Degradation pathways of anaerobic benzene-degrading bacteria

Chiho SUZUKI-MINAKUCHI

Biotechnology Research Center, The University of Tokyo

Research aims

Benzene is a carcinogenic compound, often found in gasoline-contaminated groundwater. Under aerobic conditions, benzene can be biodegraded in a short period of time. However, contamination of subsurface aquifers with benzene often results in the development of anaerobic zones. Thus, isolation and analysis of anaerobic benzene-degrading bacteria are necessary for effective bioaugmentation of benzene-contaminated groundwater.

Azoarcus sp. strain DN11 is a denitrifying bacterium that is capable of benzene degradation under aerobic and anaerobic conditions[1)]. Because the use of DN11 has been approved by both the Ministry of the Environment and the Ministry of Economy, Trade and Industry, DN11 can be used for environmental bioaugmentation in Japan. However, DN11 sometimes loses its benzene-degrading ability during cultivation. To solve this problem, the genes responsible for benzene degradation must be identified. The objective of this study was to clarify the anaerobic benzene degradation pathway of *Azoarcus* sp. strain DN11.

Methods

Three anaerobic benzene degradation pathways have been proposed: (i) an anaerobic hydroxylation of benzene, yielding phenol, (ii) a Friedel-Crafts-type methylation of benzene, yielding toluene, and (iii) a carboxylation of benzene, yielding benzoate[2)], although no initial activation enzyme has been identified. Previously, Zhang and colleagues found that *Geobacter metallireducens* can degrade toluene, phenol, and benzoate—and under anaerobic conditions, it can also degrade benzene via phenol[3)]. To

determine the genes (in the draft genome of DN11) responsible for anaerobic benzene degradation, we used the amino acid sequences of BssA (benzylsuccinate synthase, from the anaerobic toluene degradation pathway), PpsA (phenylphosphate synthase, from the anaerobic phenol degradation pathway), and BamY (benzoate coenzyme A ligase, from the anaerobic benzoate degradation pathway) of *G. metallireducens* as query molecules. Primers were constructed for the corresponding homologous genes in DN11 to check the transcription levels of those genes under anaerobic benzene-degrading conditions.

The anaerobic benzene degradation assay was performed in an anaerobic chamber containing 5% hydrogen in nitrogen. Minimal medium containing 15 μM benzene as the sole carbon source was used, and the concentration of benzene in the medium was measured using gas chromatography-mass spectrometry (GC-MS). When benzene degradation was detected, cells were harvested, RNA was extracted, and cDNA was synthesized. Reverse transcription (RT)-PCR was performed using the primers described above.

Results

When DN11 was grown under aerobic conditions, in minimal medium containing 15 μM benzene as the sole carbon source, 50–60% of the benzene was degraded, and the medium environment became anaerobic within 24 h. Because small amounts of oxygen contaminate the medium even under the anaerobic conditions, DN11 initially degrades benzene using oxygen, following which anaerobic benzene degradation occurs. Under the anaerobic conditions, the amount of benzene decreased slowly when DN11 was added to the medium (Fig. 1). We extracted RNA from the cells incubated in the anaerobic chamber for 16 d.

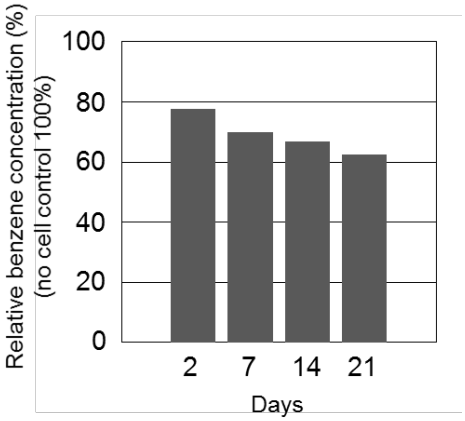

Fig. 1. Degradation of benzene by DN11 under anaerobic conditions.

DN11 was grown under anaerobic conditions, in minimal medium containing 15 μM benzene as the sole carbon source. The amount of benzene in the headspace of each vial was measured using GC-MS. Each bar shows the average of triplicate measurements.

From the draft genome of DN11, the proteins ADN11_211, ADN11_33, and ADN11_73 were found to be homologous with the proteins encoded by *bssA*, *ppsA*, and *bamY* in *G. metallireducens* (with 71%, 77%, and 49% identity in the translated amino acid sequences, respectively). We performed RT-PCR to check the expression levels for ADN11_211, ADN11_33, and ADN11_73, and found that the expression levels for ADN11_211 and ADN11_73 were much higher than those for ADN11_33 (Fig. 2).

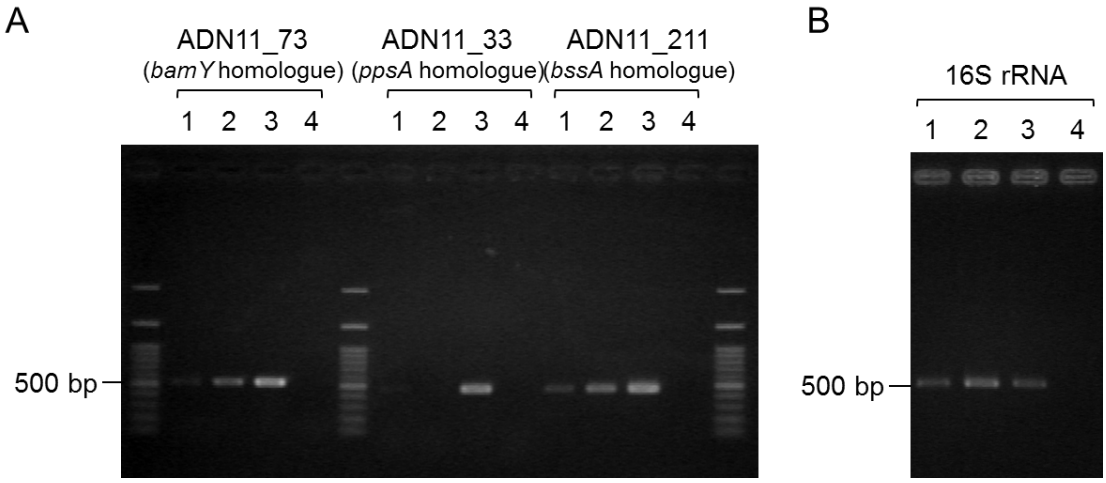

Fig. 2. Expression levels of ADN11_73, ADN11_33, and ADN11_211 under anaerobic benzene-degrading conditions.

RT-PCR amplification results for (A) ADN11_73, ADN11_33, and ADN11_211 mRNAs, and (B) 16S rRNA. Two independent cultures (indicated as “1” and “2”) were used for RNA extraction and cDNA synthesis. “3” indicates that genome DNA was used as a template. “4” indicates that no template was used.

Conclusion

DN11 is known to be capable of degrading toluene and benzoate, but not phenol, under anaerobic conditions[4)]. Our results suggest that DN11 degrades benzene via toluene and/or benzoate. Given that *G. metallireducens* degrades benzene via phenol under anaerobic conditions, the benzene degradation pathway used by DN11 should be different from that used by *G. metallireducens*. Further analyses will clarify the details of the benzene degradation pathway used by DN11 under anaerobic conditions.

References

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