Process Biochemistry 51 (2016) 220–228

Contents lists available at ScienceDirect

Process Biochemistry

journal homepage: www.elsevier.com/locate/procbio

Pseudomonas putida IOFA1 transcriptome profiling reveals a metabolic pathway involved in formaldehyde degradation

Min Jin[a,1], Xiang Yu[a,1], Xinglin Chen[a], Runying Zeng[a,b,*]

[a] State Key Laboratory Breeding Base of Marine Genetic Resource, Third Institute of Oceanography, SOA, Xiamen 361005, China
[b] Fujian Collaborative Innovation Center for Exploitation and Utilization of Marine Biological Resources, Xiamen 361005, China

ARTICLE INFO

Article history:
Received 20 July 2015
Received in revised form 29 October 2015
Accepted 23 November 2015
Available online 11 December 2015

Keywords:
Pseudomonas putida
Deep sea
Formaldehyde degradation
Transcriptome profiling
Methanol metabolism

ABSTRACT

Some strains belonging to *Pseudomonas putida* have been found to degrade formaldehyde, making them promising methylotrophic bacteria in the treatment of environmental formaldehyde. However, the pathway involved in formaldehyde degradation of *P. putida* has not been extensively investigated. *P. putida* IOFA1, a strain isolated from the deep-sea sediments of the Indian Ocean, was found to be extremely tolerant of formaldehyde (up to 10 mM), efficiently degrading formaldehyde. In this study, the transcriptome profiles of the IOFA1 strain at different stages of formaldehyde degradation were characterized. A total of 709 genes were found to be significantly altered during formaldehyde degradation. Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) analysis showed that methanol metabolism, protein synthesis, and material transportation were highly enriched during formaldehyde degradation. The expression pattern of key methanol metabolism genes, which may be responsible for formaldehyde degradation, was investigated further with the quantitative real-time chain reaction (qRT-PCR) method. Our results showed that the IOFA1 strain may adopt a metabolic strategy of formaldehyde degradation different from other reported methylotrophic microorganisms. Therefore, our study presents a novel insight into the mechanism of formaldehyde degradation, which may help in engineering *P. putida* strains with a greater ability of degrading formaldehyde.

1. Introduction

Formaldehyde is widely used in glues and resins in many industries, such as perfume, cosmetic, pharmaceutical, organic synthesis, textile, and paper- and wood-manufacturing industries, or as an active ingredient in preservatives and disinfectants [1,2]. As reported, chemical industries can discharge as much as 10 g l^{-1} of formaldehyde in wastewater [3]. The widespread use of formaldehyde has been a public concern, due to its toxic effects on human health and the environment. Formaldehyde has been shown to damage DNA critically and thus cause mutation in mammalian cells and microorganisms [4]. High levels of formaldehyde in the environment can trigger a series of symptoms including coryza, pharyngitis, headache, emphysema, nausea, and lung cancer [5]. Therefore, a suitable approach to control and prevent formaldehyde pollution in the environment is urgently required.

In general, three primary approaches are currently used to purify formaldehyde-polluted air: (1) physical absorption of formaldehyde with activated carbon or potassium permanganate filters [6], (2) chemical oxidation of formaldehyde by TiO_2 or ferrous ions while being irradiated with ultraviolet (UV) lights [7], and (3) biodegradation of formaldehyde with naturally occurring microorganisms [8,9]. For the first method, the absorbed formaldehyde may return to the environment when the adsorbent reaches saturation. The major disadvantages of the second method are its high cost and requirement for UV lights to treat formaldehyde, which may limit its wide use in the degradation of organic contaminants. The third method can efficiently and moderately degrade formaldehyde under various conditions at a low cost; therefore, it is a promising approach to rapidly decontaminate and eliminate formaldehyde from the environment [10].

To date, considerable advances have been made to apply methylotrophic microorganisms for formaldehyde degradation. Increasingly more microorganisms have been found to degrade formaldehyde, including *Pseudomonas* [10], *Bacillus* [11], fungus [12], *Mycobacterium* [13], *Rhodococcus* [14], and *Acetobacter* [15]. In spite of its microbicidal property, formaldehyde can be assimilated by methylotrophic microorganisms from the

* Corresponding author at: Third Institute of Oceanography, SOA, No. 184 Daxue Road, Siming District, Xiamen 361005, China. Fax: +86 592 2195323.
E-mail address: zeng@tio.org.cn (R. Zeng).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.procbio.2015.11.036
1359-5113/

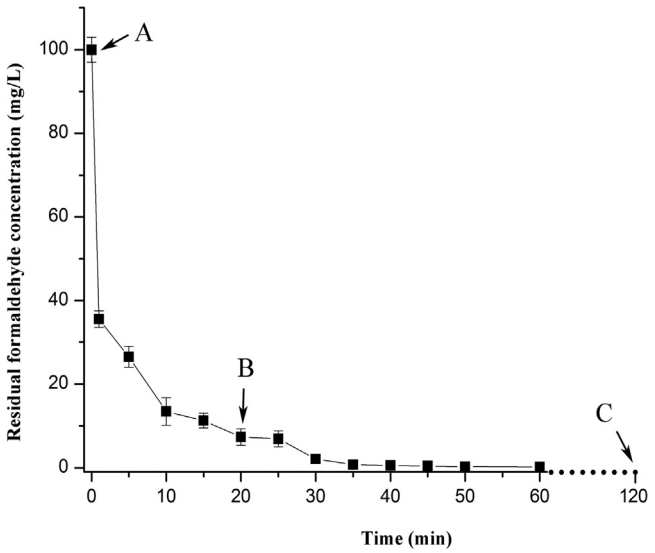

Fig. 1. Formaldehyde degradation profile of *P. putida* IOFA1.
The residual formaldehyde concentration was monitored at different intervals post IOFA1 strain addition. The samples collected for transcriptome profiling are indicated with arrows.

environment as the sole source of energy and carbon, and it may serve as a key metabolic intermediate in the methanol oxidation pathway in these microorganisms [16]. Conversely, other formaldehyde-resistant microorganisms grow in formaldehyde-polluted environments by catalyzing formaldehyde degradation with such enzymes as formaldehyde dismutase and formaldehyde dehydrogenase, instead of assimilating or dissimilating formaldehyde [17].

In this study, *Pseudomonas putida* IOFA1, a formaldehyde-degrading bacterium isolated from the deep-sea sediments of the Indian Ocean, was shown to degrade 100 mg l^{-1} of formaldehyde efficiently within 40 min (Fig. 1). Although several key enzymes in other *P. putida* strains including FM have been extensively characterized, the pathway of formaldehyde degradation in *P. putida* is not fully understood. Therefore, the pathway of formaldehyde degradation must be investigated further as a theoretical basis for producing a *Pseudomonas* strain with a greater formaldehyde-degrading ability.

2. Materials and methods

2.1. Bacterial strain and profile of formaldehyde degradation

P. putida IOFA1 was isolated from deep-sea sediments of the Indian Ocean and deposited at the China Center for Type Culture Collection (accession number: CCTCC NO. M2010280). Notably, when grown in LB media at 37 °C, *P. putida* IOFA1 efficiently degraded exogenous formaldehyde (Fig. 1). For the characterization of the IOFA1 formaldehyde degradation profile, formaldehyde was added to the IOFA1 culture (grown in Luria–Bertani (LB) media, OD_{600} = 2) with a final concentration of 100 mg l^{-1}. At different intervals post formaldehyde addition (0, 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, and 60 min post addition), the residual concentrations of the supernatant formaldehyde were measured using acetylacetone, according to the method developed by T Nash [18].

2.1.1. Characterization and identification of IOFA1 strain

The IOFA1 strain was identified as *P. putida* based on morphological and biochemical characteristics according to the methods described by Kersters et al. [19], and on the basis of the 16S rRNA gene sequence analysis. The 16S rRNA gene of IOFA1 was amplified by polymerase chain reaction using the forward primer 5′-AGTTTGATCCTGGCTCAG and the reverse primer 5′-GGCTTACCTTGTTACGACTT. Then the sequence was compared with 16S rRNAs of other bacteria using the BLAST program.

2.2. RNA isolation, complementary DNA library construction, and RNA-Seq

For transcriptome profiling, IOFA1 cells were collected in triplicate at different stages of formaldehyde degradation, that is, at the onset of degradation (0 min post formaldehyde addition, designated as sample A), during the process of degradation (20 min post addition, designated as sample B), and at the end of degradation (120 min post addition, designated as sample C), respectively (Fig. 1). For each harvested bacterial sample, the total RNA was extracted using 1 ml of TRIzol reagent (Promega) with DNase I digestion (Takara), according to the manufacturer's instructions. The RNA integrity was verified with 1% (m/v) agarose gel electrophoresis, and the amount and purity of RNA were determined spectrophotometrically by absorption at the wavelength of 260 and 280 nm on NanoDrop2000 (Thermo). After the total RNA was selected for messenger RNA (mRNA) using a Ribo-Zero Magnetic kit (Epicentre), 5 μg of mRNA was fragmented using a RNA fragmentation kit (Ambion) into RNA fragments with lengths ranging from 60 to 200 nts. Complementary DNA (cDNA) was synthesized from fragmented mRNA with a Superscript III kit (Invitrogen) using random primers. The paired-end Illumina sequencing libraries with adaptors were prepared using a TruSeq RNA sample preparation kit (Illumina), according to the manufacturer's instructions. Each sample was sequenced in a single lane on an Illumina Hiseq2000 sequencing platform.

2.3. RNA-Seq data analysis

The original RNA-Seq image data were converted into FASTQ sequence data for base calling. After the low-confidence reads were filtered with quality control steps, the high-quality data were mapped to the genome of *P. putida* IOFA1 using Bowtie2 software, and ab initio assembly was performed with Cufflinks [20]. The variance-analysis tool DEseq was used to identify differentially expressed genes from the high-throughput sequencing data [21]. Genes with *P*-value <0.05 and |log 2 (fold change)| >2 in a between-group pair-wise comparison were regarded as significantly differential genes.

The Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment table of differentially expressed genes was created by GeneMerge to determine the enriched pathway during formaldehyde degradation [22,23]. The enrichment cutoff (e-score) was set to 0.05. Gene Ontology (GO) analysis was conducted using GOatool to annotate and categorize the differentially expressed genes under three different function levels, that is, biological process, cell components, and molecular function [24].

2.4. Quantitative real-time polymerase chain reaction

To quantify the transcription of the formate transporter gene as well as the key genes involved in methane metabolism, real-time polymerase chain reaction (PCR) was performed using Power SYBR Green Master Mix (Applied Biosystems), according to the customer manual. DNA-free total RNA was extracted from IOFA1 cells collected at different intervals post formaldehyde addition (0, 5, 10, 20, 45, 60, and 120 min post addition). cDNA was synthesized from total RNA using Superscript III (Invitrogen) with random primers, which then served as templates for the amplification of real time