

ABSTRAK

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ANOTASI GEN-GEN TERKAIT RESPON TERHADAP *HEAT STRESS* DAN ANALISIS EKSPRESI GEN HSP PADA *Lactiplantibacillus plantarum* STRAIN SU-KC1A

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(xiii+ 37 halaman; 4 gambar; 4 tabel; 3 lampiran)

Lactiplantibacillus (Lpb) plantarum adalah bakteri asam laktat yang sering digunakan sebagai probiotik. Aplikasi sebagai probiotik menyebabkan *Lpb. plantarum* sering berhadapan dengan berbagai stres akibat proses manufaktur dan penanganan seperti stres suhu tinggi yang dapat berpengaruh terhadap viabilitas dan fungsionalitas probiotik. Penelitian ini bertujuan: (1) Uji ketahanan *Lpb. plantarum* strain SU-KC1a pada suhu tinggi berdasarkan viabilitas (OD dan CFU). (2) Analisis molekular terhadap ekspresi gen terkait stres suhu tinggi (gen *hsp3*). (3) Analisis hasil anotasi gen terkait stres suhu tinggi pada SU-KC1a. Metode yang digunakan dalam penelitian ini adalah: (1) Inkubasi kultur pada suhu 37°C, 42°C, 47°C dan 52°C selama satu jam untuk menentukan viabilitas sel dengan enumerasi koloni yang terbentuk. Dari hasil sebelumnya, dilakukan pertumbuhan pada suhu 37, 40 dan 42. Hasil pertumbuhan diamati melalui nilai OD. (2) RNA total dari SU-KC1a yang telah diinkubasi pada suhu 37°C, 40°C dan 42°C selama satu jam diekstraksi kemudian dikonversi menjadi cDNA. Sampel cDNA diamplifikasi menggunakan primer spesifik F(5'-TGTC AACATCAACGTCGGGTAA-3') R(5'-CGTAGTCATGGGTCGGTCCT-3') gen *hsp3*. Amplikon divisualisasi pada gel agarose 1,2%. (3) Data anotasi *whole genome sequence* SU-KC1a dibandingkan terhadap data anotasi tiga strain *Lpb. plantarum* lain (SK151, F75 dan WCFS1) untuk melihat perbandingan keberadaan gen terkait stres suhu tinggi. Hasil uji ketahanan pada suhu tinggi menunjukkan temperatur maksimal SU-KC1a adalah sekitar 42°C dengan jumlah pertumbuhan sel dari 15,8 CFU/mL menjadi 19,1 CFU/mL setelah inkubasi satu jam. Hasil visualisasi amplikon gen *hsp3* pada agarose menunjukkan penebalan pita pemisahan DNA seiring dengan peningkatan suhu. Berdasarkan hasil perbandingan gen terkait stres suhu tinggi pada keempat strain *Lpb. plantarum* (SU-KC1a, F75, WCFS1 dan SK151), terlihat bahwa gen-gen terkait stres suhu tinggi antara lain gen *groES*, *groEL*, *hsp1*, *hsp2* dan *hsp3* terkonservasi pada *Lpb. plantarum* karena bersifat esensial.

Kata Kunci : *Lactiplantibacillus plantarum*, Gen *Hsp3*, Stres Suhu Tinggi, Ekspresi *Stress Gene*

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ABSTRACT

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HEAT STRESS RESPONSE RELATED GENES ANNOTATION AND ANALYSIS OF GENE EXPRESSION IN *Lactiplantibacillus plantarum* STRAIN SU-KC1A

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Lactiplantibacillus (Lpb) plantarum is a lactic acid bacteria that commonly used as probiotics. *Lpb. plantarum* will encounter many stresses due to utilization in functional food industry as a probiotic during manufacturing and handling process such as heat stress thus will affect cell's viability and functionality. The aims of this study are: (1) Examination of SU-KC1a heat stress resistance (2) Molecular analysis of *hsp3* gene expression. (3) Analysis of heat stress related genes based on Whole Genome Sequencing annotation data. Methods used in this research were: (1) Examination of SU-KC1a heat resistance was done by incubating cultures at 37°C, 42°C, 47°C and 52°C for one hour to determine cell's viability with enumeration of colonies formed on agar plates. Based on previous result, optical density value also observed every one hour from cultures incubated at 37°C, 40°C and 42°C for three hours in total to determine growth rate of SU-KC1a during heat stress. (2) Total RNA of treated SU-KC1a extracted. Previously, SU-KC1a cultures treated by incubating at 37°C, 40°C and 42°C for one hour. After extraction, RNA samples were converted into cDNA and amplified by PCR reaction with *hsp3* gene specific primers F(5'-TGTC AACATCAACGTCGGGTAA-3') R(5'-CGTAGTCATGGGTCGGTCCT-3'). PCR amplicons visualized on 1,2% agarose gel. (3) SU-KC1a Whole Genome Sequence annotation data was compared to *Lpb. plantarum* SK151, F75 and WCFS1 annotation to identify presence and absence of heat stress related genes. Result of SU-KC1a resistance towards heat stress shows that maximum temperature of SU-KC1a is around 42°C with increased cell's viability from 15,8 CFU/mL to 19,1 CFU/mL after incubated for one hour. Growth rate of SU-KC1a incubated at higher temperature exhibit slower rate. Visualization of *hsp3* amplicon appear clearer at higher temperature indicating increase of *hsp3* gene expression. According to comparative analysis of stress related genes, heat stress response genes are conserved by reason of it is essential for survival capability.

Keywords : *Lactiplantibacillus plantarum*, *Hsp3* Gene, Heat Stress, Stress Gene Expression

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