

Characterization of the Extremely Heat-Stable Antibacterial Activity Produced by a Newly Selected Local *Bacillus* Probiotic Strain

Ali Aljohani*, Shifa A. Bahaddad, Meshal H. K. Almalki, Othman A. Alghamdi,
Hichem Chouayekh

Department of Biological Sciences, College of Science, University of Jeddah -
Kingdom of Saudi Arabia

*Corresponding author

ABSTRACT

Bacterial food poisoning and food spoilage cause significant economic losses and pose serious public health concerns. Although chemical preservatives can inhibit or kill foodborne pathogens, many have been banned due to safety concerns, increasing the need for natural, safe alternatives. Bacteriocins, ribosomally synthesized peptides with antibacterial activity, are one such promising options. Produced by several bacteria, including *Bacillus* species, bacteriocins have demonstrated broad-spectrum antimicrobial activity and potential application in food preservation. *Bacillus amyloliquefaciens*, known for its probiotic potential and production of antimicrobial substances, has attracted attention for its prebiotic, probiotic, and functional food applications. This study aimed to determine the optimal conditions for bacteriocin production and characterize the bacteriocin-like antibacterial compound produced by the newly selected extremophile probiotic strain *Bacillus amyloliquefaciens* 37. The optimal bacteriocin production conditions were investigated by evaluating the impact of preculture medium, culture medium, flask volume, and incubation time. The antibacterial compound's nature and stability were tested using enzymatic, heat, and pH treatments. The antimicrobial spectrum was assessed against multiple foodborne pathogens, and the mode of action was determined using *Staphylococcus aureus* as an indicator strain. A molecular investigation was performed to screen by PCR the genes encoding lipopeptides and bacteriocins in *Bacillus amyloliquefaciens* 37. The *Bacillus amyloliquefaciens* 37 strains produced a bacteriocin-like compound exhibiting strong activity against Gram-positive pathogens, especially *Staphylococcus aureus* and *Listeria monocytogenes*. Under optimal conditions, antibacterial activity reached 66.66 AU/ml in MHB medium. The compound showed remarkable stability across a wide pH range (pH 1–12) and extreme thermostability since it retained full activity after 15 minutes at 100 °C. Sensitivity to proteinase K confirmed the peptide nature of the compound, consistent with bacteriocins. Adding heat-killed *S. aureus* cells to the culture medium had



no stimulatory effect on bacteriocin production, indicating that production was not induced by target cells. Finally, PCR amplification products corresponding to the *acnA* gene encoding the bacteriocin amylocyclicin along with the genes *ituA*; *ituD*; *fen*; *srfA*, *Srf/lch* and *sfP* encoding the lipopeptides iturin A, iturin, fengycin and surfactin, respectively, were detected in *Bacillus amyloliquefaciens* 37 strain. The extremely heat-stable bacteriocin produced by the *Bacillus amyloliquefaciens* 37 strain presents a promising natural preservative candidate for improving food safety. Further purification and structural characterization will help confirm its potential for application in food biopreservation.

Keywords: *Bacillus*, Probiotics, Food safety, Extremely heat-stable bacteriocin, Natural antibacterial compound, Food preservation

How to Cite

Ali Aljohani, Shifa A. Bahaddad, Meshal H. K. Almalki, Othman A. Alghamdi, Hichem Chouayekh, “Characterization of the Extremely Heat-Stable Antibacterial Activity Produced by a Newly Selected Local *Bacillus* Probiotic Strain”, *AIJR Abstracts*, pp. 60-61, Mar. 2025.