

Characterization of the probiotic and stress resistance-related genes of *Lactococcus lactis* subsp. *lactis* NCDO 2118 through comparative genomics and *in vitro* assays

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Lactococcus lactis is highly important for its use in the production of many fermented products and macromolecules and its application in health improvement once it is a Generally Regarded As Safe (GRAS) species. In this scenario, *Lactococcus lactis* subsp. *lactis* NCDO 2118 (herein, NCDO2118) stands out as a xylose fermenter and GABA (gamma-aminobutyric acid) producer strain isolated from frozen peas. However, despite of those important characteristics, few is known about the mechanisms involved in probiotic effects of this strain. In this work, we have sequenced and manually curated the genome of NCDO 2118. Besides, we have compared the genome of NCDO 2118 with those of 7 additional *L. lactis* subsp. *lactis*, 6 *Lactococcus lactis* subsp. *cremoris* and 2 *Lactococcus garvieae* strains. We used the software Gegenees, Mauve and BRIG to perform phylogenomics, gene synteny and circular genome comparisons between the above mentioned species. Additionally, we have used GIPSY and Phast to predict genomic islands (GEIs) and phages, respectively. In phylogenomics analyses, a high similarity was observed between NCDO 2118 and *L. lactis* subsp. *lactis* KF147 (herein, KF147), both isolated from plants. Besides, the gene synteny analyses have clearly shown a highly conserved gene order between both strains. We have also predicted 9 genomic (GEI), 5 metabolic (MI), 4 symbiotic (SI) and 3 miscellaneous islands (MSI - region harbouring metabolic and symbiotic factors). The most prominent genomic island is MSI 2, which presents the biggest region of deletion in all *Lactococcus*, except for NCDO 2118 and KF147. We have also identified five phage regions, 3 bacteriocins (from classes I, II and III), 25 acidic and 14 bile stress resistance genes. Finally, although the strain is resistant to Vancomycin, Oxacillin and Amikacin *in vitro*, no antibiotic resistance related gene was identified in putatively horizontally acquired regions. Altogether, the high degree of similarity between all strains point that the SIs commonly shared by both NCDO 2118 and KF147 were responsible for the close relationship in phylogenomic analyses and probably for the adaptation of those strains to plants. The MIs, on the other hand, are highly conserved between all strains, which is an expected feature given the use of those strains in metabolic processes in industry. Finally, the three classes of bacteriocins may have an important role against invasion of competing strains or influencing the host immune system, which may be involved in the probiotic characteristic of this strain.

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