Phenomic profiling of *Rhodopseudomonas palustris* carbon and nitrogen use towards single-stage photofermentation H_2 production

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Rhodopseudomonas palustris is a metabolically versatile purple non-sulfur bacterium with strong potential for biohydrogen production, especially when treating organic-rich wastewater. Its ability to grow under photoautotrophic, photoheterotrophic, chemoautotrophic, and chemoheterotrophic conditions allows it to use a wide variety of organic substrates. To enable large-scale applications, single-stage photofermentation can constitute an easier solution compared to the well known two-stage dark-photofermentation H_2 production processes. However, for single-stage processes to be developed, strain-specific metabolic capabilities must be thoroughly characterized in order to select the proper substrates, *i.e.* the substrates containing the necessary carbon and nitrogen sources to sustain *R. palustris* growth and H_2 production.

This study used Biolog Phenotype MicroArrays (PMs) to evaluate the metabolic profile of *R. palustris* 42OL. The PM technique enables high-throughput analysis of nutrient utilization by monitoring the reduction of a tetrazolium dye in 96-well plates. A total of 190 carbon sources and 95 nitrogen sources were tested under four metabolic conditions: anaerobic-dark (AnD), aerobic-dark (AeD), anaerobic-light (AnL), and aerobic-light (AeL). Cultures were prepared from 15-day-old colonies and incubated for 15 days under controlled light or oxygen conditions, with growth kinetics recorded by the OmniLog system.

The bacterium showed broad carbon utilization across all conditions, particularly for amides/amines, carboxylic acids, carbohydrates, and esters. Carboxylic acids were the preferred substrates in AeL, AeD, and AnL, while carbohydrates dominated in AnD. Some substrate groups, such as alcohols and amino acids, were not used in AeL. The AnL (photoheterotrophic) condition supported the widest range of carbon metabolism, consistent with optimal growth under light and anaerobiosis.

Nitrogen utilization patterns varied: AnL data could not be interpreted due to nitrogen fixation via nitrogenase. Amino acids and inorganic nitrogen sources were consistently used across AeL, AeD, and AnD, with peptides mainly supporting growth in AnD and AeD.

Overall, the results confirm the metabolic flexibility of *R. palustris* 42OL and demonstrate the usefulness of PM technology for rapidly assessing microbial phenotypes relevant to biohydrogen production.

This research was partially funded by Ministero dell'ambiente e della sicurezza energetica (MASE), Italy, Project SPIGA "Sviluppo di una piattaforma di Produzione di Idrogeno Green mediAnte sistemi innovativi, UGOV RSH2A_000025_SPIGA_PNRRM2.C2.I3.5".