# Molecular Basis of Carbon Monoxide Utilization in *Mycobacterium* Sp. Strain JC1 DSM 3803

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## Introduction

It has been known that about $1.2\text{-}1.4 \times 10^9$ tons of carbon monoxide (CO) are added to the atmosphere each year through incomplete oxidation of fossil fuels and from various natural sources (1). The CO content of the atmosphere, however, remains constant approximately at 0.03-0.9 ppm, indicating that for atmospheric CO (unlike $CO_2$, the concentration of which is rising) there exist sinks for sufficient magnitude to balance the production. As much as of the yearly burden of CO may be converted to $CO_2$ by hydroxyl radical in the upper atmosphere. However, in the lower layers of the atmosphere and at the earth's surface, there is considerable evidence that biological oxidation of CO to $CO_2$ plays a major role, and that bulk of this oxidation is microbiological.

Carboxydobacteria are a group of aerobic bacteria which are able to grow at the expense of CO as a sole source of carbon and energy (1). The bacteria are becoming objects of considerable interest because of their potential for production of single cell protein from waste gases or mixtures of $H_2$ and $CO_2$ derived from coal, in conjunction with the reduction of CO content in the industrial waste gases. Furthermore, studies on the mechanism of bacterial CO oxidation may results in the development of nonbiological CO oxidation system which may be used to convert the toxic CO gas to useful materials. Therefore, we and other groups have studied, in order to understand the mechanism of aerobic CO oxidation, the physiological, biochemical, and molecular bases of CO oxidation in several carboxydobacteria.

*Mycobacterium* sp. strain JC1 (formally *Acinetobacter* sp. strain JC1) is a carboxydobacterium isolated from soil in Seoul, Korea (2). It is able to grow heterotrophically with several organic substrates, chemolithoautotrophically with CO, and methylotrophically with methanol and methylamine as the sole source of carbon and energy (3).

In this symposium, I am planning to present the bases for utilization of CO as the sole source of carbon and energy in *Mycobacterium* sp. strain JC1 with special emphasis on the molecular basis of the utilization.

## Physiological and Biochemical Bases of CO utilization

### Key enzyme for CO oxidation

CO dehydrogenase (CO-DH) is an enzyme responsible for the oxidation of CO to $CO_2$ in carboxydobacteria: $CO + H_2O + X(ox)\ CO_2 + XH_2(red)$. The enzyme of *Mycobacterium* sp. strain JC1 was expressed constitutively and showed no immunological relationship with those from other carboxydobacteria (4). The purified enzyme also exhibited particulate hydrogenase-like activity. The molecular

weight of the native enzyme was determined to be 380,000. The enzyme consisted of three nonidentical subunits of molecular weights 16,000, 34,000, and 85,000. One mol of native enzyme contains 2 mol each of molybdopterin cytosine dinucleotide and FAD and 8 mol each of nonheme iron and labile sulfide (4). The deduced amino acid sequences of genes for the three CO-DH subunits implied the presence of molybdopterin cofactor, FAD, and iron-sulfur centers in large, medium, and small subunits, respectively.

**Energy conservation and CO-insensitive electron transport system**

The metabolism of CO in carboxydobacteria is strictly respiratory. During growth with CO, electrons are delivered by the action of CO-DH. They are transported via CO-insensitive branch of the electron transport system containing CO-insensitive terminal oxidase (1). In the absence of CO, electrons released from organic substrates have a preference for CO-sensitive branch.

**$CO_2$ assimilation**

$CO_2$ derived from CO in carboxydobacteria is converted to cellular material via 2Calvin cycle (1).

It was found that *Mycobacterium* sp. strain JC1 contains two kinds of ribulose bisphosphate carboxylase (RubisCO), the key enzyme of Calvin cycle, which are expressed differentially under the presence of CO or methanol as a growth substrate (5). The peptide maps of RuBisCOs from cells grown on CO and methanol were found to be different. The molecular weight of the native enzymes was 520,000. The enzymes consisted of two nonidentical subunits of molecular weights 53,500 and 15,000. The enzyme showed no immunological relationship with those of other carboxydobacteria.

## Molecular Bases of CO Utilization

**Genetic organization and characteristics of the genes for CO-DH and other related genes**

Several genes including CO-DH structural genes, accessory genes, and putative regulatory genes from *Mycobacterium* sp. strain JC1 were cloned and characterized (Fig. 1).

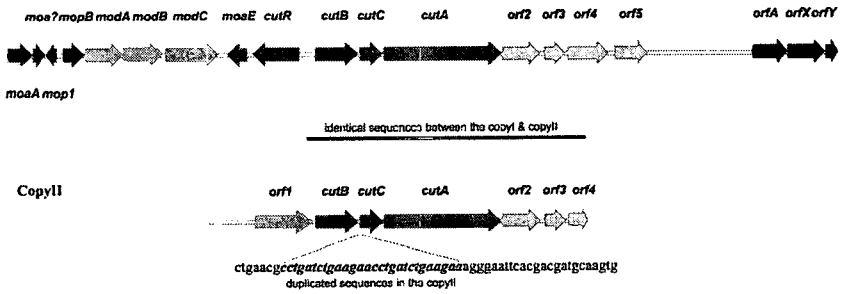


**Fig. 1.** Genetic map of two copies *cut* genes in *Mycobacterium* sp. strain JC1.

The three structural genes of CO-DH were clustered in the transcriptional order of *cutBCA*. The cloned *cutB*, *cutC*, and *cutA* genes had open reading frames of 873, 486, and 2,394 nucleotides, coding for proteins with calculated molecular weights of 30,509, 17,277, and 85,857, respectively. The homologies in the amino acid sequence of CutB, CutC, and CutA were 55.1, 38.2, and 57.7%, respectively, with *Oligotropha carboxidovorans* (6), 59.0, 36.8, and 58.0%, respectively, with *Pseudomonas thermo-*

*carboxydovorans* (7), and 57.8, 40.0, and 56.8%, respectively, with *Hydrogenophaga pseudoflava* (8). The overall identity in the nucleotide sequence of the genes with those of *Mycobacterium tuberculosis* (9), however, was over 80%. The deduced amino acid sequences of the three CO-DH subunits implied the presence of molybdenum cofactor, FAD, and iron-sulfur centers in CutA, CutB, and Cut, respectively. Primer extension analysis revealed that the transcriptional start site of the genes was the nucleotide T located 78 bp upstream of the *cutB* start codon.

It was found that the *cutBCA* and several accessory genes are duplicated in *Mycobacterium* sp. strain JC1 (Fig. 1). The duplicated genes found in copy I and copy II, in addition to the CO-DH structural genes, were *orf2*, *orf3*, and *orf4*, locating at the downstream of and running at the same direction as the *cutA*. The upstream regions of the two copies, on the other hand, did not share any identical or similar genes. Several genes possibly involved in the expression (*cutR*) and assembly (*moaE*, *modC*, *modB*, *modA*, *mopB*, *mop1*, and *moaA* homologues) of functional CO-DH were clustered in the upstream region of *cutB* in copy I. The genes were not found from the upstream region of copy II. The nucleotide sequences of *cutBCA* in copy II were identical to those from the copy I.

The cloned *cutR* had an open reading frame of 963 nucleotides, coding for proteins of LysR-type transcriptional regulator with 320 amino acids. The transcription of *cutR* separated by 314 bp from the divergently oriented *cutBCA* began at nucleotide T coinciding with start codon of *cutR*.

Analysis of the *cutR-cutB* intergenic region revealed the presence of three inverted repeated sequences. The putative -10 promoter regions of *cutR* and *cutBCA* exhibited high similarity to those of S4 and T119 promoters of *M. tuberculosis*, respectively. No conserved -35 sequences were found. The *cutBCA* promoter was not active in *E. coli*. CutR was overproduced and purified in *E. coli*, and its binding to the *cutB-cutR* intergenic region was demonstrated. Gel mobility shift assay indicated CutR bound specifically to *cutB-cutR* intergenic region. CO did not affect the CutR binding.

**Characteristics of genes for RubisCO and other related genes**

It was found that the RubisCO genes, *cbbL* and *cbbS,* of *Mycobacterium* sp. strain JC1 DSM 3803 are also duplicated (Fig. 2).

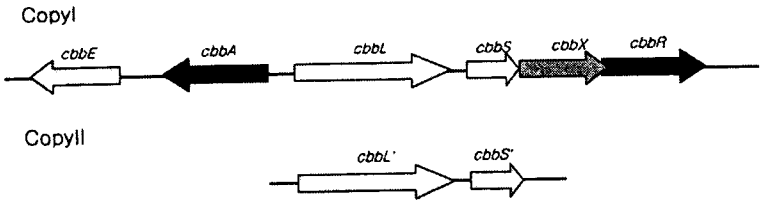


**Fig. 2.** Genetic map of two copies of *cbb* genes in *Mycobacterium* sp. strain JC1.

The first copy of RubisCO genes was clustered in the order of *cbbL-cbbS-cbbX-cbbR*. The cloned *cbbL-cbbS-cbbX-cbbR* genes had open reading frames of 1,407, 426, 1,005 and 909 nucleotides, encoding proteins with calculated molecular masses of 51,707, 15,837, 36,888 and 31,790, respectively. CbbR showed significant similarity to the LysR family of transcriptional regulatory proteins. Northern blot analysis with *cbbL*- and *cbbR*-specific probes revealed that *cbbL-cbbS-cbbX-cbbR* genes were cotranscribed as a 4-kb mRNA. Transcription of *cbbL-cbbS-cbbX-cbbR* genes began at nucleotide T located 99 bp upstream of start codon of *cbbL*. The *cbbR* gene was also transcribed independently from the three *cbb* genes as a 1.2-kb transcript.

The second copy of *cbbLS* genes of second had open reading frames of 1,419 and 429 nucleotides, encoding proteins with calculated molecular masses of 52,061 and 16,252, respectively. The *cbbR* gene was not found around the second copy of *cbbLS* genes. The deduced amino acid sequence of second copy of *cbbL* showed 88.6% identity with that of the first *cbbL* copy. The second *cbbLS* genes were cotranscribed as a 2.0-kb *cbbLS* mRNA. Transcription of the second *cbb* operon began at G located 41 bp upstream of the first codon of *cbbL*.

Comparison of the N-terminal amino acid sequences of RubisCOs prepared from cells grown on CO and methanol and those deduced from nucleotide sequences of *cbbL* genes revealed that the first and second copies of *cbbLS* genes are expressed in the presence of CO and methanol, respectively.

It was found that the conserved -10 and -35 sequences and two inverted repeats are present immediate upstream of copy I and copy II *cbbL* genes. Phylogenetic analysis of the deduced amino acid sequences of the two *cbbL* genes and *cbbR* gene indicated that *Mycobacterium* sp. strain JC1 *cbbL* and *cbbR* genes have evolved independently from those of other RubisCOs and CbbRs, respectively.

## REFERENCES


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