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Evaluación genómica, caracterización del potencial probiótico y antimicrobiano de *Lactiplantibacillus plantarum* y *Limosilactobacillus reuteri* aislados del estiércol de cerdo zungo costeño (*sus scrofa domesticus*)

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RESUMEN

La presente tesis doctoral abordó la creciente problemática de la resistencia antimicrobiana en la producción porcina, derivada del uso extensivo de antibióticos, y la consecuente necesidad de encontrar alternativas sostenibles. En este contexto, se evaluó el potencial probiótico y antimicrobiano de *Lactiplantibacillus plantarum* y *Limosilactobacillus reuteri*, bacterias ácido-lácticas (BAL) obtenidas de muestras de estiércol de cerdo zungo costeño (*Sus scrofa domesticus*), una raza criolla colombiana, buscando ofrecer una solución a esta problemática.

Se procedió al aislamiento y cultivo de las bacterias a partir de muestras de estiércol porcino, empleando agar MRS como medio selectivo. La identificación molecular de las cepas purificadas se realizó mediante PCR convencional y espectrometría de masas MALDI-TOF, técnicas que permitieron confirmar su identidad y caracterizar su perfil proteico. Se investigó *in vitro* su capacidad de resistir condiciones adversas, simulando el ambiente gastrointestinal: elevadas concentraciones de bilis y NaCl, temperaturas extremas y pH ácido, evaluando así su viabilidad como potenciales probióticos.

La caracterización de las bacteriocinas producidas, se llevo a cabo a través de métodos de ultrafiltración y precipitación química con sulfato de amonio. Se evaluó la interacción de estas bacterias con *E. coli* y *S. aureus*, patógenos comunes en la producción porcina, mediante ensayos de inhibición en placa, determinando así su potencial antimicrobiano. Se llevó a cabo un estudio genómico completo, utilizando secuenciación de nueva generación (NGS) y herramientas bioinformáticas para ensamblar, anotar y analizar los genomas de las cepas seleccionadas.

Dentro del análisis genómico, se determinó el pangenoma de *L. plantarum* HCA1, se realizó una búsqueda exhaustiva de genes de virulencia y resistencia a antibióticos, y se llevó a cabo un análisis filogenómico para establecer sus relaciones evolutivas. Se predijo la estructura tridimensional de las bacteriocinas identificadas, empleando el modelo computacional AlphaFold 3, para comprender mejor su mecanismo de acción.

Los resultados revelaron una notable adaptabilidad y resistencia de las cepas a las condiciones simuladas del tracto gastrointestinal, características esenciales para su viabilidad como probióticos. Se observó una considerable actividad antimicrobiana, atribuida a bacteriocinas estables y eficaces, entre ellas las plantaricinas de *L. plantarum*, cuyos genes clave fueron identificados y caracterizados. El estudio genómico confirmó la seguridad de las cepas, al no detectarse genes de virulencia ni de resistencia a antibióticos, y reveló un pangenoma abierto en *L. plantarum* HCA1, indicativo de diversidad genética y potencial de adaptación.

Estos hallazgos sugieren que las cepas de *L. plantarum* y *L. reuteri* estudiadas podrían constituir una alternativa probiótica y antimicrobiana a los antibióticos en la producción porcina. Las bacteriocinas, y en particular las plantaricinas, presentan propiedades que justifican una investigación más profunda para su potencial aplicación en la industria alimentaria y porcina, con el objetivo de contribuir a la producción de alimentos más seguros, una producción animal más sostenible y una mejora de la salud animal y humana. Se sientan así las bases para futuras investigaciones y el desarrollo de aplicaciones biotecnológicas de estas cepas, con un enfoque en la mejora de la salud y la producción animal.

Palabras clave:

Actividad antimicrobiana, Bacteria ácido-láctica, Industria porcina, Pangenoma abierto, Plantaricinas, Probióticos, Resistencia antimicrobiana

ABSTRACT

This doctoral thesis addressed the growing problem of antimicrobial resistance in swine production, stemming from the extensive use of antibiotics, and the consequent need to find sustainable alternatives. In this context, the probiotic and antimicrobial potential of *Lactiplantibacillus plantarum* and *Limosilactobacillus reuteri*, lactic acid bacteria (LAB) obtained from fecal samples of zungo costeño pigs (*Sus scrofa domesticus*), a Colombian Creole breed, was evaluated, seeking to offer a solution to this problem.

The isolation and cultivation of bacteria from porcine fecal samples were carried out using MRS agar as a selective medium. Molecular identification of the purified strains was performed by conventional PCR and MALDI-TOF mass spectrometry, techniques that allowed confirmation of their identity and characterization of their protein profile. Their ability to withstand adverse conditions, simulating the gastrointestinal environment (high concentrations of bile and NaCl, extreme temperatures, and acidic pH), was investigated *in vitro*, thus evaluating their viability as potential probiotics.

The characterization of the produced bacteriocins was carried out through ultrafiltration and chemical precipitation methods with ammonium sulfate. The interaction of these bacteria with *E. coli* and *S. aureus*, common pathogens in swine production, was evaluated by plate inhibition assays, thus determining their antimicrobial potential. A comprehensive genomic study was conducted, using next-generation sequencing (NGS) and bioinformatics tools to assemble, annotate, and analyze the genomes of the selected strains.

Within the genomic analysis, the pangenome of *L. plantarum* HCA1 was determined, an exhaustive search for virulence and antibiotic resistance genes was performed, and a phylogenomic analysis was carried out to establish their evolutionary relationships. The three-dimensional structure of the identified bacteriocins was predicted using the AlphaFold 3 computational model to better understand their mechanism of action.

The results revealed a remarkable adaptability and resistance of the strains to the simulated conditions of the gastrointestinal tract, essential characteristics for their viability as probiotics. Considerable antimicrobial activity was observed, attributed to stable and effective bacteriocins, including plantaricins from *L. plantarum*, whose key genes were identified and characterized. The genomic study confirmed the safety of the strains, as no virulence or antibiotic resistance genes were detected, and revealed an open pangenome in *L. plantarum* HCA1, indicative of genetic diversity and adaptation potential.

These findings suggest that the *L. plantarum* and *L. reuteri* strains studied could constitute a probiotic and antimicrobial alternative to antibiotics in swine production. Bacteriocins, and plantaricins in particular, exhibit properties that warrant further investigation for their potential application in the food and swine industries, with the aim of contributing to safer food production, more sustainable animal production, and improved animal and human health. This lays the foundation for future research and the development of biotechnological applications of these strains, with a focus on improving animal health and production.

Keywords: Antimicrobial activity, Lactic acid bacteria, Swine industry, Open pangenome, Plantaricins, Probiotics, Antimicrobial resistance.

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