UNIVERSITI PUTRA MALAYSIA

STUDIES ON THE CHARACTERISATION AND UTILISATION OF A NEW PHYTASE-PRODUCING BACTERIUM ISOLATED FROM THE RUMEN OF CATTLE

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By

LAN GANQIU

Thesis Submitted in Fulfilment of the Requirements for the Degree of Doctor of Philosophy in the Institute of Bioscience
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This thesis is dedicated to

my late parents, my loving wife, Xiao Lin and my daughter, Di Yi.

Abstract of the thesis submitted to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Doctor of Philosophy

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Chairman: Professor Tan Sri Dato' Dr. Syed Jalaludin bin Syed Salim
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Five phytase-producing bacterial strains isolated from the rumen of cattle were identified to be a new bacterial species based on their morphological, physiological, biochemical and molecular characters. The new species is named *Mitsuokella jalaludinii*. *Mitsuokella jalaludinii* hydrolysed sodium phytate rapidly and the phytase production was strongly induced by phytate present in the medium. Rice bran (RB) and soybean milk (SM) were found to be the best carbon and nitrogen sources, respectively, for phytase production by *M. jalaludinii*. Phosphate at a level of 0.05 – 0.5% in RB-SM medium had no effect on phytase production. Glucose added to RB-SM medium had a negative effect on phytase production of *M. jalaludinii*. The optimum temperature and optimum initial pH for phytase production of *M. jalaludinii* were 39 °C and about 7.0, respectively.

The activity of *M. jalaludinii* phytase was highest at 55 – 60 °C and pH 4.0 – 5.0. It was specific to phytate as a substrate, significantly stimulated by Ba^{2+}, Mn^{2+}, and Ca^{2+} and significantly inhibited by Zn^{2+}, Cu^{2+}, Fe^{2+} and Fe^{3+}. The metal ion chelators and phosphate were not the inhibitors of *M jalaludinii* phytase activity.

Acute pathogenicity tests indicated that *M. jalaludinii* was non-pathogenic to chickens and mice. Supplementation of *M. jalaludinii* culture to corn soybean meal feed for chickens significantly increased P released from the feed *in vitro* and P, DM and CP digestibilities *in vivo*. About 70% of *in vivo* response in P digestibility and 90% of *in vivo* response in DM and CP digestibilities or AME value could be predicted by the P released *in vitro*. *Mitsuokella jalaludinii* phytase was most active in the crop of broiler chickens and was inactivated in the stomach.

Supplementation of either fresh active *M. jalaludinii* culture (AMJC) or freeze-dried active *M. jalaludinii* culture (FD-AMJC) or Natuphos® phytase to low-aP diet significantly improved the feed intake, body weight gain and feed conversion ratio of broilers. The digestibilities of DM, CP, P, Ca, and Cu and the AME value of diet were significantly increased by the supplementation of AMJC. Supplementation of AMJC or FD-AMJC or Natuphos® phytase to low-aP diet significantly increased the tibia ash content and serum P concentration but significantly reduced Mn concentration in tibia ash of broiler chickens. Chicks receiving FD-AMJC had better ($P<0.05$) feed conversion rate as compared to those receiving Natuphos® phytase. FD-AMJC supplementation to low-aP diet significantly ($P<0.05$) increased the AME value of diet and the digestibilities of DM, CP, P, Ca and Cu (11 to 13-day-old and 18 to 20-day-old chicks) but Natuphos® phytase supplementation only significantly improved the digestibilities of DM, P (11 to 13-day-old and 18 to 20-day-old chicks) and Ca (11 to 13-day-old chicks). Chicks receiving low-aP diet added with AMJC or FD-AMJC or Natuphos® phytase had similar ($P>0.05$) performance as those receiving normal-aP diet but excreted less ($P<0.05$) phosphorus.

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

KAJIAN MENGENAI PENCIRIAN DAN PENGGUNAAN SPESIS BAKTERIA BARU PENGHASIL FITASE DARI RUMEN LEMBU

Oleh

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Lima strain bakteria yang menghasilkan fitase telah dipencil dan dikenalpasti sebagai satu spesis baru berdasarkan ciri-ciri morfologi, fisiokimia dan molekul. Spesis ini dinamakan *Mitsuokella jalaludinii*. Bakteria ini menghidrolisis sodium fitate dan penghasilan fitase diaruhi oleh fitate yang terdapat di dalam medium pertumbuhan. Dedak padi (RB) dan susu kacang soya (SB) merupakan sumber utama karbon dan nitrogen. Penambahan glukosa pada medium RB-SB tidak memberikan kesan positif dalam penghasilan fitase. Suhu optimum penghasilan fitase adalah 39°C dan pH optimum, 7.0. Aktiviti fitase paling tinggi pada suhu 55 - 60°C dan pada pH 4-5. Aktivitinya spesifik terhadap fitat sebagai substrat, dirangsang oleh Ba^{2+}, Mn^{2+} dan Ca^{2+}, dan dihalang oleh Zn^{2+}, Cu^{2+}, Fe^{2+} dan Fe^{3+}. Ion galian dan P didapati tidak merencat aktiviti fitase.

Ujian patogenisiti akut menunjukkan *Mitsuokella jalaludinii* tidak patogenik terhadap ayam dan tikus. Penambahan kultur *Mitsuokella jalaludinii* ke dalam makanan ayam yang mengandungi jagung dan kacang soya, meningkatkan pembebasan P secara *in vitro*, dan meningkatkan penghadaman P, DM dan CP

secara *in vivo*. Lebih kurang 70% penghadaman P *in vivo* dan 90% penghadaman DM dan CP *in vitro* atau nilai AME boleh diramalkan dari P yang dibebaskan secara *in vitro*. Fitase yang dihasilkan oleh *Mitsuokella jalaludinii* paling aktif pada tembolok ayam tetapi tidak aktif di bahagian perut.

Penambahan kultur *Mitsuokella jalaludinii* dalam bentuk kultur aktif-segar (AMJC) dan kultur aktif-kering-beku (FD-AMJC) serta 'Natuphos® phytase' ke diet rendah-fosfat meningkatkan pengambilan makanan, penambahan berat badan dan nisbah penukaran makanan ayam. Penghadaman DM, CP, P, Ca dan Cu serta nilai AME diet meningkat ($P < 0.05$) dengan penambahan AMJC. Penambahan samada AMJC, FD-AMJC atau 'Natuphos® phytase' pada diet rendah-fosfat, meningkatkan kandungan abu tibia dan P serum tetapi mengurangkan Mn abu tibia ayam. Ayam yang di beri penambahan FD-AMJC menunjukkan nisbah penukaran makanan yang lebih baik daripada ayam yang di beri penambahan Natuphos® phytase. Penambahan FD-AMJC pada diet rendah-fosfat meningkatkan ($P<0.05$) nilai AME serta penghadaman DM, CP, P, Ca dan Cu (pada ayam umur 11–13 hari dan 18 –20 hari) tetapi penambahan 'Natuphos® phytase' hanya meningkatkan penghadaman DM, P (pada ayam umur 11– 13 hari dan 18 – 20 hari) dan Ca (pada ayam umur 11– 13 hari). Ayam yang diberi diet rendah-fosfat yang ditambah AMJC atau FD-AMJC atau 'Natuphos® phytase' menunjukkan prestasi yang sama ($P > 0.05$) dengan ayam yang di beri diet normal-fosfat tetapi P kurang ($P < 0.05$) dikeluarkan.

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