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"IDENTIFICACIÓN MOLECULAR DE BACTERIAS ÁCIDO LÁCTICAS CON POTENCIAL PROBIÓTICO AISLADO DE HECES DE NEONATOS HUMANOS"

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RESUMEN

El objetivo de este estudio fue aislar e identificar molecularmente bacterias con potencial probiótico de heces de neonatos humanos y determinar su cinética de crecimiento. Se evaluó 30 muestras de heces de neonatos nacidos vía vaginal y 30 por cesárea (0-3 días). Las muestras se enriquecieron en caldo Man Rogosa Sharp (MRS) a 37°C por 24h. Se sembraron en agar MRS y se contabilizaron colonias GRAM positivas, catalasa negativa y libre de esporas. Se aislaron cepas en agar MRS modificado a 37°C por 24h, de donde se seleccionaron colonias para pruebas ***in vitro*** con sales biliares; resistencia a pH bajo y actividad antimicrobiana. Frente a *Escherichia coli* ATCC25922, *E. coli* ATCC35218, *Salmonella entérica* y *Listeria inocua* mediante ensayos de difusión en agar. La identificación molecular se realizó con amplificaciones BOX-PCR y el secuenciamiento del gen 16S rRNA. Se aislaron 48 cepas y todas presentaron resistencia a pH 3 y 0.3% sales biliares en diferente porcentaje; tres cepas mostraron actividad antimicrobiana a *E. coli* ATCC25922, 1 cepa a *E. coli* ATCC35218, 5 cepas a *L. inocua* y todas a *S. entérica*. Se obtuvieron dos perfiles BOX-PCR, de los cuales nueve cepas ($C5_2$, $C6_1$, $C7_1$, $C11_2$, $C16_2$, $C19_2$, C20, C35, y C42) presentaron 100% de similaridad a *Lactobacillus plantarum* ATCC 14917^T [ACGZ01000098] y dos (C15 y C40) 99.93% y 99.80% de similaridad a *Enterococcus faecium* CGMCC 1.2136^T [AJKH01000109]; estas cepas mostraron actividad en leche con diferencias significativas (p valor < 0.05) en cinética de crecimiento. En conclusión, se logró la identificación de cepas *Lactobacillus* y *Enterococcus* con potencial probiótico.

Palabras clave: Recién nacidos, PCR-BOX, *Lactobacillus, Enterococcus*.

ABSTRACT

The objective of this study was to isolate and molecularly identify bacteria with probiotic potential from human neonatal feces and characterize their growth kinetics. Thirty stool samples from neonates born vaginally and 30 by caesarean section (0-3 days) were evaluated. The samples were enriched in Man Rogosa Sharp broth (MRS) at 37 ° C for 24h. They were grown on MRS agar and the GRAM positive, catalase negative and spore-free colonies were counted. Strains were isolated on modified MRS agar at 37 ° C / 24h, from which colonies were selected for *in vitro* tests resistance to bile salts, to low pH and antimicrobial activity against *Escherichia coli* ATCC25922, *E. coli* ATCC35218, *Salmonella enteric* and *Listeria innocua* by diffusion in agar. The molecular identification was made with PCR-BOX amplifications and the sequencing of the 16S rRNA genes. Forty eight strains were isolated and all showed resistance to pH 3 and 0.3% bile salts in different percentages; three strains showed antimicrobial activity against to *E. coli* ATCC25922, one strain to *E. coli* ATCC35218, five strains to *L. innocuous* and all to *S. enterica*. Two BOX-PCR profiles were obtained, of which nine strains (C52, C61, C71, C112, C162, C192, C20, C35, and C42) had 100% similarity to *Lactobacillus plantarum* ATCC 14917T [ACGZ01000098] and two (C15 and C40) 99.93% and 99.80% similarity to *Enterococcus faecium* CGMCC 1.2136T [AJKH01000109]; these strains showed activity in milk with significant differences (p value <0.05) in growth kinetics. In conclusion, *Lactobacillus* and *Enterococcus* with probiotic potential were identified.

Keywords: Newborns, PCR-BOX, *Lactobacillus, Enterococcus*.