How do the bacterial members interact with each other in a pyrene-degrading consortium?

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Research objective

Polycyclic aromatic hydrocarbons (PAHs) are persistent environmental pollutants comprising various fused aromatic rings with toxic, mutagenic, and carcinogenic properties. High molecular weight PAHs containing more than three aromatic rings, such as pyrene, have high chemical stability, hydrophobicity, and recalcitrancy. Bioremediation, a set of technologies that employ bacteria to degrade target pollutants, is a powerful tool for remediating PAH-polluted sites. In our previous work, we constructed a pyrene-degrading bacterial consortium composed of strains isolated from mangrove sediments from Thailand[1]. In this consortium, the strain *Mycolicibacterium* sp. PO1 contained all the genes for the complete mineralization of pyrene to TCA cycle intermediates. In contrast, *Novosphingobium pentaromativorans* PY1 and *Brucella ciceri* PW1 contained the genes necessary for the degradation of phthalate and protocatechuate, respectively (Fig. 1).

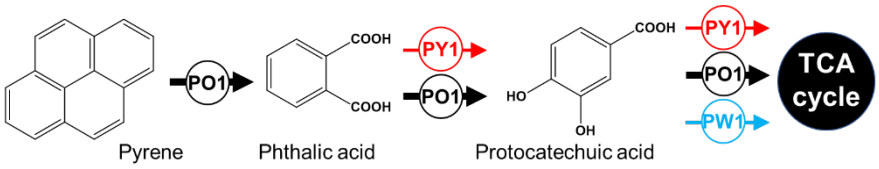

Fig. 1. Predicted flow of pyrene degradation metabolites among strains PO1, PY1 and PW1 (in circles) in our model consortium.

A consortium comprising these three strains was predicted to have enhanced pyrene degradation capability, as the flow of intermediates would be improved by synergy between different species capable of transforming them. However, such improvement was only observed when PO1 was combined with either one of the other two strains; the three-strain consortia exhibited the lowest degradation rate (Fig. 2). Using these two- and three-strain consortia, we aimed to identify factors that improve or worsen the degradation capability of degradative consortia with varying strain

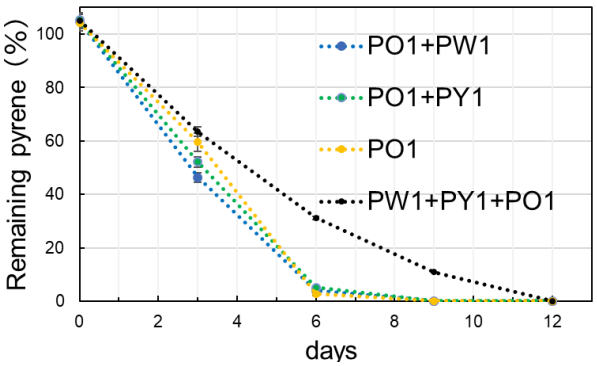

Fig. 2. Degradation of pyrene by single PO1 strain and two- or three-strain consortia. Cells were added at a concentration of 10^7 CFU/mL to a medium containing pyrene as sole carbon source.

compositions.

Methods

1. Transcriptomic analysis of different bacterial consortia

RNA sequencing was performed to evaluate how the transcriptome of each strain changed within the different consortia and affected their pyrene degradation rates. Cultures of axenic PO1, PO1 in individual combination with strains PY1 or PW1 (two-strain consortia), and with both strains simultaneously (three-strain consortium) were prepared at a cell concentration of ~10^7 CFU/mL each and exposed to pyrene as single carbon source. After three days of incubation, total RNA was extracted from the four cultures, and short-read sequencing was performed. For analysis, the RPKM values of each gene were calculated, and pairwise comparisons between the different consortia were performed. RPKM values of more than twice or less than half were considered upregulated or downregulated, respectively.

2. Analysis of metabolite flow at the single-cell level using Raman microspectroscopy

To trace the flow of metabolites during pyrene degradation within the different consortia, Raman microspectroscopy using deuterium-labeled pyrene (pyrene-d_{10}) was performed. This technique enables tracking the incorporation of a deuterium-labeled substrate into the biomolecules of individual cells when the carbon-deuterium (C-D) stretching vibration band appears in their Raman spectrum as hydrogen is replaced by deuterium. Here, axenic cultures of strains PO1, PY1, and PW1, as well as their combinations, were incubated with pyrene-d_{10} as the sole carbon source. Raman spectra of individual cells in the cultures were obtained at different time points using a Raman confocal microscope, and the number of cells in each strain exhibiting the C-D stretching band was quantified.

Results

1. Transcriptomic analysis of different bacterial consortia

Using the axenic PO1 culture for comparison, changes in PO1 gene expression levels were identified in the two- and three-strain consortia. From the previous draft genome sequence data[1], PO1 contained a total of 5574 genes. Of these, 51 and 1445 were upregulated and downregulated, respectively, in the three-strain consortium. In particular, 26 of the 30 pyrene-degradative genes were downregulated (Fig. 3, red dots). Downregulation of these degradative genes may determine the delayed pyrene degradation observed in the three-strain consortium (Fig. 2).

However, 25 of these 30 genes were also downregulated

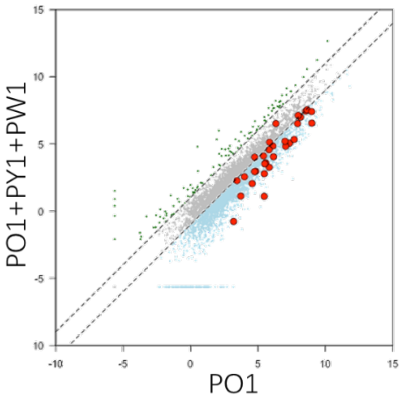

Fig. 3. Log$_2$ of RPKM values of PO1 genes in the three-strain consortium vs the axenic culture. RPKM values of twice and half the amount are shown in green and blue dots, respectively, whereas pyrene-degradative genes are shown in red.

in the PO1+PY1 consortium, whereas none of them showed significantly changed expression in the PO1+PW1 consortium, even though the pyrene-degradation rates of both consortia were enhanced. This and the fact that, in the three-strain consortia, downregulated genes also included genes related to ribosome formation, protein synthesis, and amino acid metabolism, suggest that changes in the expression of genes other than those directly associated with pyrene degradation exert an effect on the overall pyrene transformation capability of strain PO1.

2. Analysis of metabolite flow at the single-cell level using Raman microspectroscopy

Raman microspectroscopy results of PO1, PW1, and the PO1 + PW1 consortium are described in this section. PO1 cells exhibit carotenoid resonance Raman bands at 1155 and 1514 cm^{-1}. These carotenoid Raman bands were used to distinguish between PO1 and PW1 cells in the consortium when observed under the microscope. When fed pyrene-d_{10}, the individual PO1 strain displayed a C-D stretching vibration band at 2160 cm^{-1}, indicating deuterium incorporation. This band was not observed in PW1 when incubated individually with the same substrate. However, in the two-strain consortium, after 9 days of incubation, the C-D band was observed in approximately 5% of the examined PW1 cells, indicating deuterium incorporation from pyrene degradation metabolites released by PO1 strain. Currently, fine-tuning of the conditions to discriminate PY1 cells within the consortia using its Raman spectrum is being performed.

Conclusion

In this study, model pyrene-degrading consortia were analyzed using transcriptomics and Raman microspectroscopy to unveil the mechanisms involved in the enhancement or hindrance of their degradation capabilities. In the case of the consortium composed of strains PO1 and PW1, Raman spectrometric data suggested that the enhancement of its degradation capability was determined, in part, by strengthening the flow of metabolites within the consortium. However, the transcriptomic data of each strain combination indicated that the factors affecting the degradation capabilities were not limited to those involved in the expression of pyrene-degradative genes. Therefore, strain-specific metabolomic analyses or analyses of metabolites released into the culture media are necessary. The application of degradative bacteria to polluted sites has frequently been hindered by the inability of strains to sustainably display their degradation capabilities. A determining factor for this inability is the effect of the interactions between the degrader and the coexisting strains. By elucidating the causes of the negative effects caused by interspecies interactions, it will be possible to improve the application of degradative strains *in situ*.

References

1. Wanapaisan, P. *et al*. (2018). Synergistic degradation of pyrene by five culturable bacteria in a mangrove sediment-derived bacterial consortium. *J. Hazard. Mater.* **342:** 561-570.