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How do bacteria thrive the harmful effects of oxidative stress during pollutants degradation?

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Background

Bacteria are microscopic organisms that live everywhere: soil, fresh and salt water, plants and animal bodies. Some bacteria are aerobic, which means they need oxygen for respiration and growth. Although oxygen is essential for them, in aerobic environments cells form harmful molecules called reactive oxygen species (ROS) (Imlay *et al*., 2013). These molecules can damage bacterial cells by destroying important components like DNA, RNA and proteins. However, some microbes have ways of protecting themselves from this damage, allowing them to survive in difficult conditions (Méndez *et al*., 2022).

Paraburkholderia xenovorans LB400 (*P. xenovorans*) is a bacterium that was isolated from a polychlorobiphenyls (PCBs)-contaminated landfill in New York, United States. *P. xenovorans* is especially good at degrading toxic chemicals, including aromatic pollutants (Chain *et al*., 2006). However, when it processes certain substrates, it generates ROS, which can damage it. An aromatic hydrocarbon is an organic compound formed by carbon and hydrogen, where the carbon atoms are forming a ring, called "aromatic ring". Some aromatic compounds are pollutants because they are toxic, carcinogenic, and persistent in the environment.

Flavodoxins are electron shuttles that help cells to face the harmful effects of ROS Flavodoxins add electrons from its FMN (flavin mononucleotide) cofactor to oxidized compound to reduce it (Moyano *et al*., 2014). *P. xenovorans* has in its genome a gene encoding the protein called flavodoxin FldX1. Our team thinks FldX1 could help protect *P. xenovorans* from ROS while also improving its ability to break down pollutants.

Why does this matter?

Many industrial and environmental pollutants contain harmful chemicals that bacteria can help break down, naturally cleaning up polluted areas. If we can understand how bacteria manage these toxic substances, we might be able to enhance their ability to clean up pollution more efficiently.

The key questions

The study aimed to answer:

1. Do certain chemicals cause more stress to bacteria than others?
2. Can flavodoxin FldX1 help bacteria grow better when exposed to harmful chemicals?

3. Does this protein make the bacteria better at breaking down these chemicals?
4. How does the presence of this protein change what's happening inside the bacteria?

The study

Two aromatic compounds were the focus of this analysis: 3-HPA (hydroxyphenylacetate) and 4-HPA.i The difference between 3-HPA and 4- HPA is the position of the hydroxy (-OH) group on the aromatic ring. In 3- HPA, the -OH is attached to the third position, while in 4-HPA, it is at the fourth position. These chemicals are naturally found in the environment, such as soil and plants (lignin). *P. xenovorans* could use these substrates as food and energy to grow. Nevertheless, this metabolic process leads to ROS production. To study the effect of the flavodoxin FldX1 in *P. xenovorans* fitness, we used genetic engineering tools to make a bacterium that produces higher amounts of FldX1. In all the experiments, the native strain was used for comparisons.

Both strains were compared during exposure to/growth in 3-HPA and 4-HPA by measuring:

- How much ROS was produced.
- How fast the bacteria grew.
- How efficiently they degrade 3-HPA and 4-HPA.
- What changes took places as a molecular level.

Key results

- 4-HPA is more dangerous than 3-HPA: Bacteria exposed to 4-HPA produced more ROS than cells exposed to 3-HPA, meaning 4-HPA is more deleterious (higher ROS means more cell damage). This indicates that during the degradation of 4-HPA the cells face greater challenges and need stronger defense mechanisms, such as FldX1.

- Flavodoxin FldX1 improves bacterial growth and degradation capability: The bacterium that produces higher amounts of FldX1 grew faster on both 3-HPA and 4-HPA compared to the native strain and degraded them faster. This suggests that FldX1 increase bacteria tolerances to ROS.

- Flavodoxin reduces the need for stress-response proteins: When exposed to 3-HPA and 4-HPA, bacteria normally express several proteins that help to face the damage. Nevertheless, the FldX1-producing bacteria had a lower amount of stress-related proteins activated, indicating that flavodoxin alleviates the damage, making other stress responses less necessary.

- Bacteria use multiple pathways to degrade these aromatic compounds: The assay also confirmed *P. xenovorans* uses different catabolic pathways to break down 3-HPA and 4-HPA. These pathways involve specific enzymes that transform the compounds into intermediates that could be less harmful, allowing the stain to use them as carbon and energy source. For example, a previous study indicates that 3-HPA and 4-HPA are broken down through the homogentisate and the homoprotocatechuate catabolic pathways in this bacterium (Mendez et al., 2011), but our analysis suggests that 3-HPA and 4-HPA are also degraded *via* phenylacetate pathway.||

Why is this important?

These findings suggest that flavodoxin FldX1 could be used to improve the capability of the bacterium to clean up environmental pollutants, particularly those found in industrial waste, in a process called bioremediation.

Future applications

- **Bioremediation:** Bacteria with enhanced flavodoxin could be introduced into polluted environments to break down toxic substances more efficiently.
- **Industrial waste treatment:** Factories that produce harmful byproducts could use bacteria to process waste before releasing it into the environment.
- **Agriculture and wastewater treatment:** These bacteria could help degrade plant-based pollutants found in agricultural wastewater.

Conclusion

This study highlights how by just increasing the expression of native protein in bacteria, scientists could build stronger bacterial strains with powerful bioremediating skills. This could become an environmentally friendly alternative to treat polluted sites.

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