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Effects of Dissolved Oxygen Concentration and Iron Addition on Immediate-early Gene Expression of *Magnetospirillum gryphiswaldense* MSR-1

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Abstract

We report effects of dissolved oxygen (DO) concentration and iron addition on gene expression of *Magnetospirillum gryphiswaldense* MSR-1 cells during fermentations, focusing on 0.25-24 h after iron addition. The DO was strictly controlled at 0.5% or 5% O₂, and compared with aerobic condition. Uptake of iron (and formation of magnetosomes) was only observed in the 0.5% O₂ condition where there was little difference in cell growth and carbon consumption compared to the 5% O₂ condition. Quantitative reverse transcription PCR analysis showed a rapid (within 0.25 h) genetic response of MSR-1 cells after iron addition for all the genes studied, except for *MgFnr* (oxygen sensor gene) and *fur* (ferric uptake regulator family gene), and which in some cases was oxygen-dependent. In particular, expression of *sodBI* (superoxide dismutase gene) and *feoB1* (ferrous transport protein B1 gene) were markedly reduced in cultures at 0.5% O₂ compared to those at higher oxygen tensions. Moreover, expression of *katG* (catalase-peroxidase gene) and *feoB2* (ferrous transport protein B2 gene) was reduced markedly by iron addition, regardless of oxygen conditions. The data provides a greater understanding of molecular response of MSR-1 cells to environmental conditions associated with oxygen and iron metabolisms, especially relevant to immediate-early stage of fermentation.

Keywords: magnetotactic bacteria; cell growth; carbon consumption; magnetosome; oxygen; iron metabolism

Introduction

Magnetotactic bacteria are a group of Gram-negative prokaryotes capable of migrating along magnetic field lines (Blakemore 1975). This function depends on a specific intracellular structure, namely magnetosome, which contains a number of crystals of magnetic iron minerals in form of magnetite or greigite (Schüler 2002). The magnetosomes have attracted much attention for potential biomedical applications such as magnetic resonance imaging, magnetic hyperthermia and drug targeting (Alphandéry 2014), as well as the development of immunoassay and magnetic markers (Arakaki *et al.* 2008). To understand the formation of magnetosomes, *Magnetospirillum gryphiswaldense* MSR-1 has been used as a model microorganism (Schüler and Baeuerlein 1996; Heyen and Schüler 2003; Sun *et al.* 2008; Liu *et al.* 2010; Zhang *et al.* 2011; Li *et al.* 2014; Loehr *et al.* 2016), most likely as it is not a strict anaerobe, thus simplifying its cultivation. This strain was isolated from a mud sample in Germany (Schleifer *et al.* 1991), and requires a low dissolved oxygen (DO) concentration for magnetosome formation during cultivation (Heyen and Schüler 2003).

Biosynthesis of magnetosomes is controlled by a complex machinery that includes genes located in a single chromosomal region, the genomic magnetosome island (Ullrich *et al.* 2005), as well as a number of genes outside of the island. A thorough review of the biosynthesis process was recently published (Uebe & Schüler, 2016). Key genes are involved in metal ion activation and substrate recognition (Deng *et al.* 2015), iron utilization (Rong *et al.* 2008; Uebe *et al.* 2010; Rong *et al.* 2012; Qi *et al.* 2012), oxidative stress protection (Rong *et al.* 2012; Qi *et al.* 2012), as well as regulation of denitrification (Li *et al.* 2014). Expression profiles of 13 such genes were previously examined during high-iron (20 μ M ferric citrate) and low-iron (without ferric citrate) cultivations of MSR-1 cells (Wang *et al.* 2013). The finding revealed that these genes were coordinated to a balance level during magnetosome biosynthesis. Recently, the same group reported a transcriptome analysis of MSR-1 cells under aerobic (high oxygen) and micro-aerobic (low oxygen) conditions (Wang *et al.* 2016). Samples were collected at 24 h during a fed-batch fermentation and consequently the authors proposed a regulatory network to reflect the complexity of the physiological metabolism in MSR-1. On the other hand, Staniland *et al.* 2007 demonstrated rapid magnetosome formation during cultivation of MSR-1. This was observed 0.25 h after the formation was initiated by the addition of an iron source, shown by real-time x-ray magnetic circular dichroism. These studies ultimately suggested that

DO concentration and iron addition have a rapid impact on molecular and physiological response of MSR-1 cells. Despite this, precise influence of DO concentration and iron supply on cellular response of MSR-1 is not well understood. In particular, immediate-early gene expression associated with oxygen and iron metabolism has not been studied.

The purpose of this study was to investigate the effect of DO concentration and iron addition on rapid genetic response of MSR-1 cells over time. To achieve this, fermentations were conducted at DO concentration of 0.5% or 5% O₂, and compared with aerobic fermentations. The genes *sodB1*, *katG*, *MgFnr*, *feoB1*, *feoB2* and *fur* were examined at multiple time points during each set of fermentations. It is anticipated that the data presented here will provide a greater understanding of cell response to environmental conditions, potentially paving the way to strain engineering and process optimization in the future.

Materials and Methods

Bacterial strain and growth media

M. gryphiwaldense MSR-1 was purchased from Leibniz Institute DSMZ. Cells were grown and maintained on solid activated charcoal agar media as described (Schultheiss and Schüler 2003).

Liquid cultures of MSR-1 were grown in a media described previously (Heyen and Schüler 2003), containing 0.1 g/L KH₂PO₄, 0.15 g/L MgSO₄·7H₂O, 0.34 g/L NaNO₃, 3 g/L soy peptone, 3.5 g/L potassium lactate and 100 µM ferric citrate. The media was adjusted to pH 7.0 with 2 M NaOH. All chemicals were purchased from Sigma (DK). The media devoid of ferric citrate, namely DFM, was used for preparation of non-magnetic inoculum and for the initial phase of fermentation.

Preparation of non-magnetic inoculum

Non-magnetic cells were grown by three sequential transfers at a ratio of 10% (v/v) in shaking flasks containing the DFM media as described above.

Fermentation

Fermentation was carried out in 1 L BIOSAT[®] B plus bioreactors (Sartorius, DK) with a working volume of 0.8 L. Temperature was maintained at 28°C, agitation at 120 rpm, and gas flow rate was 1 L/min. pH 7.0 was controlled via a pH electrode (Mettler Toledo, USA) by addition of either H₂SO₄ (2 M) or NaOH (2 M) via peristaltic pumps. DO was measured using an oxygen probe (Hamilton, USA) and controlled by gas blending, automatically varying the proportion of nitrogen and air in the inflowing gas by cascade mode. The bioreactor was also equipped with a condenser to avoid evaporation from the media.

The bioreactors were loaded with the DFM media as described above. After sterilization (121°C for 15 min), the oxygen probe was calibrated by sparging the media with sterile nitrogen and air. When the media was saturated with air, all the fermentations were initiated by inoculation of non-magnetic cells to reach a starting OD₅₆₅ of 0.1, and fermentation was allowed to proceed with constant aeration. When the OD₅₆₅ reading reached 0.3, the DO concentration was set to 5% or 0.5% O₂ in randomly selected bioreactors, whereas aeration (*i.e.* sparging with air continuously) remained in the rest of the bioreactors as control. Once the required DO level was reached, an identical volume of sterile ferric citrate solution was added into each fermenter to give a final concentration of 100 µM. Immediately after the addition, samples were collected and the sampling point was set as '0 h'. Thereafter samples were also collected at 0.25, 2, 15 and 24 h. At each time point, cell density was measured in a spectrophotometer at a wavelength of 565 nm. Approximately 30 mL of culture was collected and placed on ice immediately. Cell fractions and spent media were separated by centrifugation at 5000 g for 5 min at 4°C (Thermo Scientific, DK). Subsequently cell pellets were prepared with RNAprotect Bacteria Reagent (Qiagen, DK) according to manufacturer's instructions and stored at -80°C, whilst the spent media was passed through a membrane filter with a pore size of 0.22 µm (VWR, DK) and stored at -20°C prior to analysis. Each fermentation condition was performed in duplicate.

Determination of iron and lactate concentration in the media

Iron content was determined by following the European Brewing Convention iron determination method (Analytica EBC 9.13.1, 2000). Briefly, ferric iron in the samples was reduced to ferrous form (Fe^{2+}) by ascorbic acid (Sigma, DK). Subsequently a complex ($[\text{Fe}(\text{bipy})_3]^{2+}$) was formed between Fe^{2+} and 2,2'-bipyridine (Sigma, DK), which was used for the colorimetric analysis of iron contents at the wavelength of 520 nm (Thermo Scientific, DK).

Concentration of potassium lactate was determined by a HPLC method: 20 μL of each sample was injected onto an HPLC column (Aminex HPX-87H, 300 \times 7.8 mm, Bio-Rad, DK) preheated to 60°C. The samples were eluted using 5 mM sulfuric acid at a flow rate of 0.5 mL/min. Peak signals were detected by a refractive index detector and analyzed using Millenium software. Quantification of compounds was carried out by comparison with known external standards.

RNA isolation and quantitative reverse transcription-PCR

Cells were disrupted using a Tissue-Lyser LT (Qiagen, DK) and total RNA was isolated using a RNeasy plus kit. The purity of the total RNA was determined using a NanoDrop Lite (Thermo Scientific, DK) and the quantity was measured with a Qubit Fluorometer (Invitrogen, DK). cDNA was generated from 0.9 μg of the total RNA using a QuantiTect Reverse Transcription Kit. The subsequent qRT-PCR was performed in a Real-Time PCR detection system (Bio-Rad, DK) by QuantiFast SYBR Green PCR Kit. PCR amplification was carried out in a volume of 20 μL with following conditions: 9°C for 5 min and 40 cycles of 95°C for 10 s and 60°C for 30 s. A melting curve from 65°C to 95°C with reads every 0.15 min was generated at the end of the program to evaluate the specificity of the PCR products. Gene *rluB* (encoding a 16Sr related synthase) was used as the internal standard for normalization of expression levels. All primers are shown in Table 1 and all kits were purchased from Qiagen, DK. The relative expression levels were approximated based on $2^{-\Delta\Delta\text{Cq}}$, with $\Delta\Delta\text{Cq} = \Delta\text{Cq}_{(\text{normalized})} - \Delta\text{Cq}_{(\text{calibrator})}$, where $\Delta\text{Cq}_{(\text{normalized})} = \Delta\text{Cq}_{(\text{target gene})} - \Delta\text{Cq}_{(\text{MGR}_{2835})}$. The calibrator Cq values are those from the cells that were collected at '0 h'. Consequently results were represented as relative expression (log₂-ratio) at 0.25, 2, 15 and 24 h compared to '0 h' when iron was added.

Transmission electron microscopy

A transmission electron microscope (FEI Titan 80-300ST, Netherlands) was used at an acceleration voltage of 120 kV to investigate the morphology and size of magnetosomes. For specimen preparation, aqueous cell samples were dropped onto carbon-coated copper grid (Agar Scientific, UK), and dried in air using an infrared lamp.

Statistical analysis

The mean and standard deviation of a data set was calculated using Excel (Microsoft, USA). Statistical analyses were performed using Origin V8.6 (Massachusetts, USA). At every time point, the expression difference of each gene between the three fermentation conditions was accessed by the Analysis of Variance (ANOVA) coupled with Tukey's test.

Results and discussion

Fermentation performance

As shown in Fig. 1A, cell density of MSR-1 reached OD_{565} of 0.3 at '0 h' of each fermentation prior to iron addition. At 2 h following the addition, similar cell growth was observed between the conditions studied; however, after 15 h cultivation, higher growth were observed in the cultures conducted at 5% and 0.5% O_2 , compared to those under aerobic conditions. At 24 h, while the latter resulted in the highest cell growth, cell densities dropped to a similar level under the two oxygen-controlled fermentations. The reduction is likely due to limitation of the carbon source. Indeed, Fig. 2A shows that the entire carbon source was consumed by 15 h in the cultures except for aerobic cultures, which showed slower carbon consumption until 24 h. This observation was consistent with DO profile of the aerobic fermentation (Fig. 1B). Here the DO level decreased gradually as cell growth increased until approximately 22 h, followed by a rapid increase when cell growth ceased. For cultures under defined oxygen conditions, the DO level was well controlled with average values of $5.0 (\pm 0.20) \%$ or $0.5 (\pm 0.07) \%$ O_2 prior to the addition of iron at '0 h'. Similar values were measured after '0 h' (Fig. 1B). The results indicate there was a minor difference in cell growth between

5% and 0.5% O₂ conditions, which in both cases was faster compared to the aerobic condition. The finding is in agreement with Heyen and Schüler 2003, who observed a prolonged lag phase under a dissolved oxygen tension of 212 mbar, corresponding to a fully aerobic condition.

Iron was only consumed under the 0.5% O₂ condition (Fig. 2B) and formation of magnetosomes was confirmed at 24 h by microscopy analysis (Fig. 3). The cell pellets obtained from this condition were observed to be totally black at the end of fermentation while the others remained cheese-white. This is expected since Zhang *et al.* 2011 reported that DO concentration < 1% is required for magnetosome formation in cultures of MSR-1. Staniland *et al.* 2007 showed rapid magnetosome formation 0.25 h after the addition of ferric quinate at a growth condition with 0.5% O₂, which was the same as we used; however, an immediate uptake of iron was not detected at 0.25 h in the current study, probably due to limitation of the iron quantification method in detecting minor changes of iron content. In addition, it should be noted that the data presented here only shows the level of external iron, since measurement of internal iron would include magnetosomes, in which the iron is not in a soluble form and thus not directly accessible for metabolism. Nevertheless, between 2 and 15 h of cultivation, a drastic uptake of iron was observed followed by a plateau until 24 h under the 0.5% O₂ condition, indicating the utilization of iron ceased after approximately 15 h in spite of the availability of iron in the media (Fig. 2B). This could probably be explained by the depletion of carbon source in the culture, since carbon is crucial for magnetosome formation as an energy source (Naresh *et al.* 2012). Indeed, carbon is particularly important in the transport process of magnetite synthesis (Schüler and Baeuerlein 1996), and is the limiting source for consumption of nitrogen and iron in the later phase of growth (Naresh *et al.* 2012).

Gene expression

Genes associated with oxygen metabolism

Oxygen is a major factor in magnetosome formation and changes in oxygen tension can induce adaptive transcriptional changes in MSR-1 cells (Wang *et al.* 2016). *katG* and *sodB* are two typical oxidative stress response genes, encoding catalase-peroxidase and superoxide dismutase, respectively (Qi *et al.* 2012).

Interestingly in the current study, the addition of iron resulted in dramatic reduction in *katG* expression throughout fermentation, regardless of oxygen conditions (Fig. 4). This observation indicated that iron repressed expression of the *katG* gene in MSR-1 cells is probably used as an indirect protection against oxidative stress and the real mechanism needs to be investigated further. In contrast, the addition of iron led to a rapid influence on *sodB* expression (Fig. 4A). Just 0.25 h after addition, the gene was *ca.* 1.5 fold overexpressed in the aerobic fermentation, *ca.* 0.5 fold reduced in the 0.5% O₂ fermentation and essentially unaffected in the 5% O₂ fermentation. These differences became less as the fermentation progressed (Fig. 4). This suggests that *sodB* is involved in the rapid response to DO concentration and this effect was triggered by iron addition, likely to be associated with iron uptake in MSR-1 cells. It would be interesting to investigate the exact role of *sodB* in the future.

MgFnr was reported as an oxygen sensor with the role of controlling magnetite biomineralization by regulation of denitrification in MSR-1 cells (Li *et al.* 2014). In the current study, however, there is no significant ($p < 0.05$) difference in expression level of *MgFnr* between the three oxygen conditions throughout fermentations. This suggests that the expression of *MgFnr* was neither affected by iron addition nor by different DO levels studied, indicating that the magnetite biomineralization may also be regulated by other unknown oxygen sensors. Our finding is in agreement with Li *et al.* 2014, where MgFnr was only involved in the expression of denitrification genes but not genes encoding oxygen respiration enzyme. The authors therefore concluded that MgFnr plays an indirect role in maintaining proper redox conditions required for magnetite biomineralization.

Genes associated with iron metabolism

Iron is another major factor during magnetosome formation. The cells accumulate a total iron content that is at least 100 times higher than that found in commonly studied bacteria such as *Escherichia coli* (Komeili 2012). Previous studies reported two ferrous iron transport genes in MSR-1, namely *feoB1* (Rong *et al.* 2008) and *feoB2* (Rong *et al.* 2012). The *feoB1* was reported to have a major role in transporting ferrous iron compared to *feoB2* (Rong *et al.* 2008). The latter only participates in magnetosome formation when the

feoB1 is deleted (Rong et al 2012). In the current study, there were significant difference across different conditions for expression of *feoB1* at $p < 0.05$; that is, the expression was increased dramatically in the aerated culture and was reduced in the 0.5% O₂ culture at each time point except for 24 h (Fig. 4). In contrast, under 5% O₂ condition the addition of iron had little impact on *feoB1* expression throughout the fermentation. These finding indicate the expression of *feoB1* is greatly affected by oxygen conditions and *feoB1* may play a significant role in sensing oxygen immediately following the presence of iron. Although the regulation mechanism is not clear, the result is partly consistent with the findings of Xu *et al.* 2016, who reported a down-regulation of *feoB1* in 24 h cells grown in low oxygen condition (0.5% dO₂) compared to highly aerated cells (30% dO₂). In contrast, expression pattern of *feoB2* showed reduced expression after the addition of iron, irrespective of oxygen conditions. At 0.25 and 2 h, the expression level was significantly ($p < 0.05$) reduced under 5% and 0.5% O₂ conditions compared to aeration cultivation, respectively, whilst the difference was less at 15 and 24 h. This result was in agreement with the observation of Rong *et al.* 2012, indicating *feoB2* is not active in the process of magnetosome synthesis in the presence of *feoB1*. Despite this, the findings here are unexpected according to previous studies, since expression levels of both *feoB1* and *feoB2* (in very low oxygen condition) were observed to be reduced following iron addition. Nevertheless, it should be noted that we use a different approach to study gene function than previous workers which might therefore give different results compared to previous studies. The difference in the results may be explained by the way of cultivating cells and controlling oxygen levels. For example, in our study gene expression was analysed after the addition of iron to non-magnetic MSR-1 cells whereas a constant iron source was present in Wang et al 2016; we conducted fermentation at controlled oxygen level of 5% or 0.5% O₂ whilst shaking flasks (*i.e.* aerobic conditions) were used in an earlier study (Wang et al 2013). In addition, Rong et al 2008 and Rong et al 2012 studied the role of *feoB1* and *feoB2* by construction of *feoB1*-deficient and *feoB2*-deficient mutants, respectively. In view of this, these findings may suggest that another level of genetic regulation may exist in MSR-1 cells to regulate the uptake and transport of ferric iron during biosynthesis of magnetosomes.

Fur was identified as a regulator involved in global iron homeostasis (Uebe *et al.* 2010) and Qi *et al.* 2012 reported that this protein can directly regulate the four genes including *feoB1*, *feoB2*, *katG* and *sodB*. The authors also demonstrated that Fur interacts with these genes via binding to their promoters. Previous studies have shown an increased expression of *fur* in a high-iron fermentation using shaking flask (Wang *et al.* 2013), and an up-regulation of a *fur* family gene (MGMSRv2_1721) in a fed-batch fermenter with constant iron supply (Wang *et al.* 2016). Unlike the experimental set-up in previous studies (Wang *et al.* 2013 and 2016), we showed the expression pattern of *fur* gene following the addition of iron in the current study, where interestingly a depressed expression of *fur* gene was observed at immediate-early stage and throughout different fermentations. In particular, at 0.25 h expression of *fur* was significantly ($p < 0.05$) decreased at 5% O₂ compared to aeration condition, whilst at 2 h and 15 h the expression was significantly ($p < 0.05$) reduced at 0.5% O₂ than aeration condition. Nevertheless, the expression was observed to become reduced in general as the fermentation progressed irrespective of oxygen conditions, indicating that the Fur protein may be more important in other cellular biochemical processes. Moreover, this may suggest that the binding of Fur to the promoters of genes as *katG* and *sodB* (Qi *et al.*, 2016) is not directly controlled by the transcription of *Fur*, but that other proteins could be involved in the immediate regulation of the binding of Fur to these promoters.

Moreover, the carbon source was depleted in all cases by 24 h, and by 15 h in the case of the 5% and 0.5% O₂ conditions (Fig. 2A). For some of the genes studied such as *sodB*, *MgFnr*, *feoB1* and *Fur*, expression in general declined during the fermentation (Fig. 4CD), and seemed to coincide with the exhaustion of carbon source, which is to be expected for many genes. In view of this, the large error observed in a couple of samples at 24 h (Fig. 4D) may be explained by cell lysis or declined cell viability. Furthermore, our study is not exhaustive, and future research should continue to refine relationships investigated in the present study. Firstly, although the present study conducted fermentations at controlled DO level of 5% and 0.5% O₂, a direct correlation of the actual concentration of oxygen with DO in the fermentation medium used would be helpful to understand the absolute oxygen level in the culture and to investigate the discrepancy of different studies. However, very sensitive oxygen electrodes would be needed for such a study. Secondly, there is a

difference in sampling handling time before quenching as we centrifuge the sample for 5 min compared to 1 min (Wang et al. 2013). This could therefore have an impact on RNA transcripts and may partly explain the differences found compared to other work. Nevertheless, the RNA samples were handled minimally and kept cold at all times. Internal reference gene was also used for normalisation. These can partly limit the effect of change during sample handling. It should also be noted other workers also seem to have longer processing times than 1 min (e.g. Schübbe, 2006 and Qi, 2012).

Conclusion

To the best of our knowledge, we show for the first time the impact of iron addition on the genetic response of *M. gryphiswaldense* MSR-1 cells during fermentations and the significance was observed from just 0.25 h following the addition of iron. In particular, expression of *sodB* and *feoB1* were dramatically reduced at 0.5% O₂ conditions compared to higher oxygen conditions, whereas no clear effect was observed on the expression of *MgFnr* and *fur*. In contrast, expression of *feoB2* and *katG* was negatively affected by iron addition, irrespective of oxygen conditions. These findings demonstrated immediate-early gene expression in MSR-1 cells associated with environmental changes (*i.e.* iron addition and DO concentration), and the specific role of these genes in bacterial magnetite biomineralization needs to be further investigated.

Conflict of interest The authors declare no conflict of interest.

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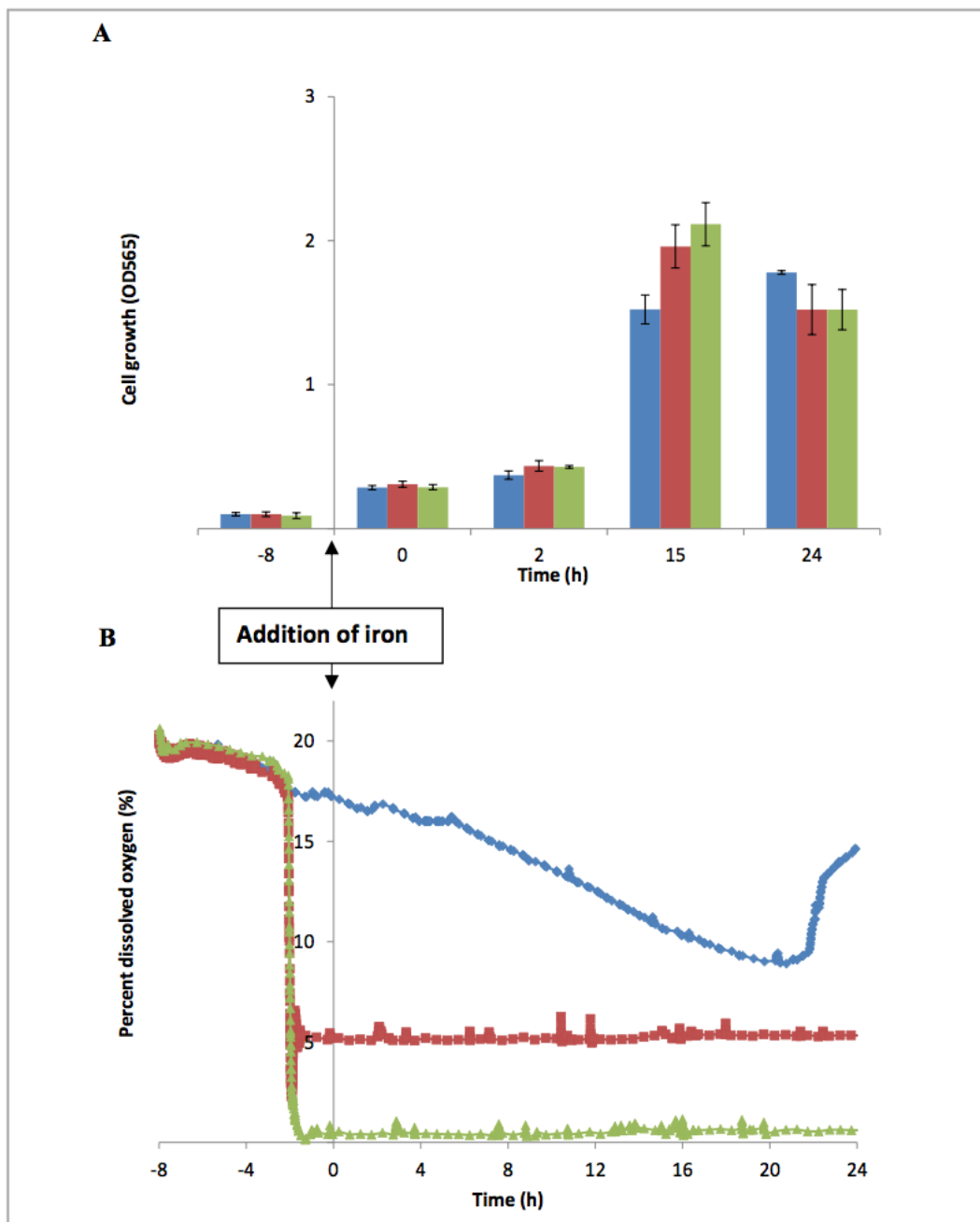


Fig. 1. Effect of dissolved oxygen (DO) concentration and addition of iron on cell growth of *M. gryphiswaldense* MSR-1. Cell growth (A) was determined using optical density reading at 565 nm, and DO was monitored continuously over time. Blue: aerobic; red: 5% O₂; green: 0.5% O₂. In each instance, agitation (120 rpm), temperature (28 °C), gas flow rate (1.0 L/min) and pH (7.0) were kept constant. Each fermentation condition was performed in duplicate.

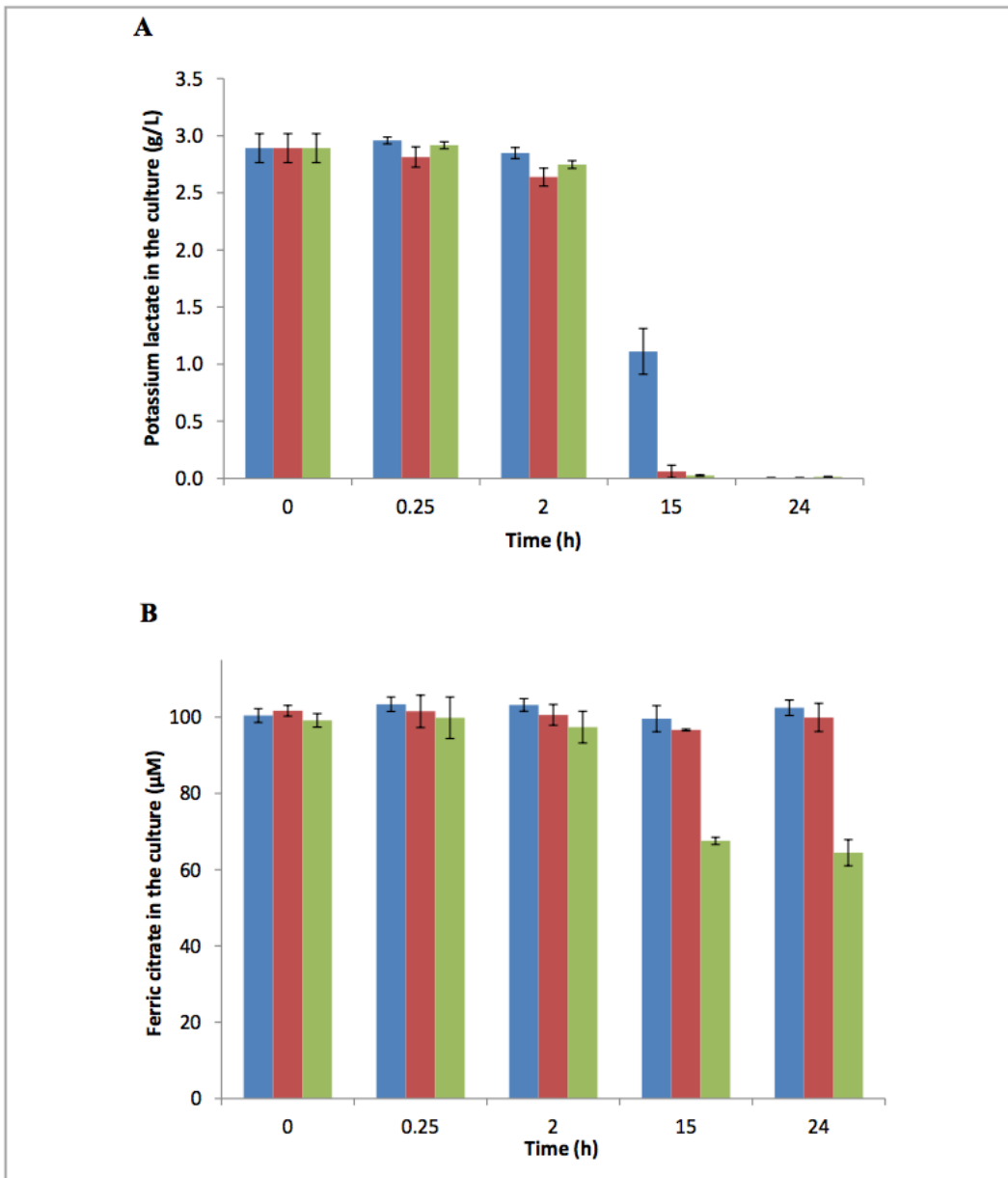


Fig. 2. Utilisation of lactate (A) and iron (B) during different fermentations. Blue bar: aerobic; red bar: 5% O₂; green bar: 0.5% O₂. Data represents the mean with range from two independent experiments, each performed in duplicate.

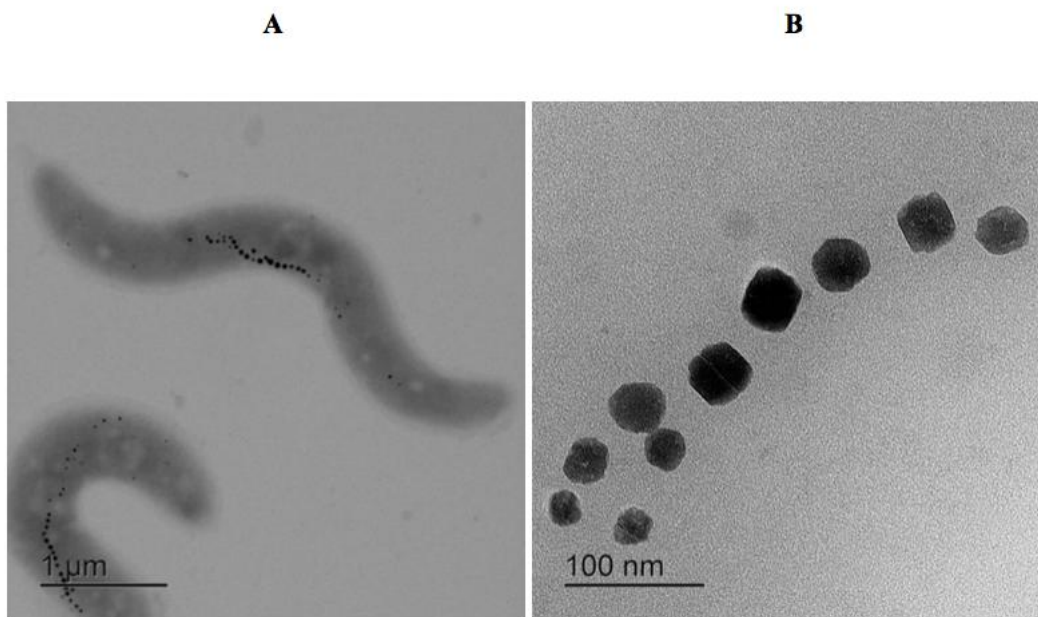
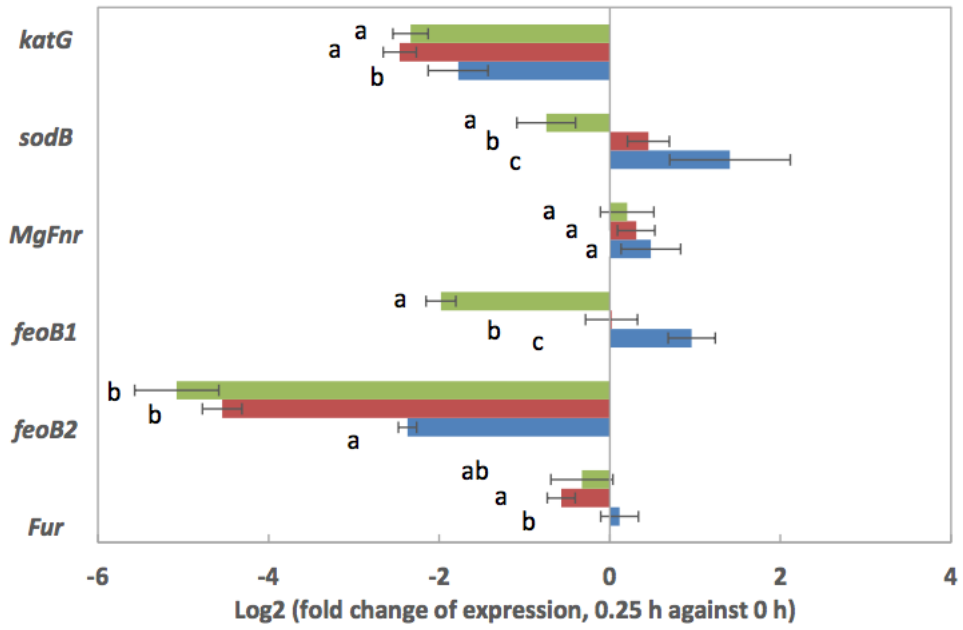
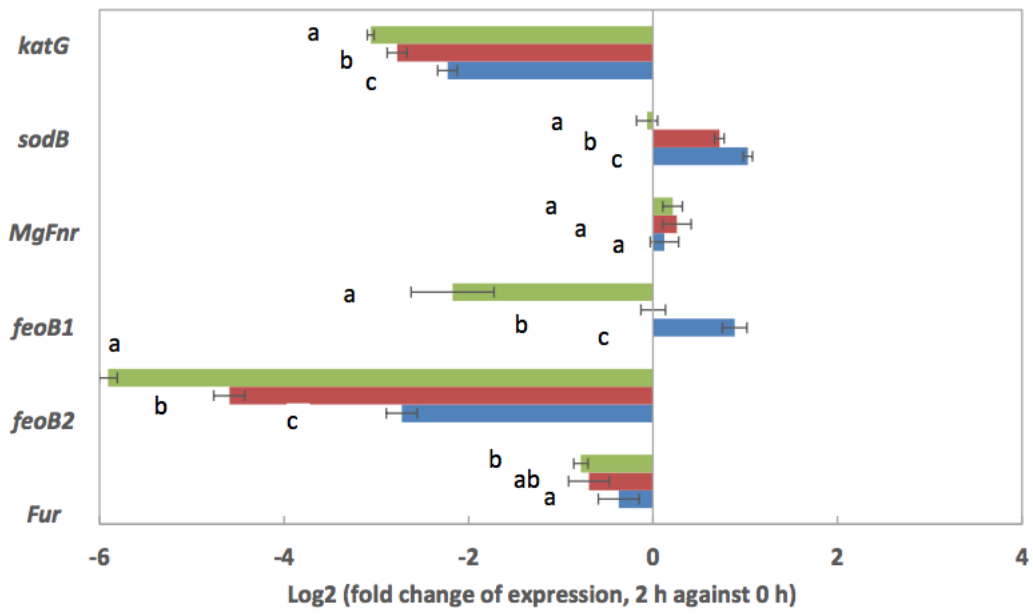


Fig. 3. TEM images of whole cells (A) and magnetosomes (B) at 24 h under 0.5% O₂ cultures. More than 20 micrographs were captured and two representative images were presented here.

A



B



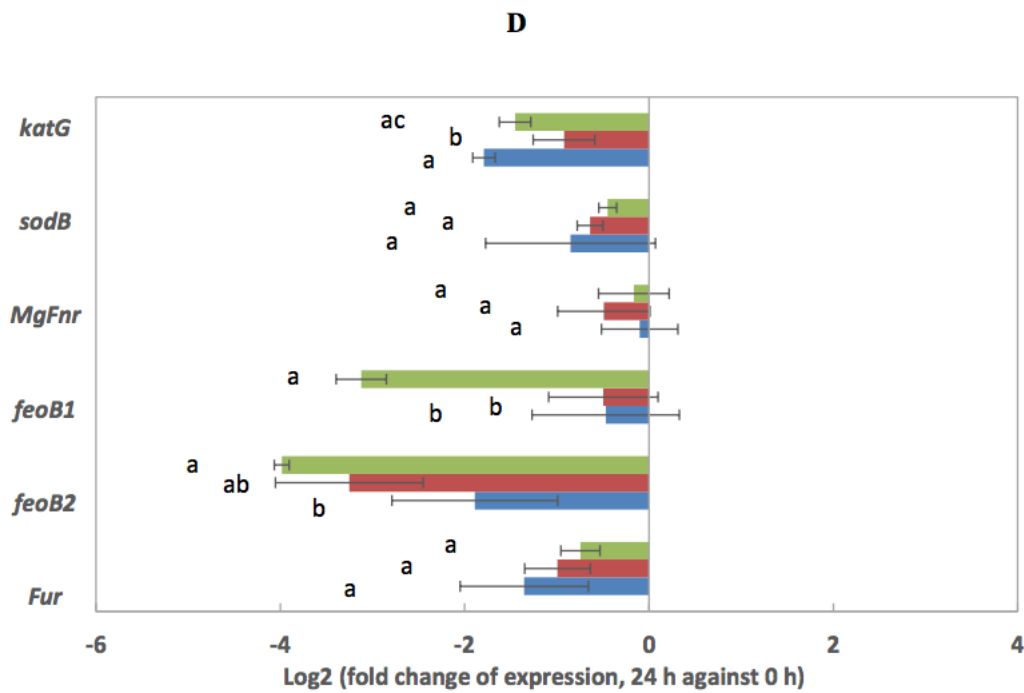
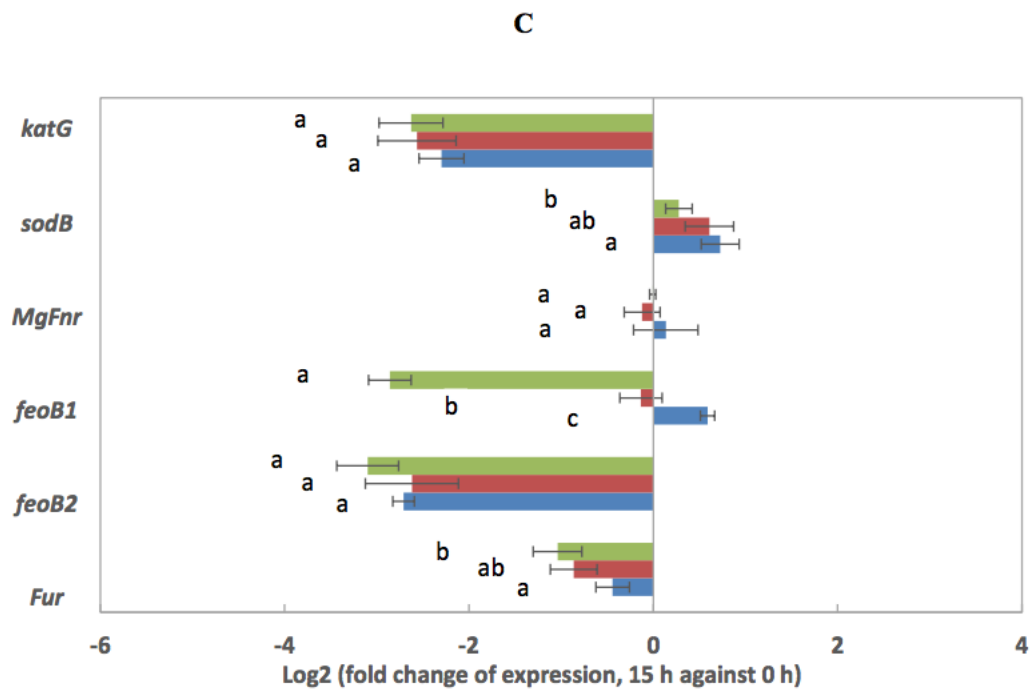


Fig. 4. Relative expression level of key genes associated with oxygen metabolism (*katG*, *sodB* and *MgFnr*) and iron metabolism (*feoB1*, *feoB2* and *fur*) at 0.25 h (A), 2 h (B), 15 h (C) and 24 h (D) under different fermentations. Blue bar: aerobic; red bar: 5% O₂; green bar: 0.5% O₂. Data represents the mean with range

from two independent experiments, each performed in duplicate. At every time point, the expression difference of each gene between three fermentation conditions was accessed by the Analysis of Variance (ANOVA) coupled with Tukey's test. Bars bearing different letters for one gene are significantly different, same letters indicate no differences ($p < 0.05$).

Table 1 Summary of genes, functions, and primer sequences (5' to 3') used in this study.

Gene	Locus tag	Forward primer sequence	Reverse primer sequence	Reference
<i>katG</i>	MGMSRv2 1805	TGAACGACGAGGAAACGGT	CCAGTCATAGCCCAACAGC	Qi, et al, 2012; Wang, et al, 2013
<i>sodB</i>	MGMSRv2 1221	CGCTCAAGATCACCAAGACC	ACGAAATCCCAGTTCACCAG	Qi, et al, 2012; Wang, et al, 2013
<i>MgFnr</i>	MGMSRv2 2946	GGAAAGCGTGTGGGCGA	AACTGGGTAAAGGTCCGGC	Li et al, 2014
<i>feoB1</i>	MGMSRv2 2312	CGCCACGAAAGCAACCAT	ATCACCCACAAAATGCCCA	Rong, et al, 2008; Wang, et al, 2013
<i>feoB2</i>	MGMSRv2 0315	CAACACCCTCTACACCCAAG	GACGACACCAGCCAGATTT	Rong et al, 2012; Wang et al, 2013
<i>fur</i>	MGMSRv2 3137	CCATCGCCACCGTTTATCG	CAACCGATGCCCAACCAG	Qi, 2012; Wang, et al, 2013
<i>rluB</i>	MGMSRv2 3279	TTGCCGCTACCGATACTCTT	CTTGTGATAACGCCAAACCC	This work