



Simposio Argentino de Jóvenes Investigadores en Bioinformática



LIBRO DE RESÚMENES

5 y 6 de Octubre
FBQyF-UNT

Tucumán - Argentina
Año 2022

Libro de Resúmenes de la 7ma edición del Simposio Argentino de Jóvenes Investigadores en Bioinformática (7SAJIB)

<https://rsg-argentina.netlify.app/conferences/sajib2022/>
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Diseño y edición: Carla Luciana Padilla Franzotti

Iniciativa del Comité Directivo del [RSG Argentina](#)
Período 2021-2022.



Phenotypic and genotypic evaluation of wakame assimilation ability in *Ligilactobacillus salivarius* strains isolated from wakame fed pigs.

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Wakame contains some bioactive components including fucoidan, alginic acid, omega 3 fatty acids, vitamins, and minerals, which has been reported to have beneficial effects in the human host. Among the beneficial effects attributed to wakame are its antibacterial, antioxidant and anti-inflammatory functions. Recently, we demonstrated that feeding pigs with wakame improved gastrointestinal immunity and induced a significant increase in the abundance of members of the Lactobacillus family, specially *Ligilactobacillus salivarius*. To determine whether the beneficial immunological effects of wakame were associated to the increment of *L. salivarius* strains, we isolated and characterized strains from wakame-fed pigs belonging to this species. The complete genome of eight selected *L. salivarius* strains (named FFIG) were sequenced and the functional and genomic characterization of those strains revealed that their immunomodulatory and adhesion capabilities are a strain-specific characteristic. In this work we aimed to further characterize the *L. salivarius* FFIG strains studying their wakame assimilation abilities by phenotypic and genotypic approaches. A prolonged fermentation experiment was designed to investigate the consumption of the saccharides by the FFIG strains in two different wakame broths (wakame leaf and wakame stalk). TLC analysis showed that the eight FFIG strains were able to utilize the saccharides contained in enzyme-treated wakame. The strains had different preferences for the two wakame broths. In wakame leaf, FFIG58, FFIG24 and FFIG63 were the strains with the highest ability to grow, whereas FFIG130 and FFIG79 were the strains with the lowest bacterial counts. In wakame stalk, the strains FFIG60, FFIG79 and FFIG130 stood out over the eight strains with the highest viable counts, whereas the FFIG58 have the lowest counts. It was reported that *L. salivarius* strains have different abundance of genes belonging to glycosyltransferases and glycosylhydrolases families, and that the set of enzymes determine the carbon sources that each strain can use for growing. Then, we evaluated the abundance of genes belonging to glycosylhydrolases families among the FFIG strains and compared them with other *L. salivarius* strains of animal origin. The clustering analysis considering the numbers and types of glycosylhydrolases showed that strains FFIG58, FFIG63, FFIG79 and FFIG124 had a significant higher abundance of enzymes from the families GH25 and GH13, when compared with all the other *L. salivarius* strains. Finally, we performed comparative genomic studies between the FFIG strains and other strains isolated from human and porcine origins. We found a core genome compound of 710 genes, and there was no correlation between the number of unique genes and the difference in the phenotypes. To the best of our knowledge, this is the first study that evaluated wakame assimilation capacity of *L. salivarius* strains using phenotypic and genomic approaches.

KEY WORDS: *Ligilactobacillus salivarius* - Wakame assimilation, Pigs, Probiotics.