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ISOLATION, PRODUCTION AND CHARACTERIZATION OF KERATINASE FROM *Bacillus* Sp. Khayat

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ISOLATION, PRODUCTION AND CHARACTERIZATION OF KERATINASE FROM *Bacillus* Sp. Khayat

By

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

ISOLATION, PRODUCTION AND CHARACTERIZATION OF KERATINASE FROM *Bacillus* Sp. Khayat

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Chairman : Associate Professor Mohd. Yunus Abdul Shukor, PhD

Faculty : Biotechnology and Biomolecular Science

The increase in demand of chicken meat products for human consumption has caused the accumulation of feather waste. In this research, seven local feather degrading bacteria have been isolated from soil and feather waste samples around Selangor and Johor, Malaysia. All the bacteria that obtained from the sampling procedure were then screened by incubating them in basal media that contained feathers as a carbon and nitrogen sources. Among the isolates, isolate E3 has shown the highest keratinolytic activity and feather degradation percentage compared to the others. Isolate E3 was then identified as *Bacillus* sp. khayat based on its 16s rRNA sequences. This strain produced keratinase optimally at a temperature of between 30 to 37^0C and at pH 8. The optimal tempature and pH for the bacterial growth were also found at 30 to 37^0C and at pH7.5 to 9 respectively. Studies using different carbon sources on keratinase production also showed that the addition of skim milk has enhanced enzyme production. The optimum concentration of skim milk for keratinase production was found at 0.2 gL^{-1}. The concentration of feather for optimum keratinase production was

determined at 1% (w/v) while for optimum growth at 0.5 to 1.5% (w/v). The bacterium was able to degrade up to 82.43% of feather in seven days with the highest keratinase production observed at the third day of incubation period. The keratinase enzyme from the bacterium was then purified through anion exchange chromatography, using DEAE cellulose as a matrix, and gel filtration chromatography, using Zorbax® column. The molecular weight analysis using SDS-PAGE gel revealed that the enzyme has a molecular weight of approximately 31.62 kDa. The optimum temperature and pH of the enzyme activity were 40^0C and pH 8 respectively. This enzyme can also retain over 80% of its original activity for one hour when preincubated at temperatures of between 20 to 45^0C and at pH 6.5 to 10. In the protease inhibitor study, the enzyme was greatly inhibited by the addition of PMSF compared to other inhibitors indicating that the enzyme is a serine type protease. The enzyme was also observed to be inhibited by the presence of all tested reducing agents such as DTNB, DTT, and 2-marcaptoethanol. All of tested metal ions such as $Zn^{2+,}$ Hg^{+}, Ag^{+}, Pb^{2+}, Mg^{2+}, Cu^{+}, K^{+}, Co^{2+}, and Ca^{2+} were found to give negative effect on keratinase activity. This keratinase was active against various types of proteinous subtrates either kerationous or unkeratinous proteins. However, the highest activity was observed when casein was used as the substrates, followed by soluble keratin, BSA, egg albumin, feather, wool, and human hair. The results of this study are very importance since they can be used to raise the potential of keratinase from *Bacillus* sp khayat in industrial applications.

Abstrak tesis yang dikemukan kepada senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PEMENCILAN, PENGHASILAN DAN PENCIRIAN KERATINAS DARIPADA *Bacillus* Sp. Khayat

Oleh

MOHD EZUAN BIN KHAYAT

Februari 2011

Pengerusi : Profesor Madya Mohd. Yunus Abdul Shukor, PhD

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Peningkatan permintaan orang ramai terhadap produk-produk berdasarkan daging ayam telah menyebabkan akumulasi sisa bulu ayam. Dalam kajian ini, tujuh bakteria pengurai bulu ayam telah dipencilkan daripada sampel tanah dan sisa bulu ayam di sekitar Selangor dan Johor, Malaysia. Kesemua bekteria yang diperolehi dari prosedur penyampelan kemudiannya disaring dengan dieramkan di dalam media asas yang mengandungi bulu ayam sebagai sumber karbon dan nitrogan. Dikalangan isolat tersebut, isolat E3 telah didapati mempunyai aktiviti keratinas dan peratusan penguraian bulu ayam tertinggi berbanding yang lain. Isolat E3 kemudiannya telah dikenalpasti sebagai *Bacillus* sp. khayat berdasarkan jujukan 16s rRNAnya. Strain ini menghasilkan keratinase secara optima pada suhu 30 hingga 37^0C dan pada pH 8. Pertumbuhan optima bakteria ini juga ditemui pada suhu diantara 30 hingga 37^0C dan pada pH 7.5 hinnga 9.0. Kajian menggunakan pelbagai sumber karbon juga menunjukkan penambahan susu skim ke dalam media meningkatkan penghasilan keratinase. Kepekatan optima susu skim untuk penghasilan keratinase tersebut ialah

0.2 gL^{-1}. Kepekatan bulu ayam untuk penghasilan optima keratinas juga telah ditemui pada 1% (w/v) manakala untuk pertumbuhan bakteria adalah pada kepekatan 0.5 hingga 1.5% (w/v). Bakteria tersebut mampu meghuraikan bulu ayam sehingga 82.43% dalam 7 hari. Penghasilan keratinas tertinggi pula ialah pada hari ketiga pengeraman. Keratinase daripada bakteria tersebut kemudian telah ditulenkan menggunakan kromatografi anion, dengan DEAE sellulosa sebagai matrik, dan kromatografi penurasan gel, menggunakan kolum Zorbax®. Analisis berat molekul meggunakan gel SDS-PAGE menunjukkan enzim tersebut mempunyai berat molekul lebih kurang 31.62 kDa. Suhu dan pH optimum untuk aktiviti enzim tersebut adalah masing-masing pada 40^0C dan pH 8. Enzim ini dapat megekalkan aktiviti asalnya lebih darpiada 80% apabila dieram dalam suhu 20 hingga 45^0C dan pH 6.5 hingga 10. Dalam kajian terhadap kesan perencat protease, aktiviti enzim ini telah direncat dengan banyaknya oleh PMSF dan ini menunjukkan ia tergolong dalam serine proteas. Aktiviti enzim ini juga didapati terencat dengan kehadiran kesemua agen-agen penurunan yang dikaji seperti DTNB, DTT, dan 2-marcaptoethanol. Kesemua ion logam yang dikaji seperti $Zn^{2+,}$ Hg^{+}, Ag^{+}, Pb^{2+}, Mg^{2+}, Cu^{+}, K^{+}, Co^{2+}, dan Ca^{2+} telah menunjukan kesan negatif terhadap aktiviti keratinas. Keratinas ini aktif terhadap berbagai-bagai jenis substrat protein sama ada protein keratin atau bukan keratin. Akan tetapi, aktiviti tertinggi telah diperhatikan apabila kasein digunakan sebagai substrat, diikuti oleh larutan keratin, albumin telur, bulu ayam, bulu kambing, dan rambut manusia. Hasil kajian ini adalah amat penting kerana dapat meningkatkan lagi potensi keratinase dari *Bacillus* sp khayat dalam aplikasi industri.

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I certify that an Examination Committee has met on date of viva voce to conduct the final examination of Mohd Ezuan Khayat on his Master of Science thesis entitled "Isolation, Production, and Characterization of Keratinase from Bacillus sp. khayat" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Putra Malaysia (Higher Degree) Regulation 1981. The committee recommends that the student be awarded the degree of Master of Science.

Members of Examination Committee were as follows:

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Name of Faculty
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This thesis was submitted to the senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently submitted for any other degree at Universiti Putra Malaysia or other institutions.

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MOHD EZUAN KHAYAT

Date: 21 February 2011

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