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Analysis of the *orf 282* Gene and Its Function in *Rhodobacter sphaeroide* 2.4.1

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The *orf282* gene of *Rhodobacter sphaeroides* is located between the *ccoNOQP* operon encoding cbb_3 terminal oxidase and the *fnrL* gene encoding an anaerobic activator, FnrL. Its function remains unknown. In an attempt to reveal the function of the *orf282* gene, we disrupted the gene by deleting a portion of the *orf282* gene and constructed an *orf282*-knockout mutant. Two FnrL binding sites were found to be located upstream of *orf282*, and it was demonstrated that *orf282* is positively regulated by FnrL. The *orf282* gene is not involved in the regulation of spectral complex formation. The cbb_3 oxidase activity detected in the *orf282* mutant was comparable to that in the wild-type sample, indicating that the *orf282* gene is not involved in the regulation of the *ccoNOQP* operon and the biosynthesis of the cbb3 cytochrome c oxidase. The elevated promoter activity of the *nifH* and *nifA* genes, which are the structural genes of nitrogenase and its regulator, respectively, in the *orf282* mutant, suggests that the *orf282* gene product acts as a negative effector for *nifH* and *nifA* expression.

Key words : *Rhodobacter sphaeroides*, nitrogenase, the *orf282* gene, the *nifH* gene, the *nifA* gene

Introduction

Rhodobacter sphaeroides is a purple, nonsulfur photosynthetic bacterium exhibiting remarkable metabolic capabilities to grow under a wide variety of environments [22, 39], capable of aerobic, anaerobic, and photosynthetic growth as well as fixing atmospheric nitrogen and carbon dioxide [14,40].

The *orf282* gene of *R. sphaeroides*, encoding for the polypeptide of 282 amino acid, is located immediately upstream of the *ccoNOQP* operon encoding the structural genes of the cbb_3 cytochrome oxidase with the opposite transcriptional orientation. *R. sphaeroides orf282* lies immediately downstream of the *fnrL* gene in contrast to the *orf277* gene (the homologue of *orf282*) of *R. capsulatus* which is far from the *fnrL* gene [41]. It was reported that several bacteria such as *R. capsulatus*, *Paracoccus denitrificans*, *Bradyrhizobium japonium*, *Azospirillum brasilense* have the *orf282*-homologue genes [6,15,33,34,42]

The role of the *orf282* gene of *R. sphaeroides* remains to be determined, but the role of the *orf282* homologue genes was suggested in a few bacteria. In *R. capsulatus*, an organism closely related to *R. sphaeroides*, Km insertion mutation of the *orf277* gene had any influence on neither activity nor assembly of the cbb_3 oxidase [15]. Revers et al. reported that Tn5 insertion mutation in the *orf280* gene of *A. brasilense* brought about an increase in nitrogen fixation activity [34]. The Orf 280 protein of *A. brasilense* was proposed to be a universal stress protein on the basis of the similarity of its C-terminal region with the UspA protein found in *E. coli* [23,34].

In this study, in order to reveal the function of the *orf282* gene of *R. sphaeroides*, the *orf282* mutant was constructed in the background of *R. sphaeroides* 2.4.1. To find out whether the *orf282* product is related to the regulation of *nif* genes, the corresponding *lacZ* transcriptional fusion plasmids were constructed and the promoter activities were measured in the *orf282* mutant (ORF282 strain) as well as wild-type (2.4.1).

Considering that the *orf282* gene is located immediately upstream and downstream of *ccoNOQP* and *fnrL*, respectively, we investigated the role of FnrL in expression of the *orf282* gene and that of Orf282 in expression of the *ccoNOQP* operon.

Materials and Methods

Bacterial strains, plasmids, and culture conditions

The bacterial strains and plasmids used in this study are listed in Table 1. *Escherichia coli* strains were grown at 37℃ in Luria-Bertani (LB) medium (BD, Sparks, MD) supplemented with antibiotics (Sigma, St Louis, MO) of following concentrations (μg/ml): ampicillin (100); tetracycline (10);

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Table 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant phenotype or genotype	Source or reference
Strains		
E. coli		
DH5α	(φ80d*lacZ*ΔM15) Δ*lacU169 recZ1 endA1 hsdR17 supE44 thi-1 gyrA96 relA1*	[13]
S17-1	Pro$^-$ Res$^-$ Mob$^+$ *recA*; integrated plasmid RP4-Tc::Mu-Km::Tn7	[37]
R. sphaeroides		
2.4.1	Wild-type	[39]
FNRL	2.4.1 derivative, Δ*fnrL*::ΩKm^r; formerly JZ1678	[41]
ORF282	2.4.1 derivative, Δ*orf282*	This study
Plasmids		
pCF1010	Sm^r/Sp^r Tc^r; IncQ	[20]
pLO1	Km^r; *sacB* RP4 – *oriT* ColE1 – *oriV*	[21]
pBBR1MCS2	broad host range, mob, Km^r	[16]
pUI8180	Tc^r; pLA2917 derivative, cosmid containing *fnrL, orf282, ccoNOQP*	[41]
pORF282lac	pCF1010 fused with a 0.65-kb *Xba*I/*Pst*I fragment containing *orf282* promoter region	This study
pPROnifH	pCF1010 fused with a 1.2-kb *Xba*I/*Pst*I fragment containing *nifH* promoter region	This study
pPROnifA	pCF1010 fused with a 0.75-kb *Not*I/*Xba*I fragment containing *nifA* promoter region	This study
pBORF282	pBBR1MCS2 fused with a 1.1-kb *EcoR*I/*Xba*I fragment containing the *orf282* gene	This study

streptomycin-spectinomycin (25); and kanamycin (50). *R. sphaeroides* strains were grown at 30℃ in Sistrom's (SIS) medium [2] supplemented with succinate (Sigma) as a carbon source and ammonium sulfate (Sigma) as a nitrogen source, and when necessary, antibiotics (Sigma) were added at the following concentrations (μg/ml): streptomycin-spectinomycin (50); tetracycline (1); trimethoprim (50) and kanamycin (25). For photosynthetic growth, cells were cultured anaerobically under incandescent light in screw-capped tubes filled with the medium.

For induction of nitrogenase, strains were first grown photosynthetically in SIS medium containing $NH_4(SO_4)_2$ as a nitrogen source for 12-14 hrs, then strains were washed with NH_4^+-free SIS medium, and induced in NH_4^+-free SIS medium containing 10 mM glutamate as N source to an optical density at 600 (OD_{600}) of 0.4-0.5 phototrophically [8]. NH_4^+-free SIS medium without $NH_4(SO_4)_2$ contains 1.61×10^{-2} mM $Na_2MoO_4 \cdot 2H_2O$ in place of $(NH_4)_6Mo_7O_{24}$. Cultivation of *R. sphaeroides* under anaerobic-dark-dimethyl sulfoxide (DMSO) (Sigma) conditions was performed in the screw-capped tubes that were completely filled with SIS medium containing 0.1% yeast extracts or NH_4^+-free SIS medium containing 10mM glutamate (Sigma) as a N source for 5~7 days at 30°C.

DNA manipulation and conjugation techniques

Recombinant DNA manipulations followed standard protocols [36] or manufacturer's instructions. *E. coli* S17-1 was used for plasmid mobilization by conjugation into *R. shpaeroides* [3].

Construction of an *orf282* mutant of *R. sphaeroides*

To delete the internal part of the *orf282* gene, a recombination PCR was performed. The two first-round PCR reactions were performed with a genomic DNA of *R. spharoides* 2.4.1 as the template. The primer pairs used for the first-round PCR were ORF-up1 (5′-ACTCGAATTCGTCTTCGCTAGG CCGACTC-3′), ORF-dn1 (5′-CGCACCGCCGTCAGCGCCT CATCGCGGGCTGGGCCGACAG-3′) and ORF-up2 (5′-CTGTCGGCCCAGCCCGCGATGAGGCGCTGACGGCGGT GCG-3′), ORF-dn2 (5′-CATATCTAGAGGCCGGCGACGAC AGCGAC-3′). The *EcoR*I and *Xba*I restriction site (underlined) were added to facilitate subcloning. The first-round PCR products were used as the template for the second round PCR using the ORF-up1 and ORF-dn2 primers. The deleted *orf282* gene (Δ*orf282*), in which 250-bp DNA fragment was deleted, was produced as the result of the second round PCR.

Following restriction of the Δ*orf282* gene with *EcoR*I/*Xba*I, it was inserted into pLO1 suicide vector. The result plasmid

pORF282Δ was introduced into *E. coli* S17-1, and transferred to the wild-type strain of *R. sphaeroides* by conjugation. The single crossovers were selected on plates with kanamycin. After the second crossover event, the strains which were sensitive to kanamycin and resistant to sucrose were isolated as the *orf282* mutants. The deletion of the portion of *orf282* was confirmed by sequence analysis.

Construction of *lacZ* transcriptional fusion plasmids

pPROnifH: The DNA fragment containing the promoter region (1.1-kb) and the 5′-portion (0.1-kb) of the *nifH* gene was amplified from the genomic DNA of *R. sphaeroides* 2.4.1 to generate a 1.2-kb fragment with *Xba*I and *Pst*I restriction sites at the ends. The primers used for PCR were NifHlac-up (5′-ATACTCCTGCAGTTCGTCGAGACGCGC-3′) and NifHlac-dn (5′-CACATATCTAGACCCATCTCGACCA-3′). The *Pst*I and *Xba*I restriction sites (underlined) were added to facilitate subcloning. The PCR product was digested with *Pst*I/*Xba*I and subcloned into the promoterless *lacZ* fusion vector pCF1010, yielding plasmid pPROnifH which was used to determine the promoter activity of *nifH*.

pPROnifA: The 0.75-kb DNA fragment containing the promoter region (0.65-kb) and the 5′-portion (0.1-kb) of the *nifA* gene was amplified from the genomic DNA of *R. sphaeroides* 2.4.1. The primers used for PCR were NifAlac-up (5′-ATACTAGCGGCCGCGGCCGTAGAGAGGATCG-3′) and NifAlac-dn (5′-CACATATCTAGAAGCTTGGCGATC TCGCA-3′). The PCR product was digested with with *Not*I/*Xba*I and subcloned into pCF1010, yielding the *nifA*::*lacZ* transcriptional fusion plasmid, pPROnifA.

pORF282lac: The DNA fragment containing the promoter region (0.55-kb) and the 5′-portion (0.1-kb) of *orf282* gene was amplified from pUI8180 using the primers ORFlac-up (5′-AGCACGTCTAGATGCGCATCGCTG-3′) with the *Xba*I site and ORFlac-dn (5′-GGAGGTCTGCAGCGGACGCAG CCG-3′) with the *Pst*I site. The PCR product containing 0.65-kb *Xba*I/*Pst*I fragment was digested and cloned the pCF1010 to construct the *orf282*::*lacZ* transcriptional fusion plasmid, pORF282lac.

Complementation of the *orf282* mutant

A 1.1-kb PCR fragment containing the entire *orf282* gene was amplified from *R. sphaeroides* genomic DNA using the primer ORFcom-up (5′-ACTCGAATTCGTCTT CGCTAGG CCGACTC) with the *EcoR*I site and ORFcom-dn (5′-CATA TCTAGAGG CCGGCGACGACAGCGAC-3′) with the *Xba*I site. After digestion with *EcoR*I and *Xba*I, the 1.1-kb PCR fragment was cloned into the corresponding sites of pBBR1MCS2, a broad host range vector. To complement the mutation of the *orf282* gene in the ORF282 strain, the resulting plasmid pBORF282 was introduced into the ORF282 strain by conjugation.

Stress conditions

R. sphaeroides harboring pORF282lac, the *orf282*::*lacZ* transcriptional fusion, was grown under aerobic conditions to an OD_{600} of 1.0~1.2 and the culture was treated with H_2O_2.

To induce nutrient starvation conditions, *R. sphaeroides* harboring pORF282lac was grown aerobically to an OD_{600} of 0.9~1.0, harvested and washed twice with NH_4^+-free or succinate-free SIS medium. The washed strains were grown in either NH_4^+-free or succinate-free SIS medium to an OD_{600} of 0.8~0.9.

Determination of the levels of spectral complexes

Crude cell-free lysates were prepared by passage through a French pressure cell (ca. 0.9-cm-diameter piston) at 90 MPa. ICM buffer (10 mM KH_2PO_4/K_2HPO_4, 1 mM EDTA [pH 7.2]) was used as the lysate solution. The levels of B875 and B800-850 spectral complexes were determined as described previously [24].

Enzyme activity assay and protein determination

The β-galactosidase assay was performed using O-nitrophenyl-β-D-galactopyranoside (Sigma) as described in the reference [26]. Cytochrome c oxidase activity was assayed spectrophotometrically by monitoring the oxidation of reduced horse heart cytochrome c (Sigma) at 550 nm and 30℃ [31]. Protein concentrations in crude cell extracts were determined by the bicinchoninic acid protein assay reagent (Pierce, Rockford, IL.) with bovine serum albumin as the standard protein.

Results and Discussion

Features of the Orf282 protein

When the amino acid sequence of Orf282 of *R. sphaeroides* was multiple aligned with the Orf282-homologues of several bacteria, the C-terminal regions of Orf282 homologues proteins are well conserved as shown in Fig. 1A. In contrast, the N-terminal regions of the proteins are not conserved. This fact implies that conserved C-terminal region plays an

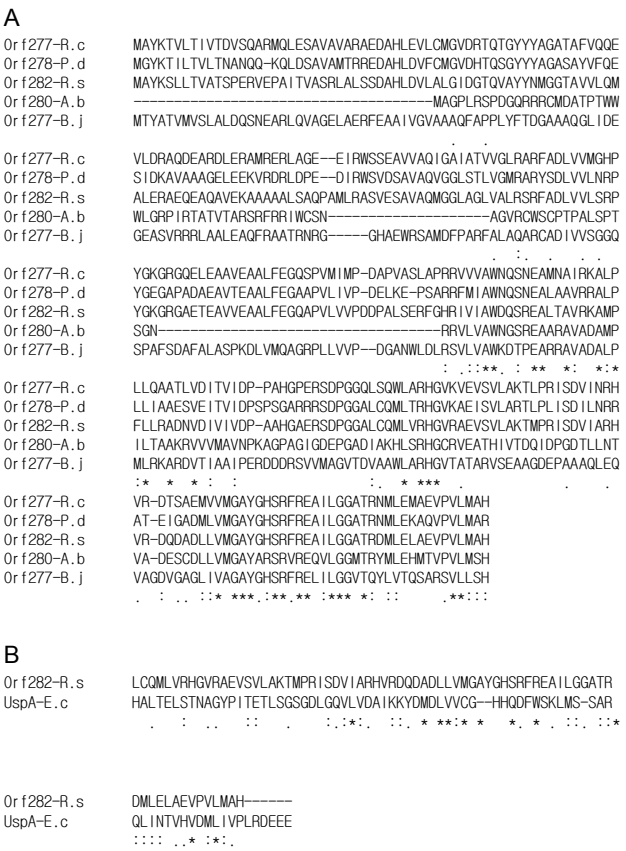

Fig. 1. A. Multiple alignment of the C-terminal regions of Orf282 homologues of *R. sphaeroides* (Orf282-R.s), *R. capsulatus* (Orf277-R.c), *P. denitrificans* (Orf278-P.d), *B. japonicum* (Orf-277-B.j), and *A. brasilense* (Orf-280-A.b). B. Two alignment of the C-terminal region of Orf282 of *R. sphaeroides* and UspA domain of *E. coli* (UspA-E.c) The alignment was generated by CLUSTALW. Identical or conservatively substituted amino acids are indicated by asterisks or colons, respectively.

important role in the function of Orf282. As shown in Fig. 1B, the C-terminal region of Orf282 of *R. sphaeroides* exhibits some similarity to UspA protein of *E. coli*.

The UspA protein in *E. coli* is a universal stress protein which required defense and survival during the growth arrest state and through various other stresses [28,29,36]. Synthesis of UspA is induced in response to a variety of stresses including the depletion of nutrients and exposure to heat shock, oxidants, and DNA-damaging agents. [10,18,28,29,36].

The UspA protein is phosphorylated at conserved serine and threonine residues under stress conditions [9]. Proteins belonging to the Usp family are distributed widely in bacteria, archaea, fungi, protozoa, and plants [1,18]. The UspA of prokaryotes includes a domain similar to the DNA-binding domain of the developmental MADS-box family and the tyrosine kinase of eukaryotes [9,25]. Relationship of the UspA domain of prokaryotes to the DNA-binding domain of eukaryotes is a clue explaining whether UspA possesses DNA-binding activity [27].

The *uspA* gene of *E. coli* is activated by a σ^{70}-dependent promoter and the production of UspA is regulated at the transcriptional level [29]. In addition to being activated by ppGpp [10,19] and RecA [4,10], the *uspA* promoter is regulated by fructose-6-phosphate of the glycolytic pathway independently of ppGpp [32]. It is repressed by FadR [7] and FtsK [5,10].

Effect of growth conditions on *orf282* expression

Using an *orf282::lacZ* transcriptional fusion, we have analyzed expression of the *orf282* gene in *R. sphaeroides* 2.4.1 grown under different growth conditions. As shown in Fig. 2A, expression of *orf282* was at the basal level under high (30%) oxygen conditions. Maximal *orf282::lacZ* expression was observed under semiaerobic (2% oxygen) conditions. Under both photosynthetic and dark-DMSO conditions, expression of *orf282* was highly induced, but slightly lower, compared with that under semiaerobic (2% oxygen) conditions. From this result, we suggest that expression of *orf282* is induced under anaerobic and oxygen-limiting conditions.

Effect of stress conditions on the *orf282* expression

Considering that the C-terminal domain of Orf282 in *R. sphaeroides* is similar to UspA protein in *E. coli*, which responds for a variety of stress conditions, we measured the activity of the orf282 promoter from the transcriptional *lacZ* fusion under nutrient depletion and oxidant treatment. As shown in Fig 2B, expression of *orf282::lacZ* fusion (pORF282lac) was increased under the condition of N and C source starvation in contrast to that of N and C rich source. Expression of the *orf282::lacZ* fusion treated with 3.5 mM H_2O_2 was increased by a factor of 1.2 when it was compared with the control culture. These results suggest that Orf282 might be one of the universal stress proteins.

Involvement of FnrL in *orf282* expression

Zeilstra-Ryalls and Kaplan predicted FnrL might be involved in the regulation of the *R. sphaeroides orf282* gene since two FNR binding consensus sequences (TTGATC CATGTCAA and TTGATCCTCATCAA) exist upstream of

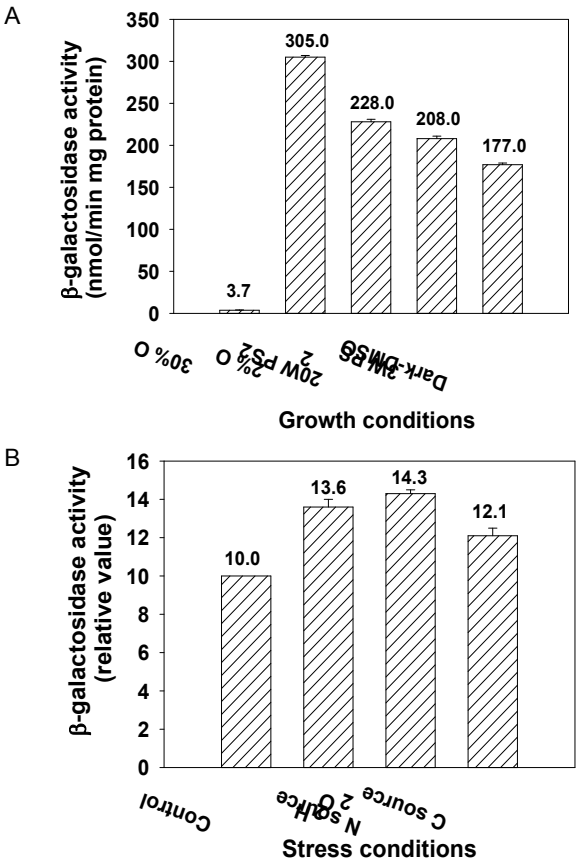

Fig. 2. Promoter activities of the *orf282* gene in *R. sphaeroides* 2.4.1 grown under different growth and stress conditions. The wild-type (2.4.1.) strain contains the *orf282*::*lacZ* transcriptional fusion plasmid, pORF282lac. A. The strains were grown aerobically (30% O_2) or semiaerobically (2% O_2) to an OD_{600} of 0.5~0.6. The strains were grown aerobically in the dark with 5% DMSO (Dark DMSO) as a terminal electron acceptor or photosynthetically (PS) in the light intensity of 20W/m^2 and 3W/m^2. B. The strains were grown aerobically to an OD_{600} of 0.5~0.6 in SIS medium (control) or in SIS medium plus H_2O_2 for toxic agent (H_2O_2), and aerobically an OD_{600} of 0.8~0.9 in NH_4^+-free SIS medium for N source starvation (N source) or succinate-free SIS medium for C source starvation (C source). All values represent the averages of two independent determinations. Error bars represent the standard deviations from the mean.

orf282 [41]. To confirm this prediction, the promoter activity of *orf282* was determined in the FnrL-minus mutant (strain FNRL) background as well as the wild type by using the *orf282-lacZ* transcriptional fusion. The strains were cultured either under 30% oxygen or 2% oxygen conditions because the FNRL strain is not able of to grow under anaerobic conditions [41]. As shown in Fig. 3, both the wild-type and

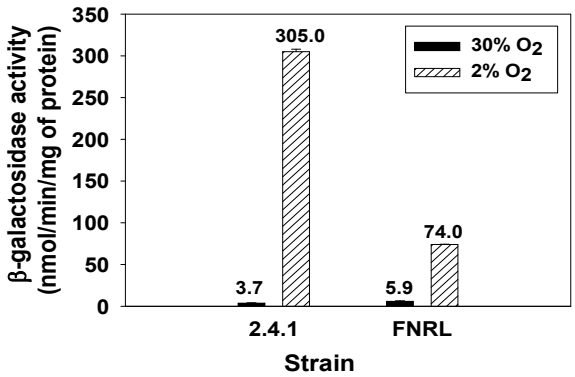

Fig. 3. Comparative β-galactosidase activity for the wild-type and FNRL mutant strain bearing the *orf282*::*lacZ* transcriptional fusion plasmid, pORF282lac. The wild-type (2.4.1) and *fnrL*-minus (FNRL) strains were grown aerobically (30% oxygen, black bars) or semiaerobically (2% oxygen, hatch bars) to an OD_{600} of 0.5~0.6. All values represent the averages of two independent determinations. Error bars represent the standard deviations from the mean.

FNRL strains exhibited basal levels of promoter activities under aerobic (30% oxygen) conditions. The promoter activity of the *orf282*::*lacZ* in the wild type grown semiaerobically (2% oxygen) was increased by a factor of 82 when compared with that found in the same strain grown aerobically. In contrast to the wild type, expression of the *orf282* gene in the FNRL strain grown under semiaerobic (2% oxygen) conditions was significantly lower than that in the wild-type strain grown under the same conditions, suggesting that the *orf282* gene is induced in an FnrL-dependant manner under semiaerobic (2% oxygen) conditions and that FnrL acts as an activator for *orf282* expression under O_2-limiting conditions. Two FNR-binding motifs exist in the intergenic region between the *orf282* and *ccoN*. It has yet to be solved which FNR-binding motif is involved in regulation of the *orf282* gene by FnrL.

Phenotypes of the *R. sphaeroides orf282* mutant

The light-harvesting (LH) complexes of *R. sphaeroides* are designated as B800-850 (LHII) and B875 (LHI), depending on their respective absorption maximum. These two pigment-protein complexes in addition with the reaction center form spectral complexes that are required for photosynthetic energy utilization of *R. sphaeroides*. As shown in Table 2, the levels of B800-850 and B875 spectral complexes synthesized in the ORF282 strain were not significantly different from those in the wild type when the strains were grown under

Table 2. Levels of spectral complexes in *R. sphaeroides* strains

Strains	Spectral complexes (nmole/mg of protein)	
	B800-850	B875
2.4.1	34.73±0.49	10.27±0.67
ORF282	35.81±0.15	9.84±0.18

The wild-type (2.4.1) and *orf282*-minus (ORF282) strains were grown under anaerobic dark-DMSO conditions.

All values represent the averages of two independent determinations.

anaerobic conditions in the dark with DMSO. This result suggests that the *orf282* gene product is not involved in the formation of spectral complexes.

The fact that the *orf282* gene of *R. sphaeroides* is located in the vicinity of the *ccoNOQP* operon encoding the cbb_3 cytochrome c oxidase led us to speculate the relationship between the *orf282* and the *ccoNOQP* operons. To ascertain whether or not the *orf282* gene product is related to the cbb_3 cytochrome c oxidase activity, the cytochrome c oxidase activity was measured in the ORF282 and wild-type strains by using reduced cytochrome c. The activity of the cbb_3-cytochrome c oxidase was determined in the *R. sphaeroides* strains grown under anaerobic dark DMSO conditions since the cbb_3 oxidase is the cytochrome c oxidase that is exclusively expressed under anaerobic conditions. In comparison with the wild type, the ORF282 strain showed virtually the same cytochrome c oxidase activity under anaerobic dark DMSO conditions, implying that Orf282 of *R. sphaeroides* is not related to expression of the *ccoNOQP* operon and assembly of the cbb_3 oxidase (Fig. 4.).

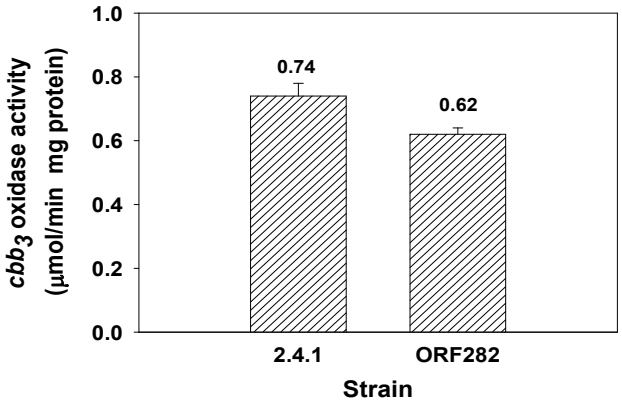

Fig. 4. Specific activity of the *cbb3* cytochrome c oxidase from the wild-type (2.4.1) and ORF282 mutant strain. *cbb3* oxidase activity was measured spectrophotometrically by monitoring the oxidation of pre-reduced horse heart cytochrome c using cells grown anaerobically under dark-DMSO conditions. All values represent the averages of two independent determinations. Error bars represent the standard deviations from the mean.

Analysis of *nifH* and *nifA* expression in the *orf282* mutant

The disruption of the *orf282* gene in *A. brasilense* led to the increase of *nifH* expression in relation to the N_2-fixing capacity, suggesting that there is a relation between the Orf282 protein and the regulation of *nifH* expression [34].

The *nifHDK* operon encodes dinitrogenase reductase (NifH) and dinitrogenase (NifDK). The *nifA* gene encodes NifA, which is involved in induction of the *nifHDK* operon [11,12,17]. To determine whether the *orf282* gene in *R. sphaeroides* is related to expression of the *nifHDK* operon and *nifA* gene or not, we constructed the *nifH::lacZ* transcriptional fusion plasmid, pPROnifH and the *nifA::lacZ* transcriptional fusion plasmid, pPROnifA. The β-galactosidase activity of the promoter was measured in the ORF282 and wild-type strains bearing pPROnifH and pPROnifA. Since expression

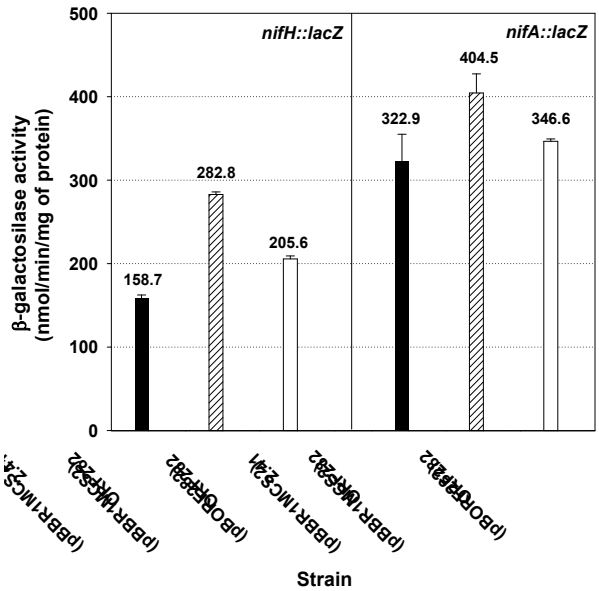

Fig. 5. The expression of the *nifH* and *nifA* in the wild-type and ORF282 mutant strain and complementation of the ORF282 strain using pBORF282. All strains contain pPROnifH(*nifH*::*lacZ*) for expression of the *niH* gene or pPROnifA (*nifA*::*lacZ*) for expression of the *nifA* gene and carry a second plasmid for the complementation test: vector pBBR1MCS2 (black bar and hatch bar); pBORF282 (pBBR1MCS2:: *orf282*) (white bar). Strains were grown photoheterophically in NH_4^+-free SIS medium with supplementation of 10 mM glutamate at a light intensity of 15 W/m^2. All values represent the averages of two independent determinations. Error bars represent the standard deviations from the mean.

of the *nif* genes is known to be derepressed in the purple nonsulfur bacteria grown under conditions with limited O_2 and NH_4^+ [17,30], the strains carrying the corresponding plasmids were grown under anaerobic photosynthetic conditions with glutamate as a nitrogen source. As shown in Fig. 5, expression of the *nifH* and *nifA* in the ORF282 strain which are grown under nitrogenase-derepressing conditions was higher than that observed for the wild type grown under the same conditions.

The result indicates that the *orf282* gene product might directly or indirectly act as either a negative effector of nifA and *nifH* expression under nitrogenase-derepressed conditions. It is also possible that ORF282 could affect the NtrBC two-component system and PII, which sense and respond to the intracellular nitrogen source in the regulatory cascade for *nif* genes [12,17].

To verify whether the phenotype of the ORF282 strain is due to the disruption of *orf 282*, a complementation experiment was performed. The plasmid pBORF282, which carries the intact *orf282* gene, was introduced *in trans* to complement the ORF282 strain. The wild-type and ORF282 strains containing the empty vector pBBR1MCS2 were included in the experiment as the controls.

The β-galactosidase activity of the ORF282 strains bearing pBORF282/pPROnifH or pBORF282/pPROnifA were restored partially to that of the wild type 2.4.1 bearing pBBR1MCS2/pPROnifH or pBBR1MCS2/pPROnifA, although the level of β-galactosidase activities were not identical quantitatively. Complementation of strain ORF282 suggests that expression of the *nifH* gene and *nifA* gene was affected by the disruption of the *orf282* gene.

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초록 : *R. sphaeroides*에서의 *orf282* 유전자의 분석과 이들의 기능

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*Rodobacter sphaeroides*에서 *orf282* 유전자는 cbb3 terminal oxidase를 암호화하는 *ccoNOQP* 오페론과 혐기적 활성자인 FnrL을 암호화하는 *fnrL* 유전자 사이에 있으며, 아직은 기능이 잘 알려지지 않았다. *orf282* 유전자의 기능을 알기 위해 우리는 *orf282*의 일부를 삭제함으로써 유전자를 붕괴시켜 *orf282*-minus mutant를 제조하였다. 두 개의 FnrL 결합 부위가 *orf282*의 upstream에 존재한다는 것이 밝혀져 있으며, *orf282* 유전자가 FnrL에 의해 양성적으로 조절된다는 것이 증명되었다. *orf282* 유전자는 B875와 B800-850 spectral complexes의 형성과 관련이 없다. *orf282* mutant에서의 cbb3 oxidase 활성을 wild type와 비교해보면 *orf282* 유전자가 ccoNOQP 오페론의 조절과 cbb3 cytochrome c oxidase의 생합성과 무관하다는 것을 알 수 있다. *orf282* mutant의 구조 유전자인 *nifH*와 조절유전자인 *nifA*의 프로모터 활성이 증가한 것은 *orf282* 유전자 산물이 *nifH*와 *nifA*의 발현에서 음성적 effector로 작용한다는 것을 시사한다.