Microbial single-cell RNA sequencing to investigate environmental triggers for ICEclc transfer competence activation in Pseudomonas

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Abstract

Horizontal gene transfer (HGT) is the process by which DNA is transferred from a donor cell to a recipient that is not part of its progeny. Mechanisms of HGT in prokaryotes include transformation, transduction, and conjugation; the latter of which transfers plasmids or chromosomally excised DNA like integrative and conjugative elements (ICEs) from a donor to a recipient in a cell-to-cell dependent contact. ICEs are widespread autonomous mobile DNA, containing the genes necessary for integration, excision, and the conjugative machinery. ICE*clc* of *Pseudomonas putida* is our experimental model and is characterized by the presence of the *clc* genes for chlorocatechol degradation. Its transfer is initiated from a subpopulation of cells (3-5%) that become transfer competent (tc). The proportion of tc cells is the highest when cultures have been grown on 3-chlorobenzoate (3CBA) as a sole carbon source and when they enter stationary phase. However, the link between growth on 3CBA and ICE*clc* transfer competence activation is unknown. Our aim is to better understand the influence of environmental factors on ICE*clc* activation, assuming that growth on 3CBA induces metabolic pathways in *Pseudomonas* which relate to tc cell formation. To identify such potential pathways, we use microbial single-cell RNA sequencing (scRNAseq) and quantify differences in transcript abundances in single cells growing on different carbon sources. We were able to differentiate tc- from non-tc cells based on their transcriptional fingerprints, and further detected carbon source and growth phase signatures. Tc cell-specific expression signatures identified by scRNAseq will be followed up in genetic and reporter studies.

Keywords: Integrative conjugative elements, ICEclc, Pseudomonas, single cell RNA sequencing

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