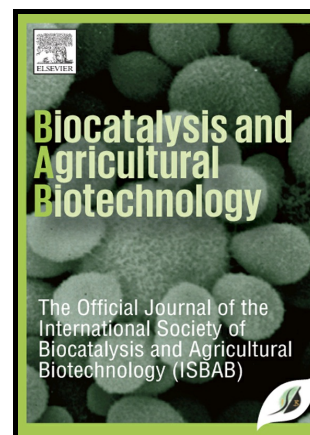


## Author's Accepted Manuscript

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**Statistical optimization for the improved production of an extracellular alkaline nuclease by halotolerant *Allobacillus halotolerans* MSP69: Scale-up approach and its potential as flavor enhancer of fish sauce**

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**Abstract**

An efficient extracellular alkaline nuclease producing halotolerant bacterium was isolated from fermented shrimp paste (*kapi*) and identified as *Allobacillus halotolerans* based on the phenotypic analysis and 16S rDNA sequence homology. The temperature and pH ranges for growth were 20-45 °C (optimum at 37 °C) and pH 6.0-10.0 (optimum at pH 8.0). A statistical optimization method was applied to enhance the alkaline nuclease production using a 20-run, 3-factor with 5-level of the central composite rotatable design (CCD) generated by design expert software. The optimum conditions for the simultaneous production of deoxyribonuclease (DNase) and ribonuclease (RNase) in a single media were determined as 17.5 g/L of yeast extract, 1.59 % (w/v) NaCl and an initial pH 8.0. The maximum productions of RNase (59.36 U/mL) and DNase (141.21 U/mL) were in agreement with the prediction values and the model was proven to be adequate. The optimized conditions from the shake flask experiments were validated in a 7 L lab scale fermenter, and the RNase and DNase production increased to 3.95-fold and 6.77-fold, respectively. Furthermore, the alkaline nuclease preliminary employed as flavor enhancer in the fish sauce fermentation process increased the aspartic acid (452.81 mg/100 mL), glutamic acid (1348.86 mg/100 mL), 5'-GMP (36.89 mg/100mL) and 5'-AMP (21.49 mg/100 mL) in fish sauce ( $P < 0.05$ ). These results demonstrate that the new alkaline nuclease showed an attractive potential for biotechnological applications as biocatalyst, especially for fish sauce production.

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