ID388 | Metabolic and Regulatory Responses of *Pseudomonas* under Oxygen-Limited Conditions

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Background: Pseudomonas species are frequently detected in anaerobic wastewater treatment systems, and their presence has been associated with the anaerobic biodegradation of lipids into methane. Their ability to thrive in oxygen-limited environments, combined with their capacity to degrade recalcitrant compounds, suggest a potential ecological role in these processes, particularly considering that strict anaerobic conditions are not always maintained in practice. Gaining a better understanding of their behavior and function is therefore essential for guiding future improvements in anaerobic digestion processes. This study aims to detect changes in gene expression by Pseudomonas species under low O2 concentrations.

Methods: Pseudomonas sp. strain I1, isolated from a micro-aerated bioreactor fed with fatty acids, was grown in sealed batch bottles using mineral medium and acetate (10 mM) as the only carbon source. The O2 levels in the headspace were adjusted to 0% (anaerobic control), 0.1%, 0.2%, 0.5%, 1%, 2%, and 21% (aerobic control). The experiment lasted around six hours. During this time, O2 and acetate concentrations were measured at 0, 3, and 6 h by chromatography. RNA was then extracted and sequenced. Gene expression analysis was performed using the MOSCA pipeline, comparing the expression levels under each condition to those at 21% O2.

Results: Significant gene expression changes were observed in oxidative stress response, TCA cycle, electron transport chain (ETC), and flagellar assembly. Under low-O2 conditions, oxidative stress genes were downregulated, with thioredoxin peroxidase achieving a 105-fold decreased at 0.2% On.Data suggest that acetate is assimilated via the ACS pathway to acetyl-CoA. Under 0.5–2% On, idh was repressed while aceA was upregulated, indicating a metabolic shift from the TCA cycle to the glyoxylate shunt. Under low O2 metabolism likely shifts to gluconeogenesis for storage compounds.

Pseudomonas spp. employ five oxygen reductases in the ETC. High-affinity types for oxygen (cbb3) and the bc1 complex were strongly upregulated under low On, while low-affinity reductases showed low expression. Flagellar assembly genes were downregulated below 0.2% On, suggesting reduced motility as energy shifts to stress survival.

Conclusions: In response to low On concentrations, Pseudomonas spp. downregulate genes involved in oxidative stress responses and motility. A metabolic shift from TCA to the glyoxylate shunt was observed.