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Comparative genomics and functional analysis of *Lactobacillus plantarum* probiotic candidates highlighted a strain-dependent capability to produce butyric acid by fatty acid biosynthesis pathway

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The genome-scale analysis of health-promoting bacteria is a fundamental approach to investigate their physiological behaviour or foresee potential probiotic and postbiotic features. Among the intensively studied LAB species, *Lactobacillus* (*L*.) *plantarum* is one of the most characterized, since it is a natural inhabitant of human gut with a wide genome rich of probiotic traits. The production of butyric acid is a metabolic activity often sought in new probiotic candidates, but the investigations of this pathway in *L. plantarum* species are few and limited to physiological observations. Therefore, the aim of the present work was to study the genomes of three *L. plantarum* strains (S2, S11 and O2) in order to understand the genetic determinants of their probiotic features. In particular, *L. plantarum* S2 and S11, but not O2, were previously shown to produce butyric acid and to inhibit the colonisation of *Listeria monocytogenes* in human gut models.

The three genomes were *de novo* assembled and reconstructed with the Mauve suite. Subsequently, they were structurally annotated with the Prokka platform, functionally annotated with Interproscan 5 suite, and compared with OrthoMCL software. An analysis of SNPs was performed and functional damages were predicted for genes of interest and their regulators. The assumptions generated from the *in silico* reconstruction and analysis were validated with targeted physiologic tests, such as the investigation of growth dynamics in different substrates and consequent production of short chain fatty acids (SCFAs).

A pool of unique gene families was shared by S2 and S11 whereas it was not present in the genome of O2, which confirmed to be phylogenetically different from the other two. Among these common genes a highly conserved region of plantaricin operons was detected. However, the reconstruction of this genomic region highlighted functional damaging and therefore the inability to actually produce plantaricins; such inability was further confirmed *in vitro*. Concerning the butyric acid, we observed that production was maximal after 48 hours of incubation (4 mM) and exerted only in presence of glucose. Interestingly, the strain O2 did not produce significant amounts of butyrate and showed a lower capability to consume glucose. Genes associated with terminal steps of common bacterial butanoate pathways were not observed in the three LAB genomes. However, we identified a type II fatty acid synthase (FASII) gene as the gene most likely

responsible for the butyric acid production in S2 and S11 strains; a search in O2 genome, for SNPs present in FASII pathway and glucose transport system revealed some functional damages.

In light of the results achieved, the inhibition of *Listeria monocytogenes* by S2 and S11 was mainly related to the production of butyric acid. This metabolic activity was correlated to the complexity and amount of sugar and nitrogen sources available, whereas the functional SNPs observed in the genome of strain O2 could explain its inability to produce butyric acid. To conclude, this study may represent a first step for understanding the complexity of the butyrate biosynthesis pathway in *L. plantarum* and provides the bases for guiding further transcriptomic investigations.