

Production, purification and characterization of an antibacterial agent from an alkaliphilic bacteria

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Background and Aims: The aim of this study was initiated in order to overcome antimicrobial resistance by evaluating the potential of alkaliphilic microorganisms as potential producers of new antimicrobial substances.

Methods: At first, a range of around 40 alkaliphilic strains has been screened to determine if they have an antibacterial activity. Then, the strain that showed pronounce activity selected and has been tried to produce the bioactive compound in large enough amount using different strategies that has been led to Solid State Fermentation (SSF) as the best method, followed by manipulation the effective factors including: supplementation with media, different salt, Na content, moisture ratio and time of cultivation .Next, the active agents purified successfully by HIC column with a proper purity which its quality was confirmed by silver-staining and then later with liquid chromatography. After that, the bioactive compound identified using the LC-MS and tandem MS/MS analyses .Finally, the synergistic effect of this bacteriocin with some antibiotics against different pathogens evaluated.

Results: The highest production of bioactive compound from the selected producer strain using SSF method was achieved after two days cultivation in the presence of 2mg Mg₂SO₄/g bran, 10mg K₂HPO₄/g bran, 10% (w/w) Na₂CO₃ and at a wheat bran/moisture ratio of 1:1.8. The MS and MS/MS analyses showed that the bioactive compound belongs to the two-component lantibiotics of haloduracin type. It is composed of two peptides with molecular weight 3043.2578 and 2330.0296. Fortunately, it demonstrates a clear synergistic effect in combination with chloramphenicol against some Gram-positive genuses such as Enterococcus and Streptococcus.

Conclusions: The production of the haloduracin under solid state fermentation and its synergy with chloramphenicol is encouraging to screen further alkaliphiles, optimize the production conditions more and further study on synergistic effect of this bacteriocin with other antibiotics to formulate very potent antimicrobial products.

Keywords: *Bacillus halodurans*; Haloduracin; Solid state fermentation; Antimicrobial synergistic activity