

**ISOLASI DAN KARAKTERISASI ENZIM β -GALAKTOSIDASE
DARI ISOLAT BAKTERI TERMOFILIK
SUMBER AIR PANAS GEDONG SONGO**

Oleh:

**Rimborani Windradi
J2C 002 163**

RINGKASAN

Enzim β -galaktosidase merupakan enzim yang dapat mengubah laktosa menjadi glukosa dan galaktosa. Berdasarkan uji kualitatif yang telah dilakukan oleh Suprapti menyatakan bahwa isolat bakteri termofilik sumber air panas Gedong Songo berpotensi menghasilkan enzim β -galaktosidase. Penelitian ini bertujuan untuk memperoleh enzim β -galaktosidase dari isolat bakteri termofilik sumber air panas Gedong Songo, memperoleh karakter enzim dan menentukan aktivitas spesifik enzim β -galaktosidase pada kondisi optimumnya.

Isolasi bakteri menggunakan media $\frac{1}{2}$ Luria Berthani ($\frac{1}{2}$ LB). Jenis bakteri diidentifikasi dengan metode pewarnaan Gram. Enzim β -galaktosidase diproduksi pada media $\frac{1}{2}$ LB selama 6 jam dengan menggunakan laktosa sebagai penginduksi. Enzim β -galaktosidase diisolasi dengan metode ekstraksi, fraksinasi amonium sulfat bertingkat dan dialisis. Uji aktivitas enzim β -galaktosidase dilakukan dengan menggunakan ONPG sebagai substratnya dan kadar protein diukur dengan menggunakan metode *Lowry*. Karakterisasi enzim meliputi pH, suhu dan waktu inkubasi optimum dari enzim β -galaktosidase yang diperoleh.

Isolat bakteri termofilik sumber air panas Gedong Songo memiliki sifat gram negatif dan berbentuk batang. Hasil karakterisasi menunjukkan bahwa kondisi optimum enzim β -galaktosidase isolat bakteri termofilik sumber air panas Gedong Songo dengan substrat ONPG adalah pada suhu 63 °C, pH 6,2 dan waktu inkubasi 30 menit. Aktivitas spesifik tertinggi pada kondisi optimum tersebut didapatkan pada F4 (60-80 %) yaitu 44,145 Unit/mg protein.

SUMMARY

β -galactosidase is an enzyme converting lactose to glucose and galactose. Suprapti through several qualitative tests concluded that thermophile bacteria isolated from Gedong Songo hot water spring has a potential of β -galactosidase. The research aim is to isolate β -galactosidase enzyme of thermophile bacteria isolated from Gedong Songo hot water spring, to characterize optimum pH, incubation time, and temperature of the enzyme and to know the specific activity of β -galactosidase in above condition.

Bacteria was isolated in $\frac{1}{2}$ Luria Berthani ($\frac{1}{2}$ LB) medium. The kind of bacteria was identified using Gram's coloring method. Production of β -galactosidase was done in $\frac{1}{2}$ LB medium for 6 hours using lactose as inducer. The β -galactosidase was isolated using extraction, fractionation and dialysis methods. Activity of enzyme fraction was tested using ONPG while protein concentration was measured using Lowry's method. Characterization done were optimum pH, temperature, and incubation time.

Bacteria isolated from Gedong Songo hot water spring had negative gram's character and bar shape. The optimum condition of β -galactosidase bacteria characterization isolated from Gedong Songo hot water spring by ONPG substrate were at 63 °C temperature, 6.2 pH and 30 minutes incubation time. Highest specific activity in above condition was obtained 44.145 Units/mg protein in F4 (60-80 %).

DAFTAR PUSTAKA

- Atlas, R. M., and Richard, B., 1993, "Microbial Ecology: Fundamental and Application", 3^{ed} ed., The Benjamin Cummings Publishing Company Inc., California.
- Ayyildiz, A., 1999, "Characterization of Catalytic Phenotype of β -Galactosidase From LacI Mutant, *E. coli* CSH-36, as a Tool For The Management of Lactase Intolerance", *Journal of Medical Sciences*, 521-527.
- Bergmeyer, H. U., 1990, "Methods of Enzymatis", *Verlag Chemie Meinhein*, Academic Press inc New York and London, vol 1, 2nd ed, 434-435.
- Beveridge, T. J., 1999, "Structure of Gram-Negative Cell Wall and Their Derived Membrane Vesicles", *J. Bacteriol.*, 181, 4725-4733.
- Brisbarre, N., Fardeau, M. L., Cueff, V., Cayol, J. L., Barbier, G., Cilia, V., Ravot, G., Thomas, P., Garcia, J. I., and Ollivier, B., 2003, "*Clostridium caminithermale* sp. nov., a slightly halophilic and moderately thermophilic bacterium isolated from an Atlantic deep-sea hydrothermal chimney", *Int J Syst Evol Microbiol* 53, 1043-1049.
- Brock, T. D., 1979, "Biology of Microorganisms", 3rd ed, Prentice-Hal Inc, New Jersey.
- Bruins, Janssen and Boom, 2001, "Thermoenzymes and Their Applications", *Applied Biochemistry and Biotechnology*, 90, 156-184.
- Carman, D. R., 2001, "Bacterial Characteristics: Introduction to Bacteriology", *Micro.*, 6, 11-23.
- Clark, Jr., John, M., and Switzen, R. L., 1997, "Experimental Biochemistry", 2nd edition, John Willey and Sons inc., USA, 116-123.
- Collins, C. H., and Lyne, P. M., 1984, "Microbiological Methods", fifth edition, Butterworths, London, 110.
- Collowick, S. P., and Kaplan, N. O., 1955, "Methods in Enzymology", Academic Press Inc, New York, 51-58, 87.
- Copeland, K. A., 1994, "Methods for Protein Analysis", Chapman and Hal, London, 24-26, 43-44.
- Drouault, S., Anba, J., and Corthier, G., 2002, "*Streptococcus thermophilus* is Able To Produce a β -Galactosidase Active during Its Transit in the Digestive Tract of Germ-Free Mice", *Journal American Society for Microbiology*, vol 68, no. 2.

- Fardiaz, S., 1993, "Analisis Mikrobiologi Pangan", edisi kelima, cetakan pertama, P.T. Raja Grafindo Persada, Jakarta, 156.
- Friedman, S. M., 1992, "Thermophilic Microorganism", Encyclopedia of Microbiology, vol 4, Academic Press Inc, 217.
- Frobisher, M., Hindstil, R. D., and Goodheart, C. R., 1974, "Fundamentals of Microbiology", Toppan and Co.LTH.
- Fukuda, W., Ismail, Y. S., Fukui, T., Atomi, H., and Imanaka, T., 2005, "Characterization of an Archaeae Malic Enzyme from The Hyperthermophilic Archaeon *Thermococcus kodakaraensis* KOD 1", volume 1, Heron Publishing, Canada.
- Gilvery, R., W., Mc., dan Goldstein, G., W., 1996, "Biokimia Suatu Pendekatan Fungsional", Airlangga University Press, Surabaya, 694.
- Hartiko, H., 1999, "Biologi Mikroorganisme Termofilik", PAU Bioteknologi UGM, Yogyakarta, 11-37.
- Inglis, TJJ., 2001, "An Introduction to Gram Positive Bacteria", *Journal Irish Veterinary*.
- Irwin, J. A. and Baird, A. W., 2004, "Extremophiles and their Application to Veterinary Medicine", *Journal Irish Veterinary*, 57(6), 348-354.
- Jakob, U., Gaestel, M., Engel, K., and Buchner, J., 1993, "Small Heat Shock Proteins are Molecular Chaperones", *J. Biol. Chem.*, 268 (3), 1517-1520.
- Johnson, R., 2003, "The Xphiles: Microorganisms at The Extremes", Grahamstown, dep. Biochemistry and Microbiology Rhodes University.
- Lee, G., J., Roseman, A. M., Saibil, H. R., And Vierling, E., 1997, "A Small Heat Shock Protein Stably Binds Heat-Denaturated Mode Substrate in a Folding Competent State", *The EMBO Journal*, 16 (3), 659-671.
- Lehninger, A. L., 1994, "Dasar-Dasar Biokimia", a. b. Maggy T., Jilid 3, Erlangga, Jakarta, 234-239.
- Ling, J., Wells, D. R., Tanguay, R. L., Dickey, L. F., Thompson, W. F., and Gallie, D. R., 2000, "Heat Shock Protein HSP101 Binds to the *Fed-1* Internal Light Regulatory Element and Mediates Its High Translational Activity", *J. American Society of Plant Physiologists*, vol. 12, 1213-1227.
- Macris, B. J., and Markakis, P., 1981, "Characterization of Extracellular β -D-Galactosidase from *Fusarium moniliforme* Grown in Whey", *Journal of Appl Environ Microbiol*, vol 41(4), 956-958.

- Macario, A. J. L., Lange, M., Ahring, B. K., and De Macario, E. C., 1999, "Stress Genes and protein in The Archaea", *Microbiol. Mol. Rev.*, 63 (4), 923-967.
- McClland, R., 2001, "Gram's Stain: The Key to Microbiology", *MLO*., 20-28.
- Moeini, H., Nahvi, I., and Tavassoli, M., 2004, "Improvement of SCP Production and BOD Removal of Whey With Mixed Yeast Culture", *Electronic Journal of Biotechnology*: Universidad Catolica De Val Paraiso, vol 7, num. 3.
- Narberhaus, 2002, "Heat Shock Proteins and Heat Adaption of The Whole Organism", α -Crystallin Type Heat Shock Proteins; Socializing Minichaperones in The Context of A Multichaperones Network", *Microbial, and mol. Biol. Rev.*, vol. 66, p. 24-93.
- Natalia, D., Yuliani, Y., Ermayadhie, Y., Putra, R., and Sindumarta, M., 2002, "Thermostable Glucoamylase Type Enzyme from *Bacillus acidocaldarius* RPI, *Biochem*", *Mol. Biol. Edu*, 30, 398-400.
- Pearson, 2006, "The Lac Operon in *E. coli*", Prentice Hall.
- Pelczar, M. J., and Chan, E. C. S., 1986, "Dasar-Dasar Mikrobiologi", UI Press, Jakarta, 83-91.
- Poedjiadi, A., 1994, "Dasar-Dasar Biokimia", UI Press, Jakarta, 142-143, 145-146, 158-162.
- Prastuti, D. W., 1999, "Purifikasi Parsial dan Karakterisasi Protease Kecambah Biji Turi (*Sesbania grandiflora*), Pasca Sarjana Jurusan Ilmu Pertanian UGM, 14-19, 43-44.
- Robert, K. M., 1997, "Biokimia Harper", a. b. Andry Hartono, Buku Kedokteran, Jakarta.
- Salle, A.J., 1973, "Fundamental Principles of Bacteriology", TMH edition, McGraw Hill, New Delhi.
- Sambrook, J., and Russell, T., 2001, "Molecular Cloning: a Laboratory Manual", 3rd ed., Cold Spring Harbour Laboratory, N. Y.
- Sastrohamidjojo, H., 1995, "Spektroskopi", Liberty, Yogyakarta, 11,15.
- Schaffer, C., Wugeditsch, T., Kahlig, H., Scheberl, A., Zayni, S., and Messner, P., 2002, "The Surface Layer (S-layer) Glycoprotein of *Geobacillus stearothermophilus* NRS 2004/3a", *Journal of Biological Chemistry*, vol. 277, No. 8, 6230-6239.
- Schlegel, H. G. dan Schmidt, K., 1984, "Mikrobiologi Umum", edisi keenam, UGM Press, Yogyakarta, 120, 208.

- Scopes, R. K., 1982, "Protein Purification, Principles and Practice", 3rd edition, Springer Verlag, New York, 85-92.
- Shahib, M. N., 1992, "Pemahaman Seluk Beluk Biokimia dan Penerapan Enzim" P. T. Citra Aditya Bakti, Bandung, 1.
- Sokatch, J. R., 1969., "Bacterial Physiology and Metabolism", Academic Press, London, New York, 62.
- Souza, A. N. and Martiens, M. L. L., 2001, "Isolation, Properties, and Kinetics of Biology Bull, 13, 2.
- Stromer, T., Ehrnsperger, M., Gaestel, M., and Buchner, J., 2003, "Analysis of The Interaction of Small Heat Shock Protein with Unfolding Proteins", *J. Biol. Chem.*, 278 (20), 18015-18021.
- Suhartono, M. T., 1989, "Enzim dan Bioteknologi", IPB, Bogor, 120-272.
- Suprpti, H. Y., 2005 "Identifikasi Fragmen Gen 16S rRNA Bakteri Termofilik Hasil Isolasi dari Sumber Air Panas Gedong Songo", Skripsi Kimia FMIPA UNDIP, Semarang.
- Susilawati, T., 2004, "Isolasi dan Karakterisasi Enzim Protease dari Isolat Bakteri Termofilik Sumber Air Panas Gedong Songo, Bawen", Skripsi Kimia FMIPA UNDIP, Semarang, 31.
- Svehla, G., 1985, "Analisis Anorganik Kualitatif", a.b. L. Setiono dan Hadyana Pudjaatmaka, bagian II, edisi kelima, PT. Kalman Media Pusaka, Jakarta, 369.
- Takai, K., Yoshihiko, S., and Aritsune, U., 1998, "Acquired Thermotolerance and Temperature-Induced Protein Accumulation in The Extremely Thermophilic Bacterium *Rhodothermus obamensis*", *J. Bacteriol.*, vol. 180, No. 10, p. 2770-2774.
- Teodoro, C. E. d. S. and Martins, M. L. L., 2000, " Culture Conditions For The Productions of Thermostable Amylase By *Bacillus sp*", *Brazilian Journal of Microbiology*, vol 31, no 4.
- Todar, K., 2004, "Structure and Function of Prokaryotic Cells", Todar's Online Textbook of Bacteriology, Wisconsin-Madison.
- Trent, J., Mette, G., Bo, J, Jan, N., and Jorgen, O., 1994, "Acquired Thermotolerance and Heat Shock Protein in Thermophiles from The Tree Phylogenetic Domains", *Journal of Bacteriology*, vol. 176, No. 19, p. 6148-6152.
- Trismilah, Deden dan Sumaryanto, 2005, 'Produksi Xilanase Pengaruh Komposisi Media Pada Produksi Xilanase dari *Bacillus stearotherophilus* DSM 22 Menggunakan Substrat Kulit Pisang', *Jurnal Saint dan Teknologi BPPT*, vol II, 66-69.

- Ullmann, A., 2001, “*E. coli* Lactose Operon”, *Encyclopedia of live sciences*, 1-6.
- Ulrich, J. T., McFeters, G. A., and Temple, K. L., 1972, “Induction and Characterization of β -Galactosidase in an Extreme Thermophile”, *Journal of Bacteriology: American Society for Microbiology*, vol 110, no. 2, USA, 691-698.
- Van den Burg, B., 2003, “Extremophiles as a Source for Novel Enzymes”, *Elsevier: Current Opinion in Microbiology*, 6, 213-218.
- Volk and Wheeler, 1993, “Mikrobiologi Dasar”, edisi kelima, jilid pertama, Erlangga, Jakarta, 18.
- Watson, J. D., Tooze, J., dan Kurtz, D.T., 1988, “DNA Rekombinan”, Erlangga, Jakarta 53-57.
- Winarno, F. G., 1983, “Enzim Pangan”, P. T. Gramedia, Jakarta, 61-62, 100.
- Wirahadikusumah, M., 1989, “Protein, Enzim dan Asam Nukleat”, ITB, Bandung, 34, 60-62.
- Yildirim, N., and Mackey, M. C., 2003, “Feedback Regulation The Lactose Operon: A Mathematical Modeling Study and Comparison with Experimental Data”, *Biophysical Society*, vol 84, 2841-2851.