



UNIVERSITI PUTRA MALAYSIA

**ISOLATION OF *STAPHYLOCOCCUS CARNOSUS* AND *BACILLUS AMYLOLIQUEFACIENS* AS POTENTIAL BIOGENIC AMINE DEGRADERS
IN FISH SAUCE**

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FSTM 2011 14

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By

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**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirements for the Degree of Doctor of Philosophy**

November 2011

Abstract of thesis presented to the Senate of Universiti Putra Malaysia
in fulfillment of the requirement for the degree of Doctor of Philosophy

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Biogenic amines are basic nitrogenous compounds formed through amino acids decarboxylation by certain microorganisms. Their presence in foods is undesirable since they could induce poisoning incidence in susceptible consumers. The aims of this study were to determine occurrence of biogenic amines in fish sauce, to isolate bacteria from fish sauce and to examine their ability to produce and degrade biogenic amines. Effect of environmental factors on the growth and histamine degrading activity of selected isolates were also studied. Furthermore, selected isolates were applied to fish sauce fermentation to investigate their role in inhibiting biogenic amines accumulation. Fish sauce samples contain 62.5-393.3 ppm of histamine, 5.6-242.8 ppm of putrescine and 187.1-704.7 ppm of cadaverine. Bacterial isolates of fish sauce belonged to the genus of *Micrococcus*, *Staphylococcus*, *Bacillus* and *Streptococcus*. Most of those isolates are observed as producers of histamine, putrescine and cadaverine in differential agar medium.

Staphylococcus carnosus (FS19) is the only isolate that did not produced those three amines. Nevertheless, several bacterial isolates exhibited biogenic amines degradation activities in a buffer system. *Bacillus amyloliquefaciens* (FS05) and *Staphylococcus carnosus* (FS19) degraded histamine of up to 59.9% and 29.1% from its initial concentration, respectively. *Staphylococcus intermedius* (FS20) and *Bacillus subtilis* (FS12) degraded putrescine and cadaverine up to 30.4% and 28.9%, respectively. Since histamine is the most active amines and often related to the biogenic amines poisoning incidence, histamine degrading bacteria is of particular interest. Histamine degrading *Staphylococcus carnosus* (FS19) and *Bacillus amyloliquefaciens* (FS05) showed tolerance to a wide range of pH, temperature and sodium chloride concentration. *Staphylococcus carnosus* (FS19) grew optimally at pH 8, 35 °C in the presence of 9% sodium chloride. While *Bacillus amyloliquefaciens* (FS05) grew optimally at pH 7, 40 °C in the presence of 9% sodium chloride. *Staphylococcus carnosus* (FS19) optimally degrade histamine at pH 6, in the presence of 9% sodium chloride and at 40 °C. While *Bacillus amyloliquefaciens* (FS05) optimally degrade histamine at pH 7, in the presence of 12% sodium chloride and at temperature 35 °C. The effectiveness of *Staphylococcus carnosus* (FS19) and *Bacillus amyloliquefaciens* (FS05) as starter culture in inhibiting biogenic amines accumulation during fish sauce fermentation was further investigated. The pH value increased in all treated (with starter culture) and control (without starter culture) samples, while salt concentration remained constant throughout fermentation. Aerobic bacteria count decreased of about 3 log cycles during fermentation of fish sauce. Proteolytic bacterial count also decreased of about 2.5 log cycles during fermentation with no significant

difference ($p>0.05$) among the samples. The presence of histamine producing bacteria in fish sauce samples was considered to be indigenous from raw material or contamination during fermentation, since both cultures were non histamine producers. Concentration of amino acid histidine, arginine, lysine and tyrosine decreased at different rates throughout fermentation because they were converted into their respective biogenic amine. In general, histamine, putrescine, cadaverine and tyramine increased throughout the fermentation. However, their concentration were markedly higher ($p<0.05$) in control (without starter cultures) as compared to samples treated with starter cultures. Histamine concentration was reduced by 27.7% and 15.4% by *Staphylococcus carnosus* (FS19) and *Bacillus amyloliquefaciens* (FS05), respectively. Both cultures could also reduce other amines during fermentation. After 120 days of fermentation, the overall biogenic amines concentration was reduced to around 15.9% and 12.5% by *Staphylococcus carnosus* (FS19) and *Bacillus amyloliquefaciens* (FS05), respectively. These findings emphasized that the application of starter cultures with amines oxidase activities during fish sauce fermentation is effective in reducing accumulation of biogenic amines in the end product. Moreover, exploration of other biogenic amines degrading bacteria that exhibit the optimal growth and activities at much higher salt concentration will be more useful for the purpose.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PEMENCILAN *STAPHYLOCOCCUS CARNOSUS* AND *BACILLUS*
AMYLOLIQUEFACIENS SEBAGAI PENGURAI AMINA BIOGENIK
BERPOTENSI DALAM SOS IKAN**

Oleh

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Amina biogenik adalah sebatian nitrogen yang terhasil melalui aktiviti dekarboksilasi asid amino oleh mikroorganisma tertentu. Kewujudan mereka tidak diingini dalam makanan kerana boleh menimbulkan keracunan kepada pengguna. Matlamat kajian ini adalah untuk mengetahui kewujudan amina biogenik dalam sos ikan, untuk memencil bakteria dari sos ikan dan menguji kemampuan mereka dalam penghasilan dan penguraian amina biogenik. Kajian ini juga dimaksudkan untuk mengkaji pengaruh pelbagai julat faktor persekitaran terhadap tumbesaran dan aktiviti penguraian histamin oleh pencilan terpilih. Kajian diteruskan untuk mengkaji keupayaan pencilan terpilih untuk mengurai amina biogenik selama fermentasi sos ikan. Keputusan ujikaji menunjukkan bahawa sos ikan mengandungi histamin dalam julat 62.5-393.3 bahagian per juta (bpj), putresin dalam julat 5.6-242.8 bpj dan kadaverin dalam julat 187.1-704.7 bpj. Pencilan mikrobial yang dihasilkan dari sos ikan adalah tergolong dalam genus

Micrococcus, *Staphylococcus*, *Bacillus* and *Streptococcus*. Sebahagian besar mikrobia tersebut dikenal pasti sebagai penghasil histamin, putresin dan kadaverin. *Staphylococcus carnosus* (FS19) adalah satu-satunya pencilan yang tidak menghasilkan ketiga-tiga amina tersebut. Namun demikian, beberapa pencilan lain menunjukkan kebolehan mengurai amina biogenik dalam sistem bafer. *Bacillus amyloliquefaciens* (FS05) dan *Staphylococcus carnosus* (FS19) mampu mengurai histamin, masing-masing 59.9% dan 29.1% dari kepekatan awalnya dalam sistem bafer. Manakala, *Staphylococcus intermedius* (FS20) and *Bacillus subtilis* (FS12) mampu mengurai putresin dan kadaverin, masing-masing 30.4% dan 28.9% dari kepekatan awalnya. Oleh kerana histamin adalah amina yang paling aktif dan sering menjadi punca keracunan makanan, bakteria pengurai histamina menjadi kajian pilihan. *Staphylococcus carnosus* (FS19) dan *Bacillus amyloliquefaciens* (FS05) yang merupakan pendegradasi histamin mempamerkan toleransi terhadap pH, suhu, dan kepekatan garam dalam julat yang luas. *Staphylococcus carnosus* (FS19) mampu tumbuh secara optimal pada pH 8, suhu 35 °C dan kepekatan garam 9%. Manakala *Bacillus amyloliquefaciens* (FS05) tumbuh secara optimal pada pH 7, suhu 40 °C dan kepekatan garam 9%. *Staphylococcus carnosus* (FS19) mengurai histamin secara optimal pada pH 6, suhu 40 °C dan kepekatan garam 9%. Manakala *Bacillus amyloliquefaciens* (FS05) mengurai histamin secara optimal pada pH 7, suhu 35 °C dan kepekatan garam 12%. Kajian ini dilanjutkan untuk mengkaji keupayaan kedua-dua kultur bakteria dalam merencat akumulasi amina biogenik semasa fermentasi sos ikan. Nilai pH bagi semua sampel sos ikan didapati meningkat, manakala kepekatan garam adalah tetap sepanjang fermentasi. Jumlah kiraan bakteria aerob berkurang

dalam anggaran 3 kitaran log semasa proses fermentasi. Bakteria proteolitik juga mengalami penurunan bilangan dalam anggaran 2.5 kitaran log. Kewujudan bakteria penghasil histamin adalah berpunca dari bahan mentah atau pencemaran semasa fermentasi kerana kedua kultur bakteria bukan penghasil histamin. Keputusan ujian asid amino menunjukkan corak penurunan yang berbeza dalam kepekatan histidin, arginin, lisin dan tirosin kerana mereka diubah menjadi amina biogenik yang terkait. Manakala kepekatan histamin, putresin, kadaverin dan tiramin menunjukkan corak peningkatan semasa fermentasi. Namun, kepekatan ketiga-tiga amina nyata lebih tinggi pada sampel kawalan berbanding dengan sampel yang diinokulasi dengan kultur bakteria. Kepekatan histamin berkurang sebanyak 27.7% dan 15.4% pada sampel yang diinokulasi dengan *Staphylococcus carnosus* (FS19) dan *Bacillus amyloliquefaciens* (FS05) masing-masing berbanding dengan sampel kawalan. Kedua-dua kultur juga boleh mengurangkan amina biogenik lain semasa fermentasi. Selepas 120 hari fermentasi, kepekatan amina biogenik secara keseluruhan menurun sekitar 15.9% dan 12.5% pada sampel yang diinokulasi dengan *Staphylococcus carnosus* (FS19) dan *Bacillus amyloliquefaciens* (FS05) masing-masing berbanding dengan sampel kawalan. Penemuan ini menekankan bahawa penggunaan kultur pemula dengan aktiviti oksidase amina dalam fermentasi sos ikan didapati berkesan dalam mengurangkan pengumpulan amina biogenik. Pencarian bakteria pengurait histamin yang lebih aktif pada kepekatan garam tinggi adalah penting dan bermanfaat dalam memenuhi tujuan tersebut.

ACKNOWLEDGEMENT

In the name of Allah, most Gracious, most Merciful. All praise and gratitude be to Allah for His blessing and love that allow me to complete this piece of work.

I would like to express my sincere gratitude to my supervisor, Professor Dr. Fatimah Abu Bakar, for all her invaluable advices and continuous guidance throughout my study and through the time I share. Thank you very much for your kindness, encouragement and for being so patient and understanding.

I would also like to express my sincere gratitude to my co-supervisor, Professor Dr. Jinap Selamat, for her advice and encouragement which help me to finish my study. Thank you very much for your care and for allowing me to work in your laboratory. My sincere gratitude also goes to Professor Dr. Jamilah Bakar as my co-supervisor for her continuous advice, support and motivation during my study.

I would never forget to express my heartfelt thanks to my beloved parents. To my late father, thank you very much for all your invaluable advice and teaching when I was young. Thanks for always inspiring and encouraging me to seek knowledge along lifetime. May Allah always shower your *ruh* with His blessing and love, Amin. To my beloved mother, thank you so much for your unconditional love, support and understanding. I will never have moved this far in my life without your blessing and endless love. To my sister and my late brother, bunches of thanks for supporting, accompanying me and being a good friend.

My thankfulness to Mrs. Mawar from LKIM for her kind helps to collect fish sauce samples from manufacturer site. My appreciations also go to Mr. Abdul Halim Abdul Rahman, Mr. Zulkefli Nordin, Mr. Hamezan Muhammad, Mrs. Norliza Othman and Mrs. Suraya Saad for their help and cooperation during laboratory experiments. I also extend my sincere thanks to all staff of Faculty of Food Science and Technology for whatever helps during my study.

My sincere thanks goes to all my dearest friends in the Laboratory of Food Safety and Quality II, especially Ang Swi See, pak Willy Pranata Widjaja and late pak Asep Edi Kusnadi for their friendship, sharing and helping hand during my study. I will always remember the laughter, jokes and sweet memories with all of you.

My warmest appreciation goes to my housemate Syaiful Bahri Panjaitan, Masanto Masyahit, Mohamad Shamzi Mohamed and Ahmad Nafi as well as my Indonesian fellows in UPM for all the nice moments to share and precious supports to give.

I would like to acknowledge the Ministry of Science, Technology and Innovation (MOSTI) of Malaysia for the financial support through the Universiti Putra Malaysia Graduate Research Fellowship (GRF).

Last but not least, a special appreciation and gratitude to anyone else whose name is not mentioned here for their invaluable help and encouragement making this piece of work feasible.

I certify that a Thesis Examination Committee has met on 14 November 2011 to conduct the final examination of Muhammad Zukhrufuz Zaman on his thesis entitled “Isolation of *Staphylococcus carnosus* and *Bacillus amyloliquefaciens* As Potential Biogenic Amines Degradar In Fish Sauce” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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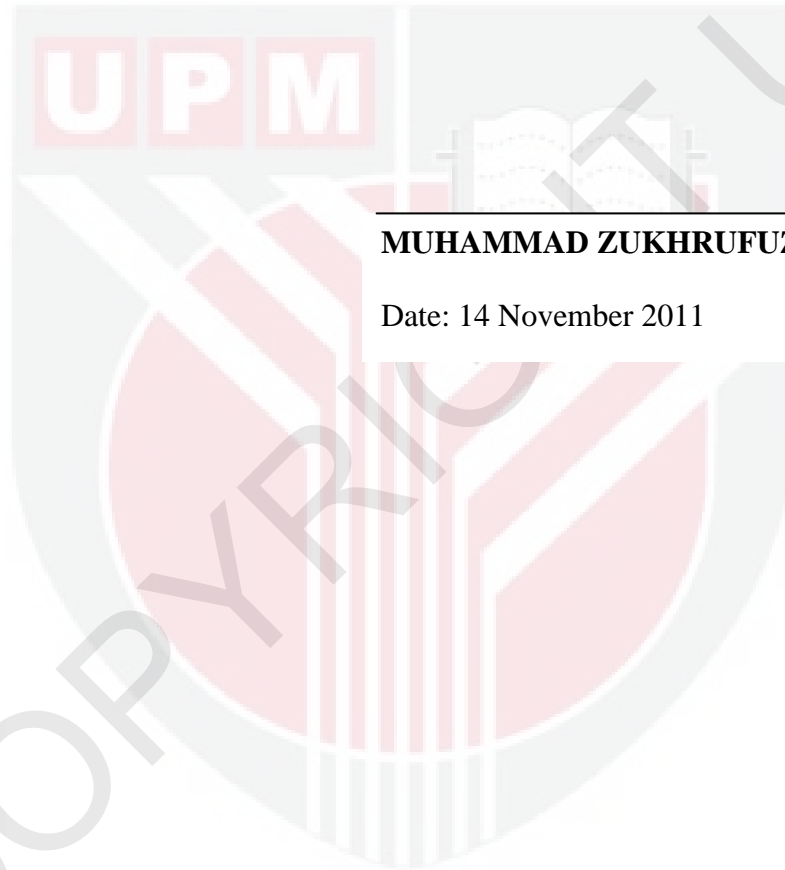
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DECLARATION

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 at any other institution.



MUHAMMAD ZUKHRUFUZ ZAMAN

Date: 14 November 2011



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