Characterization of antimicrobial compounds from Weissella cibaria strains isolated from artisanal cheese-production regions

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Antimicrobial activity of lactic acid bacteria has been observed in many food fermentation processes. Organic acids (OA) produced during the growth of these microorganisms play a major role in antagonism towards pathogenic bacteria. The present study characterizes OA compounds produced during the growth of Weissella cibaria strains previously isolated from artisanal cheese-producing regions. Strains of W. cibaria W21, W25, and W42 were used to produce antimicrobial compounds in de Man, Rogosa, and Sharpe broth (Oxoid) for 24h at 32 °C. The cell-free supernatant (CFS) was obtained by centrifugation and filtration. The microdilution assays were used to determine antagonism against Listeria monocytogenes, Staphylococcus aureus, Salmonella enterica, and Escherichia coli. Target strains were cultured in a brain heart infusion (Oxoid) at 37 °C for 24h, and aliquots (100 µL, 104 CFU/mL) were transferred to 96-well microtiter plates. Then, 50 µL aliquots of the selected CFS were added to each well. The microplates were incubated at 37 °C for 24h in a Multiskan™ GO Microplate Spectrophotometer (Thermo Fisher). The cultures' optical densities (OD) were measured (λ = 600 nm). The inhibition values were expressed in % of inhibition. After, OA were quantified with a high-performance liquid chromatograph. The supernatant was processed. The samples were separated on a Biohad HPX 87H column, 300 x 7.8 mm, maintained at 45 ºC using a Shimadzu Prominence 20A chromatograph coupled to a refractive index detector. The mobile phase was 5.0 mM sulfuric acid with a 0.7 mL/min flow rate. Crotonic acid was used as an internal standard in the samples and calibration curve. Strain W21 inhibited 88.1% of L. monocytogenes, 37% of S. aureus, 77.3% of S. enterica, and 71.1% of E. coli. Strain W25 inhibited 44% of L. monocytogenes, 22.2% of S. aureus, 49.9% of S. enterica, and 18.6% of E. coli. Strain W42 inhibited 89.1% of L. monocytogenes, 88.5% of S. aureus, 80.3% of S. enterica, and 86.2% of E. coli. Each CFS contained high concentrations of lactic and acetic acids. The W21 supernatant also contained propionic, valeric, isovaleric, butyric, and malic acids. The OA compositions of W25 CFS were the same as the W21 strain except for the malic acid. The OA composition of W42 CFS was lactic, acetic, propionic, butyric, and malic acids. Lactic and acetic acids are effective against Gram-positive and Gram-negative bacteria, providing a significant basis for antimicrobial activity. Propionic acid is effective, especially against some Gram-positive bacteria. Its presence suggests its contribution to the antimicrobial activity. Valeric and isovaleric acids have antimicrobial activity but are generally less potent. Their presence may contribute to the antimicrobial activity but does not explain the high efficacy itself. Butyric acid has antimicrobial properties that can complement the action of other acids suggesting a supportive role in bacterial inhibition. Malic acid correlates with greater antimicrobial efficacy, especially against Gram-positive bacteria such as S. aureus and L. monocytogenes. Malic acid can act synergistically with other acids. Therefore, the presence of malic acid, together with other acids such as lactic and acetic, appears to be decisive for strong antimicrobial activity. The absence of malic acid and the presence of less potent acids such as valeric and isovaleric

may explain the lower antimicrobial efficacy of the W25 strain.

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