Short Communication

The cbbL Gene is Required for Thiosulfate-Dependent Autotrophic Growth of Bradyrhizobium japonicum

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Bradyrhizobium japonicum is a facultative chemolithoautotroph capable of using thiosulfate and H2 as an electron donor and CO2 as a carbon source. In B. japonicum USDA110, the mutant of cbbL gene encoding a large subunit of ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO) was unable to grow using thiosulfate and H2 as an electron donor. The cbbL deletion mutant was able to grow and oxidize thiosulfate in the presence of succinate. These results showed that the major route of CO2 fixation for thiosulfate-dependent chemotrophic growth is the Calvin-Benson-Bassham cycle involving RuBisCO in B. japonicum.

Key words: Bradyrhizobium japonicum, CO2 fixation, chemotroph, cbb

Bradyrhizobium japonicum, a nitrogen-fixing endosymbiont of soybean nodules (6), is a facultative chemotroph utilizing thiosulfate (15), H2 (5, 8, 13) and CO2 (11, 14) as an electron donor and CO2 as a carbon source. However, the genes relevant to CO2 fixation during the chemotrophic growth of B. japonicum have yet to be identified.

There are four major pathways for CO2 fixation; the Calvin-Benson-Bassham (CBB) cycle, reductive tricarboxylic acid (rTCA) cycle, 3-hydroxypropionate (3-HP) cycle and reductive acetyl coenzyme A (acetyl-CoA) pathway, in bacteria and archaea (17). A search for the genes related to CO2 fixation during the chemoautotrophic growth on the genome of B. japonicum strain USDA110 revealed the presence of structural genes for the CBB cycle and rTCA cycle.

Activity of ribulose 1,5-bisphosphate carboxylase (RuBisCO) was biochemically detected in a crude cell extract of B. japonicum strain USDA122 grown chemotrophically with H2 as an electron donor under a gas mixture (v/v) of 84% N2, 5% CO2, 1% O2, and 10% H2 (13). RuBisCO was also purified from USDA122 cells (18). In addition, the expression of cbbLS encoding RuBisCO was enhanced with H2 as a sole electron donor, as compared with that under heterotrophic conditions in B. japonicum strain USDA110 (5).

Recently, Masuda et al. (15) found that B. japonicum USDA110 is able to fix ambient CO2 during chemotrophic growth using thiosulfate at quite low concentrations of CO2 (0.03–0.07% [v/v]) in contrast with previous reports of H2-dependent chemotrophic growth (5% [v/v] CO2) (5, 8, 13). Generally, RuBisCO enzymes have low affinity for CO2 and require higher CO2 concentrations (1). Therefore, we examined whether cbb encoding RuBisCO is required for thiosulfate-dependent chemotrophic growth at ambient concentrations of CO2 in the air.

The bacterial strains and plasmids used in this study are listed in Table 1. B. japonicum strains were cultured aerobi-
yielding pSAC50 (Table 1). The Tc′-cassette was isolated from p34S-Tc at the Smal site (3), and inserted into the AatII site of pSAC50, yielding pSAC51. It was conjugated into B. japonicum strain USDA110 by triparental mating using pRK2013 as a helper plasmid (20) and Tc′Kmr transconjugants were selected. PCR was performed using primers P1 (5′-ACTACACGCCAAAGGACACC-3′) and P2 (5′-GAACTTCACGTCCTTCCAGA-3′), and total DNA of the transconjugants as templates (Fig. 1B and C). One of the transconjugants, strain USDA110ΔcbbL, which showed the expected gene replacement (Fig. 1C), was used as the cbbL mutant throughout this study (Fig. 1A and C).

The wild-type strain USDA110 and the cbbL mutant were subjected to growth experiments on the H2-uptake medium under an atmosphere containing 5% CO2 and 10% H2 (5, 8, 13). The cbbL mutant showed markedly weak growth compared to the wild type for 28 d (Fig. 2A). The growth of cbbL mutant was similar to that of strain USDA110 on HM plates supplemented with arabinose (Fig. 2B). These results suggested that the cbbL gene is required for H2-dependent chemoautotrophic growth of B. japonicum USDA110, supporting the previous biochemical observation that RuBisCO activity increased during chemoautotrophic growth (5, 8, 13).

Subsequently, the wild type and the cbbL mutant were examined for chemoautotrophic growth using thiosulfate as an energy source. The cbbL mutant showed a growth defect compared to the wild type on H2-uptake agar medium (A) and HM medium (B) for 28 d under a gas mixture (v/v) of 84% N2, 10% H2, 5% CO2 and 1% O2.

Table 1. Bacterial strains and plasmids used in this study

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Relevant characteristicsa</th>
<th>Reference or source</th>
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<tr>
<td>Strains</td>
<td>B. japonicum USDA110 wild type (9)</td>
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<tr>
<td></td>
<td>USDA110 ΔcbbL; Tc′; Tet</td>
<td>This study</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>DH5α cloning host strain Toyobo</td>
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<td>Plasmids</td>
<td>pSAC50; pK18mob carrying 3.6-kb cbbALSX fragment of brc04278; Km′; This study</td>
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<tr>
<td></td>
<td>pSAC51; pK18mob carrying cbbL::Tc′ cassette; Tc′ Km′; This study</td>
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<tr>
<td></td>
<td>brc04278; pS800 carrying cbb operon; Km′; (9)</td>
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<tr>
<td></td>
<td>p34S-Tc; Plasmid carrying 2.1-kb Tc′ cassette; Tc′ Km′; (3)</td>
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<tr>
<td></td>
<td>pK18mob; integration vector; oriV, oriT, mob; Km′; (21)</td>
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<td>pRK2013; ColE1 replicon carrying RK2 transfer genes; Km′; (4)</td>
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a Tc′, tetracycline resistant; Km′, kanamycin resistant.
b Toyobo, Osaka, Japan.
an electron donor under an ambient CO\textsubscript{2} level in the air. The \textit{cbbL} mutant was unable to grow and oxidize thiosulfate under the condition over 27 d (Fig. 3B), whereas the wild type was able to grow chemolithoautotrophically using thiosulfate as an electron donor (Fig. 3A). The \textit{cbbL} mutant was able to grow and oxidize thiosulfate like strain USDA110 in the presence of succinate (Fig. 3C and D). These results indicate that the \textit{cbbL} gene is required for chemolithoautotrophic growth using thiosulfate under ambient CO\textsubscript{2} levels in the air.

Therefore, we concluded that the major route of CO\textsubscript{2} fixation is the CBB cycle, not the rTCA cycle, during chemolithoautotrophic growth using thiosulfate and ambient CO\textsubscript{2} in the air.

The \textit{cbbL} gene was required for chemolithoautotrophic growth using H\textsubscript{2} (Fig. 2A) or thiosulfate (Fig. 3A and B). Thus, it is possible that the CBB cycle plays a major role also in chemolithoautotrophic growth using other inorganic electron donors such as CO in \textit{B. japonicum} strain USDA110. However, we cannot exclude the possibility that the rTCA cycle makes a minor contribution to CO\textsubscript{2} fixation, because the \textit{cbbL} mutant showed weak growth on the H\textsubscript{2}-uptake medium (Fig. 2A).

A thiosulfate-oxidizing capability is frequently found along with hydrogenase (H\textsubscript{2} oxidation) activity in the \textit{Bradyrhizobiaceae} (15). A study of the genomic sequences of \textit{Rhodopseudomonas palustris} CGA009 (12), \textit{Bradyrhizobium} sp. BTAi1 (7) and \textit{Bradyrhizobium} sp. ORS278 (7), which show thiosulfate-oxidizing activity, revealed that they carried structural genes for RuBisCO (rpa1559–1560 for CGA009; BBta0451–0452, BBta2641–2642, and BBta6396–6397 for BTAi1; and BRADO1659–1650 and BRADO2274–2275 for ORS278). Thus, it is possible that the CBB cycle is commonly required for chemolithoautotrophic growth in other members of the \textit{Bradyrhizobiaceae}.

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**References**


