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GC-MS-Based Untargeted Metabolomics to Study the Metabolism of Pectobacterium Strains

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Abstract : Pectobacterium spp. were previously classified into the Erwinia genus founded in 1917 to unite at that time all Gram-negative, fermentative, nonsporulating and peritrichous flagellated plant pathogenic bacteria. After work of Waldee (1945), on Approved Lists of Bacterial Names and bacteriology manuals in 1980, they were described either under the species named Erwinia or Pectobacterium. The Pectobacterium genus was formally described in 1998 of 265 Pectobacterium strains. Currently, there are 21 species of Pectobacterium bacteria, including Pectobacterium betavasculorum since 2003, which caused soft rot on sugar beet tubers. Based on the biochemical experiments carried out for this, it is known that these bacteria are gram-negative, catalase-positive, oxidase-negative, facultatively anaerobic, using gelatin and causing symptoms of soft rot on potato and sugar beet tubers. The mere fact of growing on sugar beet may indicate a metabolism characteristic only for this species. Metabolomics, broadly defined as the biology of the metabolic systems, which allows to make comprehensive measurements of metabolites. Metabolomics, in combination with genomics, are complementary tools for the identification of metabolites and their reactions, and thus for the reconstruction of metabolic networks. The aim of this study was to apply the GC-MS-based untargeted metabolomics to study the metabolism of P. betavasculorum in different growing conditions. The metabolomic profiles of biomass and biomass media were determined. For sample preparation the following protocol was used: extraction with 900 µl of methanol: chloroform: water mixture (10: 3: 1, v: v) were added to 900 µl of biomass from the bottom of the tube and up to 900 µl of nutrient medium from the bacterial biomass. After centrifugation (13,000 x g, 15 min, 4oC), 300µL of the obtained supernatants were concentrated by rotary vacuum and evaporated to dryness. Afterwards, two-step derivatization procedure was performed before GC-MS analyses. The obtained results were subjected to statistical calculations with the use of both uni- and multivariate tests. The obtained results were evaluated using KEGG database, to asses which metabolic pathways are activated and which genes are responsible for it, during the metabolism of given substrates contained in the growing environment. The observed metabolic changes, combined with biochemical and physiological tests, may enable pathway discovery, regulatory inference and understanding of the homeostatic abilities of P. betavasculorum.

Keywords : GC-MS chromatograpfy, metabolomics, metabolism, pectobacterium strains, pectobacterium betavasculorum
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