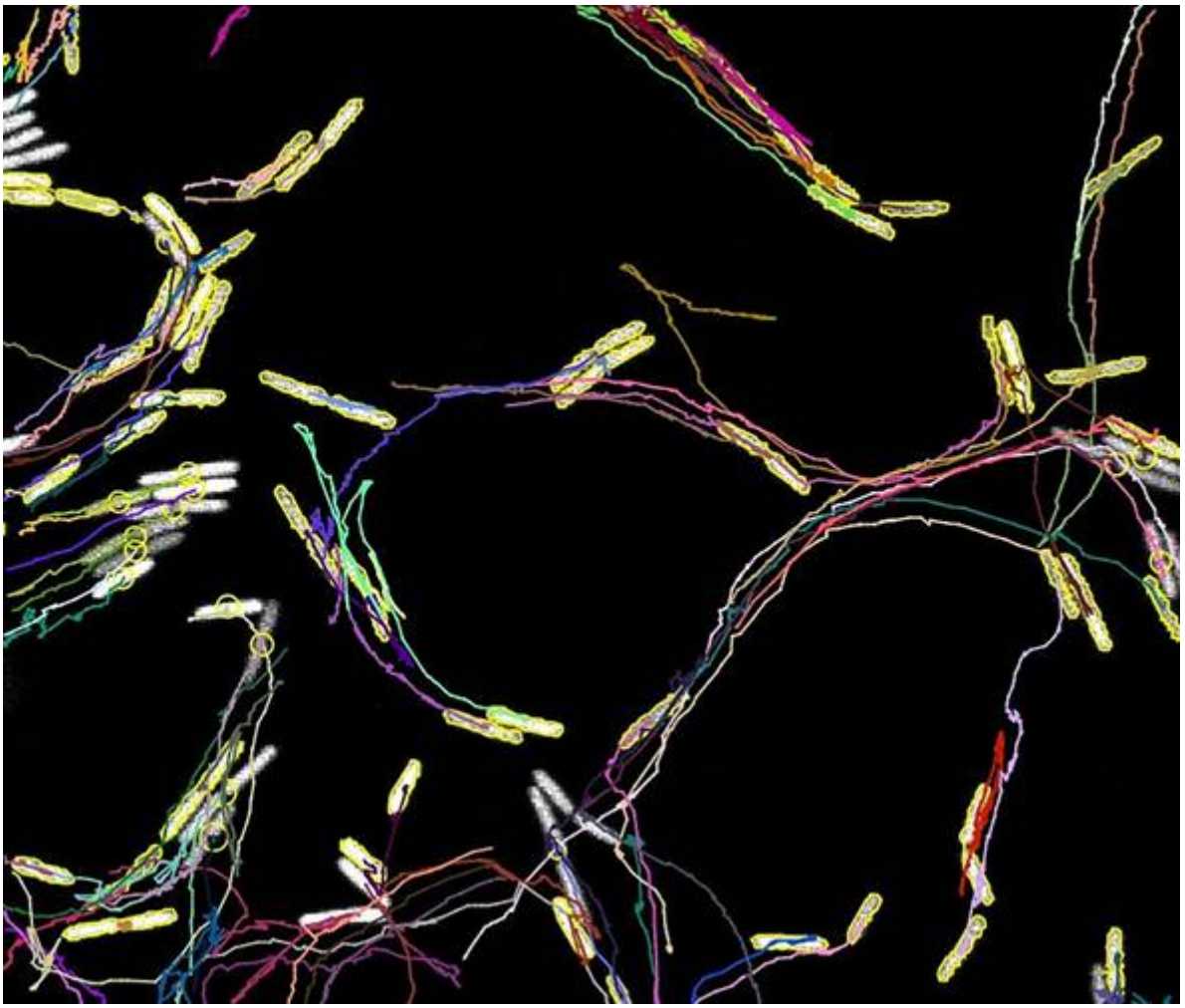
Sociedad Argentina de Microbiología General

XIX CONGRESO DE LA SOCIEDAD ARGENTINA DE MICROBIOLOGÍA GENERAL

22 al 25 de octubre del 2024
Centro cultural y Pabellón Argentina de la Universidad Nacional de Córdoba, Córdoba, ARGENTINA.



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POLYHYDROXYBUTYRATE (PHB) GRANULE-ASSOCIATED PROTEINS IN *Halomonas titanicae* KHS3

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Polyhydroxyalkanoates (PHAs) are biodegradable polymers produced by microorganisms under conditions of nutrient imbalance, frequently due to nitrogen limitation. To enhance the economic viability of PHA production, it is essential to develop more efficient PHA-producing strains and to gain a comprehensive understanding of the PHA biosynthetic pathways. PHAs accumulate in the cell, forming granules containing the PHA core and a layer of "PHA-granule-associated proteins" (PGAPs). Phasins, Pha synthases, and Pha depolymerases are central PHA metabolic proteins and are typically identified as PGAPs. The recent advancements in protein identification technologies have revealed that many other proteins, unrelated to PHA metabolism, might be interacting with these PHA granules in a specific way. In our research group, we are focused on studying polyhydroxybutyrate (PHB) using the environmental strain *Halomonas titanicae* KHS3 (HtKHS3), which can accumulate up to 50-60% (w/w) PHB using different carbon sources such as glucose, waste glycerol or phenanthrene. In the genomic sequence of HtKHS3, a complete set of genes potentially involved in PHAs metabolism was identified. As an initial exploratory approach to advance our understanding of PGAPs in HtKHS3, we conducted a proteomic analysis of cytosolic, membrane and PHA granules-enriched fractions in HtKHS3 cells grown under PHB accumulation conditions. Preparation of native PHA granules-enriched fractions was set up for HtKHS3 according to the protocol previously described in the bibliography for non-halophilic bacteria. Mass spectrometry analysis of the three fractions allowed us to identify a total of 602 proteins from the 4493 predicted proteins in the genomic sequence of HtKHS3. A comparative analysis of proteins present in cytosolic, membrane and PHA granules-enriched fractions identified 52 proteins out of the 602 detected proteins, which were found exclusively or at higher levels in the PHB enriched fraction. From these 52 putatively PGAPs, only 3 seemed to be reliably involved in PHB metabolism (a phasin, a 3-hydroxyalkanoate synthase and depolymerase). The finding of the 3-hydroxyalkanoate synthase was specially interesting since in the HtKHS3 genomic sequence it was predicted as hypothetical protein. However, several other proteins were predominantly or exclusively detected in HtKHS3 PHB granules, with no clear relationship to PHB metabolism. When compared with available literature for other bacteria, some of these proteins were also identified as putative PGAPs, such as the case of pyruvate dehydrogenase E1 component. Our preliminary proteomic analysis of

HtKHS3 has identified several proteins associated with PHB granules, some of which are likely involved in PHB metabolism, while others may not be directly related. To address the specificity of the identified PGAPs, in vivo subcellular localization of fluorescent PGAP fusion proteins and functional validation of HtKHS3 PGAPs will be carried out.

Palabras clave: Polyhydroxybutyrate - Halomonas - Bioplastic - Proteomics