UNIVERSITI PUTRA MALAYSIA

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

MUHAMMAD ZUKHRUFUZ ZAMAN

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By

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

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Faculty : Food Science and Technology

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 produce 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.
Amino 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 mikrobia yang dihasilkan dari sos ikan adalah tergolong dalam genus...
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 menujukkan 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.
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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 Degrader 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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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

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