

## THE OPTIMIZATION OF GROWTH CONDITION OF THE BACTERIA PRODUCING COLD-ACTIVE PROTEOLYTIC ENZYME FROM THE ANTARCTIC REGION

MUHAMMAD ASYRAF ABD LATIP<sup>1,2</sup>, NOOR FAIZUL HADRY NORDIN<sup>3\*</sup>,  
SITI AISYAH ALIAS<sup>4,7</sup>, JERZY SMYKLA<sup>5</sup>, FARIDAH YUSOF<sup>2</sup>,  
AND MOHD AZRUL NAIM MOHAMAD<sup>6</sup>

<sup>1</sup>Mariculture Research Division, Fisheries Research Institute Langkawi, Kompleks Perikanan Bukit Malut, 07000 Langkawi, Kedah, Malaysia

<sup>2</sup>Biotechnology Engineering Department, Kulliyah of Engineering, International Islamic University Malaysia, Jalan Gombak, 53100 Kuala Lumpur

<sup>3</sup>International Institute for Halal Research and Training (INHART), Block A, Level 3, KICT Building, International Islamic University Malaysia, Jalan Gombak, 53100 Kuala Lumpur

<sup>4</sup>Institute of Ocean and Earth and Sciences, C308, Level 3, Block C, Institute for Advanced Studies Building, 50603 Kuala Lumpur, Malaysia

<sup>5</sup>Institute of Nature Conservation, Polish Academy of Sciences, al. A. Mickiewicza 33 PL-31-120 Krakow, Poland

<sup>6</sup>Department of Biotechnology, Kulliyah of Science, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, Bandar Indera Mahkota, 25200 Kuantan, Pahang, Malaysia

<sup>7</sup>National Antarctic Research Centre, B303, Level 3, Block C, Institute for Advanced Studies Building, 50603 Kuala Lumpur, Malaysia

\*Corresponding author: [faizul@iium.edu.my](mailto:faizul@iium.edu.my)

(Received: 30<sup>th</sup> May 2022; Accepted: 9<sup>th</sup> September 2022; Published on-line: 4<sup>th</sup> January 2023)

**ABSTRACT:** The growth conditions of bacteria producing cold-active protease isolated from an Antarctic sample were screened using one-factor-at-time (OFAT). Then, crude protease of the strain was extracted during the late logarithmic phase for enzymatic assay. A strain that showed the highest enzyme activity was selected for optimization via response surface method (RSM). The parameters studied were incubation temperature (4 – 36 °C), pH media (4 – 10) and NaCl concentration (0 – 8%). Based on the OFAT results, all eight strains showed the highest growth rate at 20 °C, pH 7 and 4% (w/v) NaCl. The assay showed that the crude enzyme extracted from strain SC8 exhibited significantly higher activity (0.20 U and 0.37 U) than the positive control (0.11 U and 0.31 U) at -20 °C and 20 °C. RSM suggested that the optimized setting for growth of SC8 were at 20.5 °C, pH 6.83 and 2.05% (w/v) of NaCl with the results of the bacterial growth rate value was  $3.70 \pm 0.06 \times 10^6$  cells/hr. Optimal growth conditions of SC8 from this study are useful for the large-scale production of cold-active protease in future.

**ABSTRAK:** Keadaan pertumbuhan bakteria yang menghasilkan enzim protease aktif sejuk daripada sampel Antartika disaring menggunakan satu faktor pada masa (OFAT). Kemudian, enzim protease ini diekstrak pada lewat fasa logaritma untuk ujian enzimatik. Strain yang menunjukkan aktiviti enzim tertinggi telah dipilih untuk tujuan pengoptimuman melalui kaedah permukaan tindak balas (RSM). Parameter yang dikaji ialah suhu penderaman (4 – 36 °C), pH media (4 – 10) dan kepekatan NaCl (0 – 8%). Berdasarkan OFAT, kesemua lapan bakteria menunjukkan kadar pertumbuhan tertinggi pada 20 °C, pH 7 dan 4% NaCl. Hasil ujian enzimatik menunjukkan bahawa enzim protease yang diekstrak daripada SC8 mempamerkan aktiviti yang jauh lebih tinggi (0.20 U dan 0.37 U) daripada kawalan positif (0.11 U dan 0.31 U) pada -20 °C dan 20 °C.