

Effects of a Strong Static Magnetic Field on Bacterium *Shewanella oneidensis*: An Assessment by Using Whole Genome Microarray

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The effect of a strong static 14.1 T magnetic field on log phase cells of bacterial strain *Shewanella oneidensis* MR-1 was evaluated by using whole genome microarray of this bacterium. Although differences were not observed between the treatment and control by measuring the optical density (OD), colony forming unit (CFU), as well as post-exposure growth of cells, transcriptional expression levels of 65 genes were altered according to our microarray data. Among these genes, 21 were upregulated while other 44 were downregulated, compared with control. Bioelectromagnetics 26, 2005. © 2005 Wiley-Liss, Inc.

Key words: DC; magnetic field; gene expression; transcription expression

INTRODUCTION

High static field magnets are widely used in medical and research laboratories such as magnetic resonance imaging (MRI) and nuclear magnetic resonance (NMR). Studies of the biological effect of strong magnetic field have been intensified in recent years due to its possible harmful or beneficial effects on many eukaryote organisms, including human beings [Sakuria et al., 1999; Emura et al., 2003; Iwasaka and Ueno, 2003; Schiffer et al., 2003]. In our previous studies, we found that the strong static magnetic fields had an apparent effect on insect egg hatching, the hatching was delayed by the strong static magnetic fields and the delay non-linearly increased with the intensity of the magnetic field [Pan, 1996; Pan and Liu, 2004]. The larval development in the strong magnetic field was slower than that in the geomagnetic field. Denegre et al. [1998] reported that a strong magnetic field altered the cleavage planes in frog eggs.

As relatively simple living organisms, bacteria are important research subjects in this field too. Kohno et al. [2000] studied the effect of static magnetic field on bacteria *Streptococcus mutans*, *Staphylococcus aureus*, and *Escherichia coli*. They found that the ferrite magnet caused strength-dependent decreases in the growth rate and maximum number of bacteria for *S. mutans*, *S. aureus* when cultured under anaerobic conditions, but their growth was not inhibited under aerobic conditions. Their finding suggested that oxygen related to the growth in the cases of *S. mutans* and *S. aureus*.

However, no growth effects were detected in *E. coli* cultures. Stansell et al. [2001] found that exposure of *E. coli* to static magnetic field significantly increased its antibiotic resistance. Horiuchi et al. [2001] found that a high magnetic field (5.2–6.1 T) promoted survival rate of *E. coli* B cells of stationary phase, since the colony forming unit (CFU) number under the high magnetic field (5.2–6.1 T) was 100 000 times higher than that under a geomagnetic field. The same authors also reported that the amount of S factor encoded by the *rpoS* gene under the high magnetic field was larger than that

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under control, and thus the activity of the *rpoS* gene was affected by the high magnetic field [Horiuchi et al., 2001].

Shewanella oneidensis MR-1 is a gram-negative, facultative anaerobe bacterial strain. This bacterium can anaerobically respire numerous organic compounds such as fumarate and dimethyl sulfate oxide, and many metal oxides such as Fe(III), Mn(IV), Cr(VI), and U(VI) [Myers and Myers, 1992, 1993a,b; Leblanc et al., 2001; Marier and Myers, 2001]. Because of its versatile respiration pathways, which may be applied to the immobilization of environmental pollutants in soil and groundwater (i.e., chromium and uranium), this strain has been intensively investigated as an excellent metal reducing cellular system for studying redox mechanisms [Myers and Myers, 1993; Fries et al., 1994; Glasauer et al., 2001; Johnson et al., 2001; Arnesano et al., 2002; Giometti et al., 2003]. Sequencing the whole genome of this strain is one important step for thoroughly understanding of this complex organism [Glasauer et al., 2001]. In this study, we report our studies of the effect of 14.1 T static magnetic field on the growth of log phase cells of bacterial strain *Shewanella oneidensis* MR-1, as well as the transcriptional activities of genes evaluated through a whole genome microarray.

EXPERIMENTAL METHOD

Bacterial Cell Preparation

A single colony grown on Luria Broth (LB) agar was picked up and transferred to 10 ml LB liquid medium for culturing overnight at 30 °C. The next morning, 1 ml of culture was pipetted and transferred to 100 ml LB liquid medium in a 250 ml flask, followed by shaking at 150 rpm and 30 °C. When growth reached mid-log phase, cells were inoculated at the ratio 1:100 into each of two sterilized glass vials containing 15 ml fresh LB liquid medium for magnetic field exposure experiment.

14.1 T Magnetic Field Treatment

The glass vials were mounted on a mechanical shaking device that was specially designed for this experiment. One vial was located at the center of the 14.1 T magnet (Varian INOVA 600 NMR spectrometer with Oxford 14.1 T magnet) and the other vial, used as a control, was located at the bottom of the 14.1 T magnet where the fringe field was about 22.8 mT (228 G). The magnetic field at the center was well shimmed by cryoshim coils built into the superconducting magnet; the homogeneity of the magnetic field meets the requirement for high resolution NMR experiments.

Due to the nature of the experiment in the 14.1 T magnetic field, the shaker was made of plastic and copper materials, and all moving parts were made of plastic materials to avoid the eddy current effect; therefore, the effect on the homogeneity of the magnetic field in the center of the magnet is negligible, if any, even during the shaking. We assumed that the fringe field at the bottom of this magnet has no apparent biological effect, as indicated by our previous results [Pan and Liu, 2004] and the temperatures at the both locations were same.

Two experiments were conducted. In the first experiment, the MR-1 was exposed to 14.1 T magnetic field for 1.5 h; and in the second experiment, the MR-1 was exposed to the same magnetic field for 12 h. The samples were mechanically shaken at 120 rpm during the entire period in the magnetic field at a room temperature of 23 °C. The shaking permits bacteria growth under aerobic conditions. However, due to a technological limit of our system, we set the rate of shaking as 120 rpm, not as 150 rpm as before exposure. The ideal growth temperature for MR-1 is 30 °C. Due to the same technical difficulty, the temperature for exposure experiment was set as room temperature 23 °C.

After treatment of the 14.1 T magnetic field, the optical density (OD) value of both the exposed and control samples were measured at wavelength 600 nm using a spectrometer (Spectronic 20D+, Thermo Spectronic). The exposed and control samples (0.5 ml) were transferred to 9.5 ml fresh LB liquid medium for further culturing. The culture conditions were the same as that described in cell preparation. The OD value was measured every 2 h. The OD value is proportional to the amount of bacterial cells in the liquid. Meanwhile, both the treatment and control samples were diluted to 10^{-7} and 0.1 ml of each dilution spread on LB agar plates for CFU counting. Colony counting was performed after the plates were incubated at 30 °C for 2 days.

Microarray Hybridization and Data Analysis

Based on the known sequence [Heidelberg, 2002], we designed a gene-specific primer set for all *S. oneidensis* MR-1 genes whose full or almost full length has less than 75% similarity to another gene. For those genes with more than 75% similarity to another genes, we designed a 50-mer oligo for the region whose similarity to another sequence is less than 75% using the software PRIMEGENS [Xu et al., 2002]. We successfully PCR-amplified and fabricated the whole-genome scale microarray of *S. oneidensis* MR-1 that has a 95% of genome coverage [Liu et al., 2003].

Liquid-cultured cells in exponential growth phase were inoculated at the ratio 1:100 into a vial containing

15 ml fresh LB liquid medium and then subjected to 14 T magnetic field exposure for 12 h at room temperature 23 °C. The samples were mechanically shaken during the entire exposure period in the magnetic field. Cell cultures were harvested from each sample after 12 h exposure followed by a brief centrifugation, resuspended in RNeasy lysis solution, and stored at -70°C for later RNA extraction. Microarray fabrication, hybridization, probe labeling, image acquisition, and processing were carried out as described in references [Liu et al., 2003].

Gene expression analysis was performed using three independent microarray experiments, with each slide containing two replicate array of *S. oneidensis* MR-1 genome, a collection of 4608 distinct ORFs. Total cellular RNA from 14.1 T magnetic field exposed and control samples was isolated and purified using the TRIzol Reagent (Gibco BRL) according to the manufacturer's instruction. The ratios of the exposed samples over the control were normalized using Pooled-Common Error model provided by the statistical analysis software ArrayStat v. 2.0. Data was gained by the software IMAGINE, normalized by trimmed geometric mean, and statistically analyzed by the software ARRAYSTAT after all outliers were removed [Liu et al., 2003].

RESULTS AND DISCUSSION

Cell Growth

Initially, we started with our 1.5 h exposure to 14.1 T magnetic field of MR-1, and no significant results were obtained, as reflected by OD measurement and colony forming unit (CFU) counting, as well as a growth experiment of cells post-exposure (data not shown). Then we extended the exposure time to 12 h. If there is any apparent effect of 14.1 T magnetic field on the cell growth, then the number of cells in the center of magnetic field would be significantly different from that at the bottom of the magnet after such 12 h exposure, such difference would be detected by the OD measurement and CFU counting, as well as post-exposure growth. Our results indicate that 12-h exposure of the 14.1 T magnetic field has no detectable effect on the cell growth of *S. oneidensis* MR-1, as reflected by OD measurement and CFU counting. Both the treatment and control reached an OD value about 1.0 after 12 h exposure, and the CFU counting showed that the viable cells of both treatment and control reached about 10^{10} /ml. Furthermore, the post-exposure growth curves of both exposed and controlled cells of *S. oneidensis* MR-1 also failed to show an effect, as indicated in Figure 1. The first point at 0 h represents the

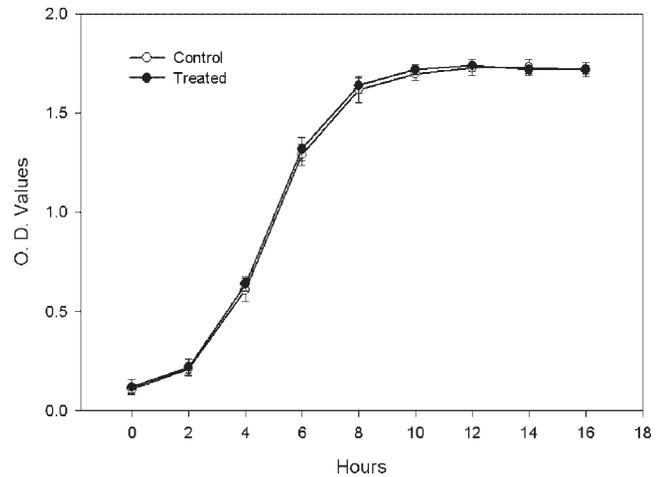


Fig. 1. The post-exposure growth of the *Shewanella oneidensis* MR-1 cells after exposure to 14.1 T magnetic field for 12 h. The initial optical (OD) of post-exposure growth of both treatment and control samples began at about 0.1. The OD value was measured at wavelength 600 nm.

inoculated amount of cells, including both living and dead, just taken out of the magnet.

Our result is different from the results by Horiuchi et al. [2001] on *E. coli* cultured in the 5.2–6.1 T

TABLE 1. Hypothetical Genes With Significant Changes in Gene Expression after Exposure to 14.1 T Magnetic Field for 12 h

Gene	Expression ratio ^{a,b}	
	AVG	P-value
SO0362	-1.555	*
SO0683	+3.626	*
SO0938	-2.096	*
SO1419	-3.861	*
SO1757	-1.580	*
SO1831	-1.548	*
SO2591	-1.595	*
SO2603	-1.473	*
SO2688	+2.657	*
SO2770	+2.102	*
SO2832	-1.460	*
SO2884	-1.502	*
SO2970	+2.107	*
SO3062	+5.069	*
SO3074	+2.086	*
SO3198	-1.988	*
SO3313	-1.669	**
SO3475	+2.734	*
SO4008	-1.560	*
SO4560	-1.479	*

^aThe average (AVG) expression ratio of the treatment sample to the control was calculated from 12 replicates together, a $P = .05$ standard *t*-test result. All genes are significant in expression changes (*, $P < .05$; **, $P < .01$; probability in *t*-test).

^bFold increases is marked as (+) and decreases (-).

TABLE 2. Known Functional Genes With Significant Changes in Gene Expression after exposure to 14.1 T Magnetic Field for 12 h

Gene	Symbol	Putative function	Expression ratio ^a	
			AVG	<i>P</i> -value ^b
Energy metabolism				
SO0053	gpsA	Glycerol-3-phosphate dehydrogenase	-1.965	***
SO0608	petA	Ubiquinol-cytochrome c reductase	-1.645	*
SO1778	omcB	Decaheme cytochrome c	-1.757	*
SO2362	ccoQ	Cytochrome c oxidase, cbb3-type	-1.980	**
SO2421	ansA	L-asparaginase I	-1.587	*
SO2513		NqrDE/RnfAE family protein	-1.541	*
Transport and binding proteins				
SO0157		Proton/glutamate symporter	+1.527	*
SO1112	bfr1	Bacterioferritin subunit 1	-1.701	*
SO2786		Sulfate permease family protein	+2.124	*
Cellular process				
SO0913	fic	Cell filamentation protein Fic	-1.701	*
SO2578	mine	Cell division topological specificity factor MinE	-1.848	*
SO3890		Methyl-accepting chemotaxis protein	+3.152	*
DNA metabolism				
SO1457		Type I restriction-modification system, M subunit	-1.527	*
SOA0013	umuD	UmuD protein	+1.531	*
Cell envelope				
SO3191		Chain length determinant protein	-1.520	*
SO3193		Polysaccharide biosynthesis protein	-1.585	*
SO3524	pilE	Type IV pilus biogenesis protein PilE	-1.701	*
SO4321		OmpA family protein	+1.526	*
Amino acid biosynthesis				
SO0248	rpsE	Ribosomal protein S5	-1.508	*
SO0249	rpmD	Ribosomal protein L30	-1.582	*
SO2300	infC	Translation initiation factor IF-3	-1.527	*
SO3798	rluA-2	Ribosomal large subunit pseudouridine synthetase A	-1.721	**
SO3939	rpsL	Ribosomal protein S9	-1.425	*
Signal transduction				
SO3306		Sensor histidine kinase	-1.634	*
Regulatory function				
SO0864		Transcriptional regulator, LuxR family	+1.562	*
SO1965		Transcriptional regulator, LysR family	+1.418	*
SO2640		Transcriptional regulator, MarR family	+1.737	*
SO4388		DNA-binding response regulator	-1.653	*
SO4571		Transcriptional regulator, LysR family	-1.770	*
Transcription				
SO1209	pnp	Polyribonucleotide nucleotidyltransferase	+1.284	*
SO2571		ATP-dependent RNA helicase, DEAD box family	-1.629	*
Purines, pyrimidines, nucleosides, and nucleotides				
SO1142	carB	Carbamoyl-phosphate synthase, large subunit	-1.435	*
SO1217	deoC	Deoxyribose-phosphate aldolase	+1.566	*
SO3803	hpt-2	Hypoxanthine phosphoribosyltransferase	-1.600	*
Amino acid biosynthesis				
SO3175	asnB	Asparagine synthetase, glutamine hydrolyzing	-1.742	*
SO3414	thrB	Homoserine kinase	+1.497	*
SO4309	lysA	Diaminopimelate decarboxylase	+1.768	*
Fatty acid and phospholipid metabolism				
SO1679		Acyl-CoA dehydrogenase family	+2.227	*
Protein fate				
SO2223		Peptidase, putative	-1.587	*
SO0260	ccmD	Heme exporter protein CcmD	-1.605	*
Others				
SO1756		Glyoxalase family protein	-1.912	***
SO3810		OmpA-like transmembrane domain protein	-1.637	*
SO4238		TPR domain protein	-1.618	*
SOA00164		Iron-containing alcohol dehydrogenase	+1.468	*
SOA00136		ISSod3, transposase	-1.538	*

^aThe average (AVG) expression ratio of the treatment sample to the control was calculated from 12 replicates together, a $P = .05$ standard *t*-test result. All genes are significant in expression changes (*, $P < .05$; **, $P < .01$; ***, $P < 0.001$; probability in *t*-test.)

^bFold increases is marked as (+) and decreases (-).

magnetic field. In that study, Horiuchi et al. [2001] found that the number of viable cells of *E. coli* B in the stationary phase after 48 h under the magnetic field of 5.2–6.1 T was 100 000 times higher than that under a geomagnetic field. Such a difference may be due to different experiment design, since our experiment only observed growth of cells exposed under strong magnetic field for 12 h and the bacteria is still in its log phase stage, whereas Horiuchi's experiment lasted over two days at a different magnetic field strength. The physiological status of bacteria between log phase and stationary phase is very different, and bacterial cells in stationary phase much more easily to give rise to mutants adaptive to stress environments. Indeed, this may explain why the effect of strong magnetic field on viable cell of *E. coli* only appeared during stationary phase in the study of Horiuchi et al. [2001].

Gene Expression

Among a total of 4583 genes assayed following 12 h, 14.1 T magnetic field exposure, total 65 genes were significantly changed in expression, compared to the non-exposed control. The magnitude of change ranges from 1.5 to 5 times. Out of these 65 genes, 21 were upregulated whereas the other 44 genes were repressed. According to TIGR gene annotation of *S. onedensis* MR-1, 20 of these 65 genes encode conserved hypothetical proteins and thus their exact functions are unknown (Table 1). Table 2 lists all other genes whose functions are known and that were significantly changed in the gene expression against the control. Five genes are identified as transcriptional regulator and they include SO1965 (LysR family member), SO0864 (LuxR family member), SO4571 (LysR family member), SO4388 (DNA-binding response regulator), and SO2640 (MarR family member). Among these transcriptional regulators, SO1965, SO0864, and SO2640 were upregulated, whereas both SO4571 and SO4388 were downregulated under the magnetic stress condition. Previously, a couple of studies reported that *rpoS* gene, which encodes a sigma factor and plays a role as a transcriptional regulator of some genes, had increased activities in stationary stage [Tsuchiya et al., 1999; Horiuchi et al., 2001]. Here, we reported the activities of other transcriptional regulators were affected by strong static magnetic fields under log phase stage of bacterial growth. However, the mechanism underpinning such expression alterations is not clear.

Under the category of energy metabolism, six genes showed altered expression levels under the magnetic stress (Table 2). They are SO1778 (*omcB*, encoding decaheme cytochrome *c*), SO0608 (*petA*, encoding iron sulfur subunit of ubiquinol-cytochrome

c reductase), SO0053 (*gpsA*, glycerol-3-phosphate dehydrogenase), SO2513 (encoding a NqrDE/RnfAE family protein), SO2421 (*ansA*, L-asparaginase I), and SO2362 (*ccoQ*). It is worthwhile to note that SO1778 (*omcB*), SO0608 (*petA*), and SO2362 (*ccoQ*) are iron-containing proteins. Similarly, SO1112 (*bfr1*), encoding bacterioferritin subunit I was also transcriptional suppressed under the same condition. In contrast, SOA00164, which encodes an iron-containing alcohol dehydrogenase was upregulated under the same condition. Since the expression of these iron-containing proteins involved in energy metabolism was affected by the strong magnetic field, it is reasonable to suppose that the strong magnetic field perhaps influenced the iron metabolism and then energy metabolism of MR-1, although the exact mechanism remains unknown at this moment.

As shown in Table 2, genes encoding other functional proteins also showed altered expression level. For instance, under the category of transport and binding protein, SO0157 and SO2578, encoding a proton/glutamate symportor and sulfate permease family protein, respectively, were upregulated. In contrast, several genes involved in protein biosynthesis (SO0248, 249, 2300, 3798, and 3939) were downregulated. However, the mechanism underpinning such changes is not clear. Also, the changes at transcriptional levels were not reflected at organism level, as our growth experiments showed. This may suggest that log phase cells of MR-1 were capable of maintaining their physiological homeostasis under such stress, even though they had to experience some transcriptional changes at molecular level.

CONCLUSION

We studied the possible effect of 14.1 T static magnetic field on the growth and transcriptional expression of log phase cells of bacterium *Shewanella oneidensis* MR-1. The results conclude that while little effects on cell growth at log phase were observed, apparent changes at transcriptional levels were detected in some genes of *S. onedensis* MR-1. However, mechanism underpinning such changes was not clear.

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