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Biodegradation of Phenazine-1-Carboxylic Acid by Rhodanobacter sp. PCA2 Proceeds via Decarboxylation and Cleavage of Nitrogen-Containing Ring

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Abstract : Phenazines are a large class of nitrogen-containing aromatic heterocyclic compounds, which are almost exclusively produced by bacteria from diverse genera including Pseudomonas and Streptomyces. Phenazine-1-carboxylic acid (PCA) as one of 'core' phenazines are converted from chorismic acid before modified to other phenazine derivatives in different cells. Phenazines have attracted enormous interests because of their multiple roles on biocontrol, bacterial interaction, biofilm formation and fitness of their producers. However, in spite of ecological importance, degradation as a part of phenazines' fate only have extremely limited attention now. Here, to isolate PCA-degrading bacteria, 200 mg L-1 PCA was supplied as sole carbon, nitrogen and energy source in minimal mineral medium. Quantitative PCR and Reverse-transcript PCR were employed to study abundance and activity of functional gene MFORT 16269 in PCA degradation, respectively. Intermediates and products of PCA degradation were identified with LC-MS/MS. After enrichment and isolation, a PCA-degrading strain was selected from soil and was designated as Rhodanobacter sp. PCA2 based on full 16S rRNA sequencing. As determined by HPLC, strain PCA2 consumed 200 mg L-1 (836 μM) PCA at a rate of 17.4 μM h-1, accompanying with significant cells yield from 1.92 × 105 to 3.11 × 106 cells per mL. Strain PCA2 was capable of degrading other phenazines as well, including phenazine (4.27 μM h-1), pyocyanin (2.72 μM h-1), neutral red (1.30 μM h-1) and 1-hydroxyphenazine (0.55 μM h-1). Moreover, during the incubation, transcript copies of MFORT 16269 gene increased significantly from 2.13 × 106 to 8.82 × 107 copies mL-1, which was 2.77 times faster than that of the corresponding gene copy number (2.20 × 106 to 3.32 × 107 copies mL-1), indicating that MFORT 16269 gene was activated and played roles on PCA degradation. As analyzed by LC-MS/MS, decarboxylation from the ring structure was determined as the first step of PCA degradation, followed by cleavage of nitrogen-containing ring by dioxygenase which catalyzed phenazine to nitrosobenzene. Subsequently, phenylhydroxylamine was detected after incubation for two days and was then transferred to aniline and catechol. Additionally, genomic and proteomic analyses were also carried out for strain PCA2. Overall, the findings presented here showed that a newly isolated strain Rhodanobacter sp. PCA2 was capable of degrading phenazines through decarboxylation and cleavage of nitrogen-containing ring, during which MFORT 16269 gene was activated and played important roles.

Keywords : decarboxylation, MFORT16269 gene, phenazine-1-carboxylic acid degradation, Rhodanobacter sp. PCA2
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