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PHENOTYPIC AND GENOTYPIC FEATURES OF SPONTANEOUS VARIANTS FROM THE PLANT BIOSTIMULANT *Pseudomonas pergaminensis* 1008T EMERGED DURING LONG-TERM INOCULANT STORAGE

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Pseudomonas pergaminensis 1008T (hereafter Pp1008) is a wheat rhizospheric isolate commercialized as the active ingredient of liquid suspensions for crop seed treatment. Pp1008 increases 8% on average the crop yield of wheat in the field when applied to seeds before sowing. During long-term shelf storage of the commercial formulation, three different colony morphotypes were isolated and confirmed by 16S sequencing to be stable variants derived from the original wild type strain Pp1008. Here, we present the physiological and genomic characterization of these three emerged stable phenotypic variants (namely, PV1, PV2 and PV3) in comparison with the parent Pp1008. PV1 colonies were more translucent and expanded faster than the wild type; PV2 colonies were characterized by a wrinkly surface, whereas PV3 colonies were highly mucoid. All variants were studied in terms of growth under different carbon sources, autoaggregation, motility in semisolid medium, cellular features under the electron microscope, production of extracellular compounds (surfactants, pyoverdine, auxin, protease, phospholipase), biofilm formation, phosphate solubilization from CaHPO₄, and functionality of the Gac-Rsm global regulatory cascade. Genome sequencing revealed that PV1 had 35 nucleotide replacements including loss-of-function mutations in genes encoding the DNA mismatch repair subunit MutL and the global post-transcriptional regulatory sensor kinase GacS. PV2, instead, had a single and specific internal 16-bp deletion in the *wspF* gene, which encodes a reported regulator of cellulose production, swimming motility and biofilm formation. Finally, PV3 showed a nonsense point mutation affecting the mucoidy regulatory gene *mucA*. In all cases, the identified mutations could explain most of the distinctive phenotypic features of each spontaneous variant. The emergence of these stable phenotypic variants may represent an adaptative pathway to persist under the long-term storage conditions of the liquid formulation. It would be interesting to understand the impact of these genotypic and phenotypic variations in the capacity of the variants to colonize the roots of bacterized seeds and to promote plant growth yield.

Palabras clave: *Pseudomonas pergaminensis* - phenotypic variants –
phenotyping - genome sequencing