant *Magnetospirillum magneticum AMB-1* Enz. Microb. Tech. 29:13-19.

Yang C, Takeyama H, Matsunaga T (2001b) Iron Feeding Optimization and Plasmid Stability in Production of Recombinant Bacterial Magnetic Particles by *Magnetospirillum magneticum AMB-1* in Fed-Batch Culture J. Bioscience Bioeng. 2:231-216.

FIGURES

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

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

Figure 3: (a) The growth curves of magnetotactic bacteria cultivated either in the absence of rhodamine B or in the presence of 0.4 μM, 4 μM, 40 μM or 400 μM of rhodamine B. The growth curves represent the absorption of the different suspensions of magnetotactic bacteria at 565 nm (left y scale) or the number of MTB per ml (right y scale) measured at the different days of growth (day 0, day 1, day 3, day 4). (b) The Magnetic moments of the suspensions of magnetotactic bacteria cultivated either in the absence or in the presence of 0.4 μM, 4 μM, 40 μM or 400 μM of rhodamine B, measured under the application of a magnetic field of 1000 Oe during the fourth day, the seventh day, the eighth day or the ninth day of bacterial growth.

Figure 4: (a), (b) The magnetosome size distribution of the magnetosomes contained within the magnetotactic bacteria for the bacteria cultivated either in the absence, (a), or in the presence of 4 μM rhodamine B, (b). (c), (d) The magnetosome chain length distribution of the chains of magnetosomes contained within the magnetotactic bacteria for the bacteria cultivated either in the absence, (c), or in the presence of 4 μM rhodamine B, (d).

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

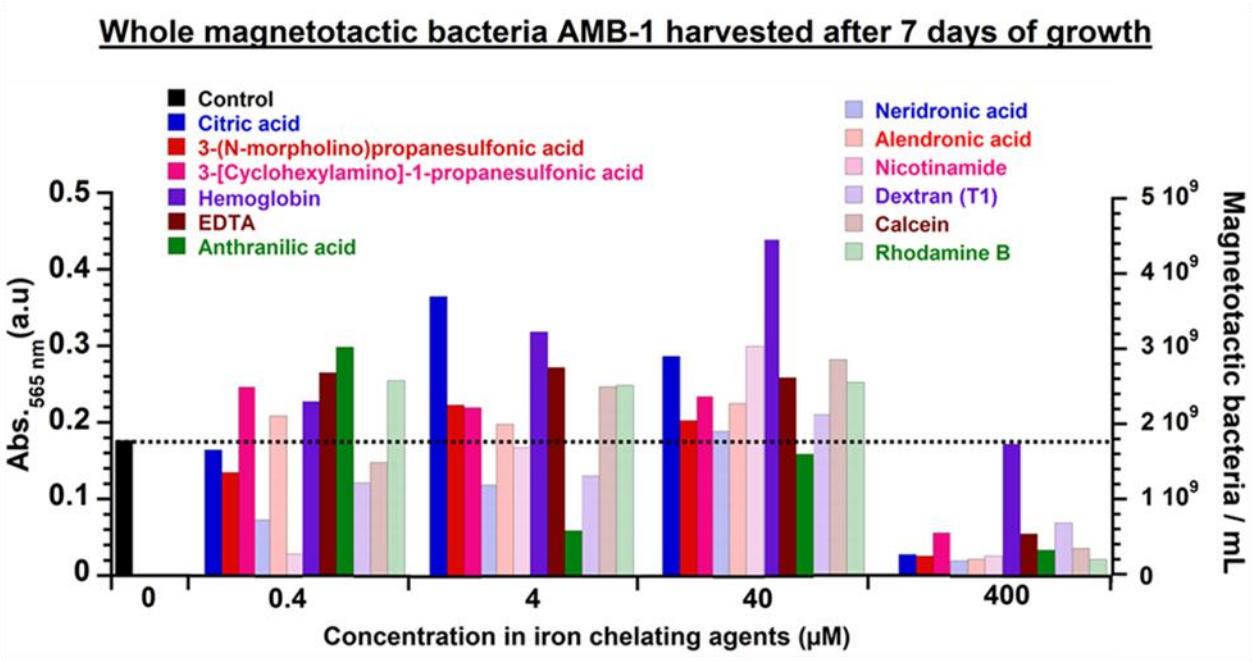
Whole magnetotactic bacteria AMB-1 harvested after 7 days of growth
Control
Citric acid
3-(N-morpholino)propanesulfonic acid
3-[Cyclohexylamino]-1-propanesulfonic acid
Hemoglobin
EDTA
Anthranilic acid
Neridronic acid
Alendronic acid
Nicotinamide
Dextran (T1)
Calcein
Rhodamine B
Abs.565 nm (a.u)
Magnetotactic bacteria / mL
0
0.1
0.2
0.3
0.4
0.5
1 10^9
2 10^9
3 10^9
4 10^9
5 10^9
0
0.4
4
40
400
Concentration in iron chelating agents (µM)

FIG. 1

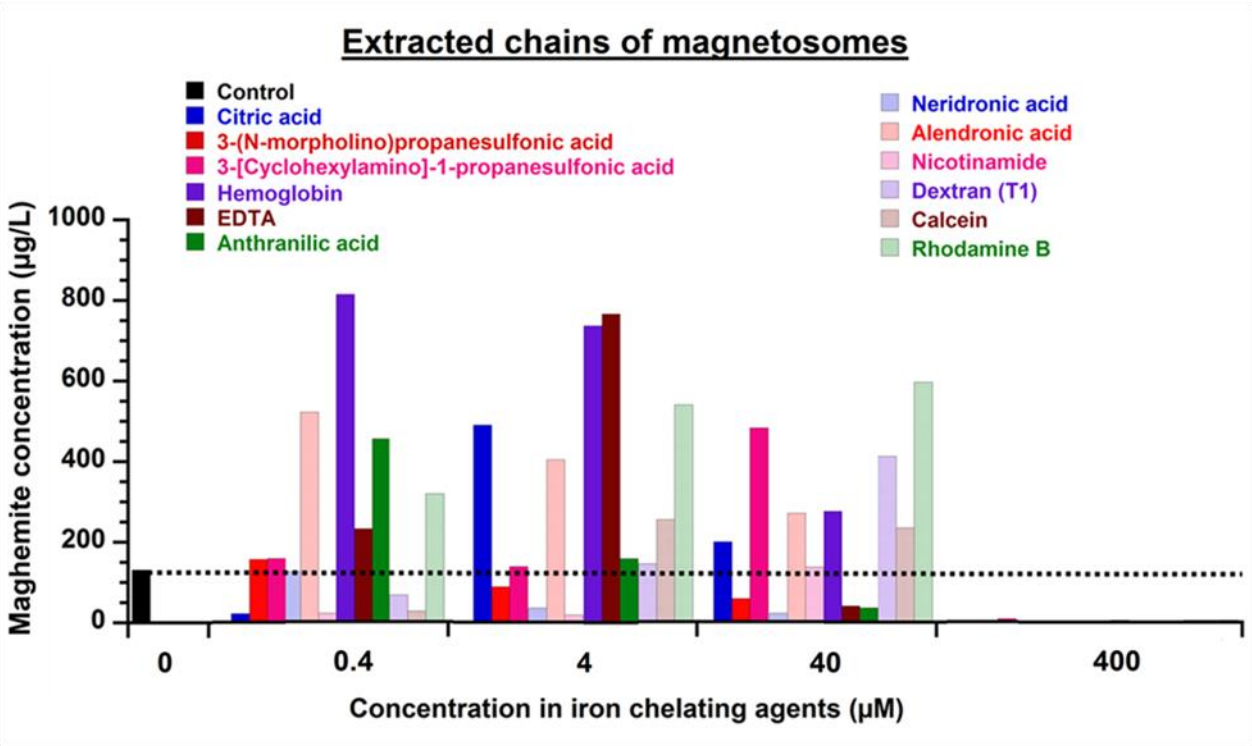
Extracted chains of magnetosomes
Control
Citric acid
3-(N-morpholino)propanesulfonic acid
3-[Cyclohexylamino]-1-propanesulfonic acid
Hemoglobin
EDTA
Anthranilic acid
Neridronic acid
Alendronic acid
Nicotinamide
Dextran (T1)
Calcein
Rhodamine B
Maghemite concentration (µg/L)
1000
800
600
400
200
0
0
0.4
4
40
400
Concentration in iron chelating agents (µM)

FIG. 2

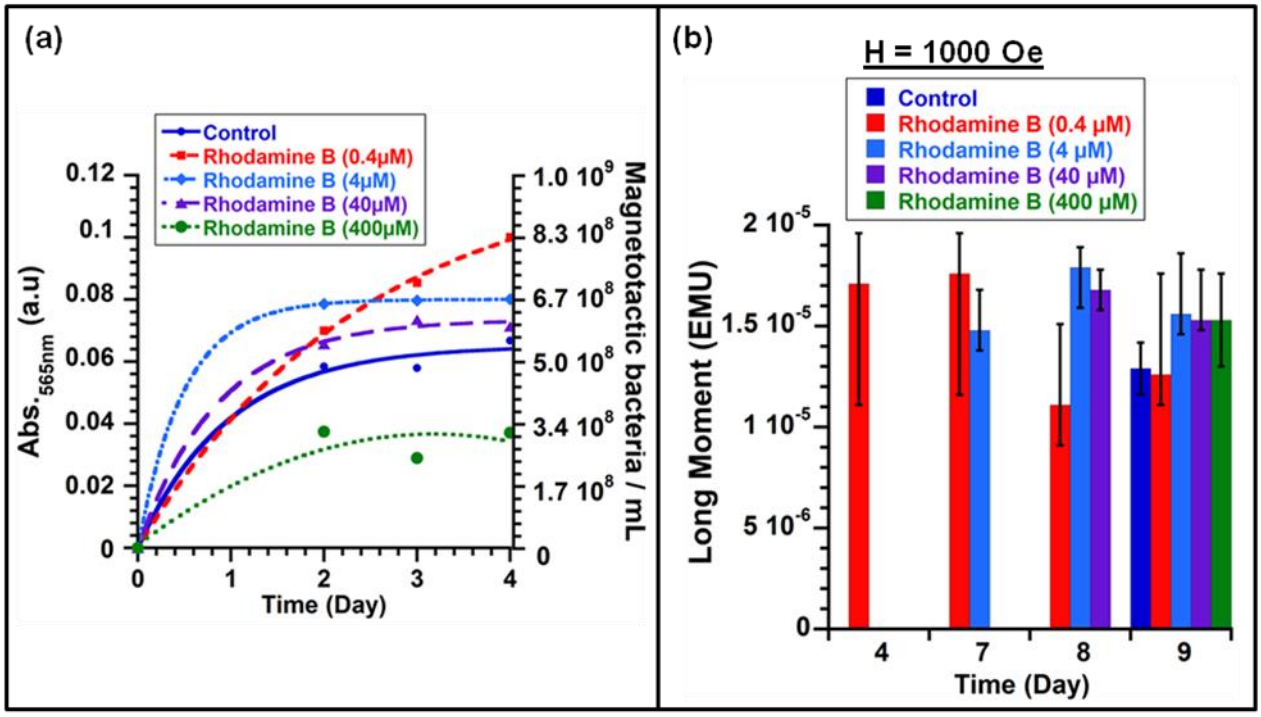
(a)
Control
Rhodamine B (0.4μM)
Rhodamine B (4μM)
Rhodamine B (40μM)
Rhodamine B (400μM)
Abs.565nm (a.u)
Magnetotactic bacteria / mL
Time (Day)
(b)
H = 1000 Oe
Control
Rhodamine B (0.4 μM)
Rhodamine B (4 μM)
Rhodamine B (40 μM)
Rhodamine B (400 μM)
Long Moment (EMU)
Time (Day)

FIG. 3

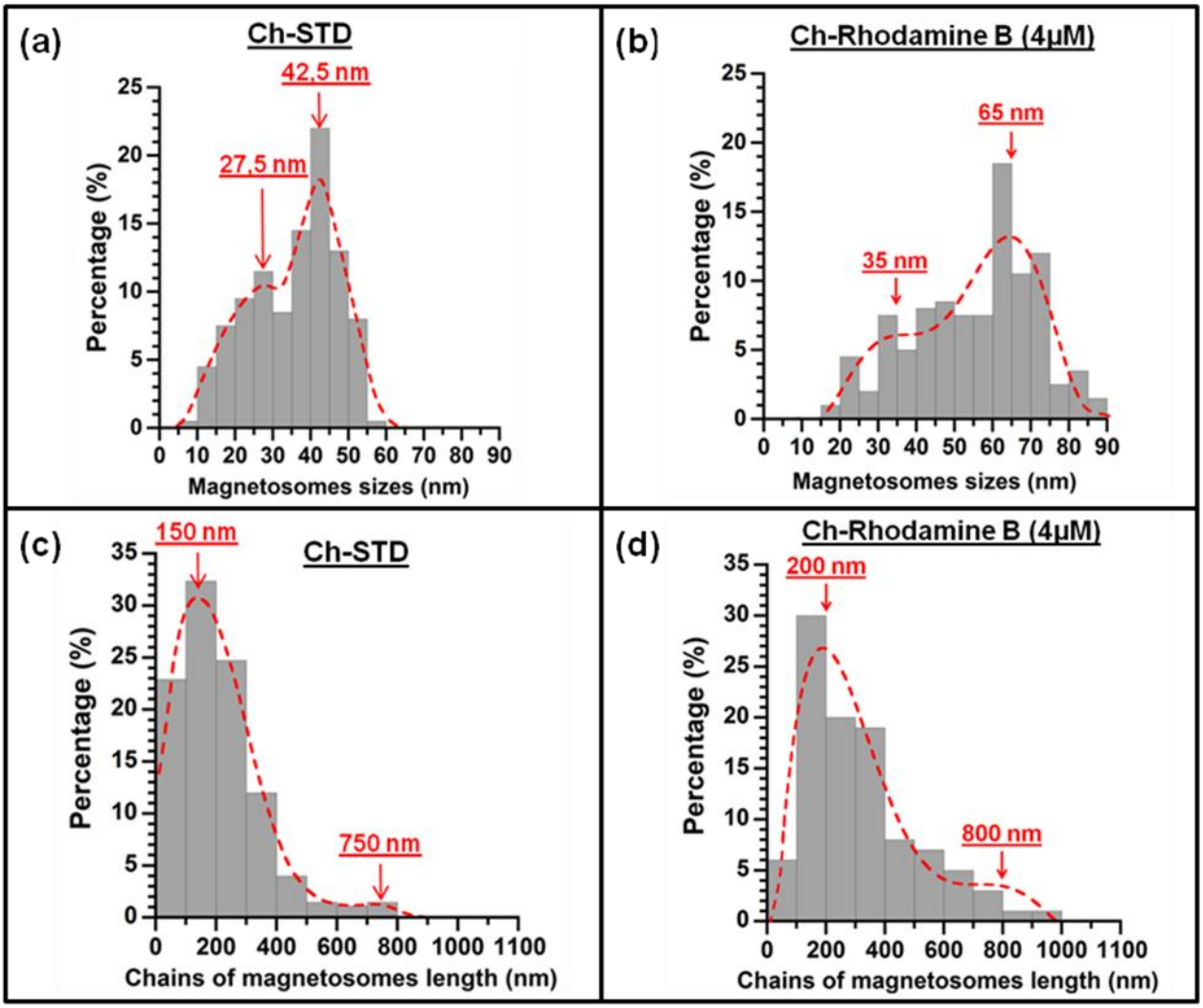
(a)
Ch-STD
42,5 nm
27,5 nm
Percentage (%)
Magnetosomes sizes (nm)
(b)
Ch-Rhodamine B (4µM)
65 nm
35 nm
Percentage (%)
Magnetosomes sizes (nm)
(c)
Ch-STD
150 nm
750 nm
Percentage (%)
Chains of magnetosomes length (nm)
(d)
Ch-Rhodamine B (4µM)
200 nm
800 nm
Percentage (%)
Chains of magnetosomes length (nm)

FIG. 4

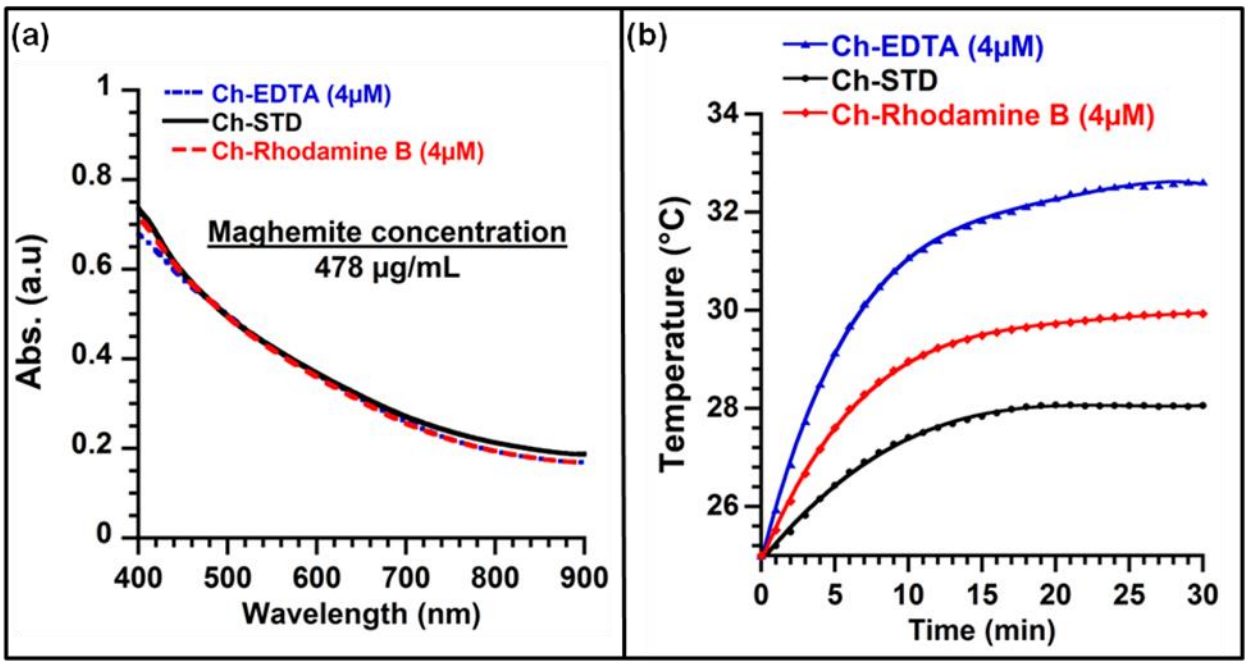
(a)
Ch-EDTA (4μM)
Ch-STD
Ch-Rhodamine B (4μM)
Maghemite concentration
478 μg/mL
Abs. (a.u)
Wavelength (nm)
(b)
Ch-EDTA (4μM)
Ch-STD
Ch-Rhodamine B (4μM)
Temperature (°C)
Time (min)

FIG. 5