เอกสารแนบที่ 1

บันทึกข้อความ

ส่วนราชการ คณะวิศวกรรมศาสตร์ มหาวิทยาลัยอุบลราชธานี โทร.3343

ที่ ศธ 0529.8.3/219 **วันที่** 25 ธ.ค. 2552

เรื่อง ขออนุมัติงบประมาณสนับสนุนเพื่อเสนอบทความวิจัยในงานประชุมวิชาการนานาชาติ

เรียน รองคณบดีฝ่ายวิจัยและบริการวิชาการ

ตามหนังสือที่ ศธ 0529.8.1/6260 ลงวันที่ 2 ธันวาคม 2552 เรื่องขออนุมัติรับทุนการเสนอผลงานวิจัย/ผลงานทางวิชาการ แบบ Oral Presentation ในต่างประเทศ ปีงบประมาณ 2553 มหาวิทยาลัยโดยอธิการบดีได้อนุมัติและสนับสนุนงบประมาณให้ ดร.กรรณิกา รัตนพงศ์เลขา เข้าร่วมเสนอบทความวิจัย ในระหว่างวันที่ 26-28 กุมภาพันธ์ 2553 (โดยเดินทางออกจากมหาวิทยาลัยตั้งแต่วันที่ 25 กุมภาพันธ์ 2553 และเดินทางกลับถึงมหาวิทยาลัยวันที่ 1 มีนาคม 2553) ตามความทราบแล้วนั้น

ทุนดังกล่าวอนุมัติเป็นจำนวนเงิน 40,000 บาท (-สี่หมื่นบาทถ้วน-) แต่จากประมาณการค่าใช้จ่ายการนำเสนองานในครั้งนี้มีค่าใช้จ่ายรวมเป็นเงิน 83,530 บาท (แปดหมื่นสามพันห้าร้อยสามสิบบาทถ้วน) ดังเอกสารแนบ ดังนั้น ภาควิชาวิศวกรรมเคมี จึงใคร่ขออนุมัติงบประมาณเพิ่มเติมโดยเบิกจ่ายตามจริงแต่ไม่เกิน 40,000 บาท เพื่อให้การนำเสนอผลงานครั้งนี้ราบรื่นและสร้างชื่อเสียงแก่มหาวิทยาลัยอุบลราชธานีและประเทศไทยต่อไป

จึงเรียนมาเพื่อโปรดพิจารณา

(ผู้ช่วยศาสตราจารย์ ดร.ไพรัตน์ แก้วสาร)
รักษาราชการแทนหัวหน้าภาควิชาวิศวกรรมเคมี

บันทึกข้อความ

ส่วนราชการ โครงการจัดตั้งกองส่งเสริมการวิจัย ฯ สำนักงานอธิการบดี โทร.3035,3042

ที่ ศธ 0529.1.4/ 2755 **วันที่** 14 ธ.ค. 2552

เรื่อง ขอแจ้งผลการพิจารณาขอรับทุนการเสนอผลงานวิจัย/ผลงานทางวิชาการ แบบ Oral Presentation ในต่างประเทศ ประจำปีงบประมาณ 2553

เรียน คณบดีคณะวิศวกรรมศาสตร์

ตามบันทึกข้อความคณะวิศวกรรมศาสตร์ที่ ศธ 0529.8.1/6260 ลงวันที่ 2 ธันวาคม 2552 หน่วยงานของท่านได้เสนอขอรับทุนการเสนอผลงานวิจัย/ผลงานทางวิชาการ แบบ Oral Presentation ในต่างประเทศของ ดร.กรรณิกา รัตนพงศ์เลขา ความละเอียดทราบแล้วนั้น

มหาวิทยาลัยอุบลราชธานี ยินดีให้ทุนดังกล่าว **ในวงเงินตามที่จ่ายจริงไม่เกิน 40,000 บาท (สี่หมื่นบาทถ้วน)** ตามประกาศมหาวิทยาลัยอุบลราชธานี เรื่อง การรับสมัครทุน และหลักเกณฑ์การใช้เงินกองทุนวิจัย มหาวิทยาลัยอุบลราชธานี ประจำปีงบประมาณ 2553 **จะสามารถเบิกค่าใช้จ่ายได้ตามประกาศฯ คือ 1) ค่ายานพาหนะระหว่างประเทศ 2) ค่าลงทะเบียน และ 3) ค่าเช่าที่พักในต่างประเทศเท่านั้น**

ทั้งนี้ ผู้ได้รับทุนจะต้องปฏิบัติตามเงื่อนไขผู้ได้รับทุน และดำเนินการตามขั้นตอนทุนการเสนอผลงานวิจัย/ผลงานทางวิชาการ แบบ Oral Presentation ในต่างประเทศ รายละเอียดและแบบฟอร์มสามารถ Download ได้ที่ http://www.ubu.ac.th/~research/index_research.php?page=form หัวข้อ "แบบเสนอขอทุนเผยแพร่ผลงานวิจัย"

จึงเรียนมาเพื่อโปรดทราบ และโปรดแจ้งผู้เกี่ยวข้องทราบเพื่อดำเนินการ

(ผู้ช่วยศาสตราจารย์ ดร.กังวาน ธรรมแสง)
รองอธิการบดีฝ่ายวิจัยและบริการวิชาการ

(นางรุจิรา โชคสวัสดิ์)
เจ้าหน้าที่บริหารงานทั่วไป
15 ธ.ค. 2552

แบบ วจ-1ค

บันทึกข้อความ

ส่วนราชการ..... โทร. .....

ที่ศธ 0529.2.01/ 6660 วันที่ 2 S.A. 2552

เรื่อง ขออนุมัติรับทุนการเสนอผลงานวิจัย/ผลงานทางวิชาการ แบบ Oral Presentation ในต่างประเทศ ประจำปีงบประมาณ 2553

1) เรียน รองอธิการบดีฝ่ายวิจัยและบริการวิชาการ (ผ่านคณบดี)

ด้วยข้าพเจ้า น.ส.กรรณิการ ชัยหงวนเอก ได้รับการตอบรับให้นำเสนอผลงานวิจัย/ผลงานทางวิชาการ เรื่อง The importance of Lactobacilli in contemporary food and pharmaceutical industry และ Investigation of a suitable growth strategy for optimization of intensive propagation and lactic acid production of selected strain of Lactobacillus yeni. แบบ Oral Presentation ในการประชุมวิชาการนานาชาติ 2010 International Conference on Chemical Engineering and Applications (ICCEA2010) ระหว่างวันที่ 26-28 กุมภาพันธ์ 2553 ประเทศ ฮ่องกง

จึงใคร่ขออนุมัติรับทุนการเสนอผลงานฯ จำนวน 64,480 บาท โดยมีค่าใช้จ่ายที่ให้การสนับสนุนตามประกาศ ดังนี้

☑ 1. ค่าลงทะเบียน จำนวนเงิน 24,500 บาท

☑ 2. ค่ายานพาหนะระหว่างประเทศ จำนวนเงิน 9980 บาท

☑ 3. ค่าเช่าที่พักในต่างประเทศ จำนวน 4 คืน อัตรา 7500 บาท/คืน จำนวนรวม 30,000 บาท

ข้าพเจ้าขอรับรองว่าผลงานเรื่องดังกล่าว ไม่เป็นส่วนหนึ่งของผลงานระดับบัณฑิตศึกษา และเป็นการนำเสนอผลงานในรูปแบบ Oral Presentation และจะมีการตีพิมพ์บทความของข้าพเจ้าใน Proceeding/ วารสารวิชาการระดับนานาชาติ ทั้งนี้ หากตรวจสอบทราบในภายหลัง ข้าพเจ้ายินดีคืนเงินที่ได้รับการสนับสนุนทั้งหมด พร้อมได้แนบหลักฐานประกอบการพิจารณา จำนวน 1 ชุด ตามลำดับ ดังนี้ ☑ 1. สำเนาหลักฐาน/หนังสือตอบรับการเข้าร่วมเสนอผลงาน แบบ Oral Presentation ☑ 2.สำเนาหลักฐานการรับผลงานเพื่อตีพิมพ์ใน Proceeding/ วารสารวิชาการ ☑ 3. หลักฐานการจัดประชุม ☑ 4. กำหนดการนำเสนอผลงานแบบ Oral Presentation ☑ 5. บทความที่จะนำไปเสนอต่อที่ประชุม

จึงเรียนมาเพื่อโปรดพิจารณา

(ลงชื่อ)..... ผู้ขอทุน
(น.ส.กรรณิการ ชัยหงวนเอก)
ตำแหน่ง อาจารย์

(ลงชื่อ).....
(รองศาสตราจารย์ พ.อ.สถาพร ...)
คณบดีคณะวิศวกรรมศาสตร์/ผู้บังคับบัญชา

2) เรียน รองอธิการบดีฝ่ายวิจัยและบริการวิชาการ ผ่านหัวหน้างาน ตรวจสอบแล้ว เห็นควร ☑ เสนออธิการบดีเพื่อพิจารณาอนุมัติทุน ☐ แจ้งกลับต้นสังกัด เนื่องจากขัดประกาศฯ ..... (ลงชื่อ)..... ผู้ตรวจสอบ (นางสาวปัญจิรา ศุภดล) ตำแหน่ง นักวิเคราะห์นโยบายและแผน วันที่ 3 ธ.ค. 2552	3) เรียน รองอธิการบดีฝ่ายวิจัยและบริการวิชาการ เพื่อโปรดพิจารณา เห็นควร ☑ เสนออธิการบดีเพื่อพิจารณาอนุมัติให้ทุนจากกองทุนวิจัย จำนวน 40,000 บาท ☐ แจ้งกลับต้นสังกัด เนื่องจาก ขัดประกาศฯ (ลงชื่อ)..... (นายสุภวัฒน์ โสวรรณี) ตำแหน่ง รักษาการในตำแหน่งหัวหน้ากองส่งเสริมการวิจัยฯ วันที่ 3 ธ.ค. 2552
☐ 4) แจ้งกลับต้นสังกัด ☑ 4) เรียน อธิการบดี เพื่อโปรดพิจารณาเห็นควรอนุมัติให้ทุน จากกองทุนวิจัย จำนวน 40000 บาท (ลงชื่อ)..... (ผู้ช่วยศาสตราจารย์ ดร.กังวาน ธรรมแสง) ตำแหน่ง รองอธิการบดีฝ่ายวิจัยและบริการวิชาการ วันที่ 3 ธ.ค. 2552	5) อนุมัติตามเสนอ (ลงชื่อ)..... (ศาสตราจารย์ ดร.ประกอบ วิโรจนกูฏ) ตำแหน่ง อธิการบดีมหาวิทยาลัยอุบลราชธานี วันที่ 1 1 ธ.ค. 2552

ประมาณการค่าใช้จ่ายการนำเสนอผลงานวิจัย

รายการ	จำนวนเงิน (บาท)
1. ค่าโดยสารเครื่องบิน อบ.-กทม.-อบ. (จ่ายตามจริง)	7,050
2. ค่าโดยสารเครื่องบินระหว่างประเทศ กทม.-สิงค์โปร์-กทม.(จ่ายตามจริง)	9,980
3. ค่าเดินทางภายในต่างประเทศ (ตามที่จ่ายจริง)	3,000
4. ค่าพาหนะรับจ้างระหว่างบ้านพัก-สนามบินในประเทศ	600
5. ค่าเช่าที่พักในต่างประเทศ (7,500 บาท x 4 วัน)	30,000
6. ค่าเบี้ยเลี้ยงในต่างประเทศ (2,100 บาท x 4 วัน)	8,400
7. ค่าลงทะเบียน (700 USD)	24,500
รวมค่าใช้จ่ายทั้งสิ้น	**83,530**

เอกสารหมายเลข 5.1.1

Notification of Acceptance CCEA 2010

February 26-28, 2010, Singapore

http://www.iacsit.org/ccea/index.htm

Dear M.P.Zacharof, R.W. Lovitt and K. Ratanapongleka,

Paper ID : A002

Paper Title : Investigation of a suitable growth strategy for optimisation of intensive propagation and lactic acid production of selected strains of *Lactobacillus* genus

The review process of the 2010 International Conference on Chemical Engineering and Applications (CCEA 2010) has been completed. Based on the recommendations of the reviewers and the Technical Program Committee, about 35% of the total submissions have been accepted for publication. Based on the recommendations of the reviewers and the Technical Program Committee, we are pleased to inform you that your paper identified above has been accepted for oral presentation. You are cordially invited to present the paper orally at CCEA 2010 to be held on February 26-28, 2010, Singapore.

Your paper, if registered, will be published in the CCEA 2010 Conference Proceedings, which will be indexed by Thomson ISI, British Library and Nelson.

Besides the conference proceedings, 20 Papers will be selected from the registered ones, which should be with at least 30% new content and different titles, and at least 6 pages, to be published in the International Journal of Chemical Engineering and Applications (IJCEA. ISSN: 2010-0221) free of charge. The selection result of these papers will be announced after the registration process.

This notification email serves as our formal acceptance of your paper as well as an invitation to present your work at CCEA 2010. Please note that this email will be sent to your co-author(s) (if any), as well.

(Important) Only registered papers will be published in the conference proceeding. So in order to register the conference successfully, you must finish following FIVE steps.

1. Fill the Copyright Release Form.
http://www.iacsit.org/ccea/CCEA%20Copyright%20Form.doc

2. Format your paper according to the Template carefully.
http://www.iacsit.org/ccea/CCEA.template.doc

3. Download and fill the Registration Form.
http://www.iacsit.org/ccea/CCEA%20Registration.doc

4. Finish the payment of Registration fee at the Bank. (The bank transfer information can be found in the Registration form)
http://www.iacsit.org/ccea/CCEA%20Registration.doc

5. Send your filled copyright form, final papers (both .doc and .pdf format), filled registration form (.doc format), the scanned payment (in jpg format) to us at ccea@vip.163.com. (Before December 15, 2009).

Maybe some unforeseeable events could prevent a few authors not to attend the event to present their papers, so if you and your co-author(s) could not attend CCEA 2010 to present your paper for some reasons, please inform us. And we will send you, the official receipt of registration fee, proceedings, and/or other materials after CCEA 2010 free of charge.

Please strictly adhere to the format specified in the conference template while preparing your final paper. If you have any problem in preparing the final paper, please feel free to contact us via ccea@vip.163.com. For the most updated information on the conference, please check the conference website at http://www.iacsit.org/ccea/index.htm. The Conference Program will be available at the website in Late January, 2010.

Finally, we would like to further extend our congratulations to you and we are looking forward to meeting you in Singapore.

Yours sincerely,

CCEA 2010 Organizing Committees
Singapore
http://www.iacsit.org/ccea/index.htm

เอกสารหมายเลข 5.1-1

Notification of Acceptance CCEA 2010

February 26-28, 2010, Singapore

http://www.iacsit.org/ccea/index.htm

Dear Myrto-Panagiota Zacharof, Karnika Ratanapongleka and Robert W. Lovitt,

Paper ID : A003

Paper Title : The importance of Lactobacilli in contemporary food and pharmaceutical industry

The review process of the 2010 International Conference on Chemical Engineering and Applications (CCEA 2010) has been completed. Based on the recommendations of the reviewers and the Technical Program Committee, about 35% of the total submissions have been accepted for publication. Based on the recommendations of the reviewers and the Technical Program Committee, we are pleased to inform you that your paper identified above has been accepted for oral presentation. You are cordially invited to present the paper orally at CCEA 2010 to be held on February 26-28, 2010, Singapore.

Your paper, if registered, will be published in the CCEA 2010 Conference Proceedings, which will be indexed by Thomson ISI, British Library and Nelson.

Besides the conference proceedings, 20 Papers will be selected from the registered ones, which should be with at least 30% new content and different titles, and at least 6 pages, to be published in the International Journal of Chemical Engineering and Applications (IJCEA. ISSN: 2010-0221) free of charge. The selection result of these papers will be announced after the registration process.

This notification email serves as our formal acceptance of your paper as well as an invitation to present your work at CCEA 2010. Please note that this email will be sent to your co-author(s) (if any), as well.

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1. Fill the Copyright Release Form.
http://www.iacsit.org/ccea/CCEA%20Copyright%20Form.doc

2. Format your paper according to the Template carefully.
http://www.iacsit.org/ccea/CCEA.template.doc

3. Download and fill the Registration Form.
http://www.iacsit.org/ccea/CCEA%20Registration.doc

4. Finish the payment of Registration fee at the Bank. (The bank transfer information can be found in the Registration form)
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5. Send your filled copyright form, final papers (both .doc and .pdf format), filled registration form (.doc format), the scanned payment (in jpg format) to us at ccea@vip.163.com. (Before December 15, 2009).

2.2 Inoculum source

All the *Lactobacilli, ,Lactobacillus casei NCIMB 11970 Lactobacillus plantarum NCIMB 8014, Lactobacillus lactis NCIMB 8586* and *Lactobacillus delbruckii subsp.bulgaricus NCIMB 11778* were provided in a lyophilised form by National Collection of Food and Marine bacteria(NCIMB) , Aberdeen , Scotland.

2.2 Growth Experiments

2.2.1 Preliminary Growth experiments

Pyrex glass pressure tubes sealed with butyl rubber stoppers and aluminium seals were used to test the effect of basal and optimum on *Lactobacilli* growth. The tubes were prepared under aseptic and anaerobic conditions. The media recipe for the basal medium is glucose 2% w/v, yeast extract 1.5% w/v, peptone 1% w/v, sodium acetate, 0.5% w/v, tri-sodium citrate 0.2%, potassium hydrogen phosphate 0.2% w/v, MgSO4 0.02% w/v , MnSO4 0.002% w/v and resazurin dye 0.0005% v/v. The same recipe was followed for *L.delbruckii* although glucose was replaced with lactose, yeast and Tween 80 was added to the medium due to the extensive auxotrophic needs of the bacterium.
Each component was tested separately so to certify its influence on growth in a range of concentrations between 0% w/v to 4% w/v.
All the components were combined and an optimised medium was fabricated. The medium's recipe is glucose 2% w/v, yeast extract 2 w/v, sodium acetate, 1% w/v w/v,tri-sodium citrate 1% w/v, potassium hydrogen phosphate 0.5 w/v and resazurin dye 0.0005% v/v. For *L.delbruckii,* yeast extract 1%, peptone 1%, lactose 2% and Tween 80 0.1%.

2.2.2 Bench Device (Stirring Tank Reactor, STR)

A 2L Pyrex glass fermenter has been selected for the procedure. The fermenter was equipped with an hydrargiric thermometer for temperature control, a pH probe (Fischer Scientific, UK) for pH control, a magnetic stir bar for agitation, an glass aeration port, a sampling and inoculation port, a gas flow stainless steel port connected with a filter for gas sterilisation (Polyvent filter, 0.2μm, Whatman Filters, UK), a port for alkali/acid feed and stainless steel coils for heat emission. All the ports were made of stainless steel and were connected with plastic tubes of several lengths. The working volume of the reactor was set at 1.5L. The pH probe was connected with a pH controller apparatus (Electrolab FerMac 260, UK) which was calibrated with suitable acidic and alkali solutions (pH 4 and pH 7) to adjust the pH range. The gas filter was connected with a gaseous nitrogen flask via rubber tubes and the flow was set up at 50 ng/ml. The alkali feed port was connected with a plastic bottle containing an alkali solution of 100 ml of NaOH 1M which was placed on an electronic scale (Ohaus portable advanced, Switzerland) o to measure the volume of alkali/acid used for pH maintenance. The coils were connected with a water bath (Grant Water bath, UK) and a pump (Watson Marlow Digital, 505S, UK) for continuous preservation of steady temperature The fermenter was placed on a magnetic stirring plate (SM1, Stuart Scientific, UK) and was constantly stirred at 150 rpm as being anaerobic bacteria

2.4 Analytical methods

2.4.1 Measurement of cellular growth and biomass

The cellular growth was measured by placing the pressure tubes into a spectrophotometer fitted with a test tube holder (PU 8625 UV/VIS Philips, France) at 660 nm. The tube had a 1.8 cm. light path.

2.4.2 Measurement of Lactic acid amount and rate

Lactic acid productivity rate and the amount of lactic acid produced by each strain during the pH and temperature control fermentation are indirectly determined by the following theorem: 1 M of NaOH neutralises the effect of 1 M Lactic acid: According to this equation the amount of lactic acid produced is directly proportional to the amount of sodium hydroxide consumed during the fermentation process.

The rate of lactic acid produced is indirectly calculated by the following equation:

$$\frac{dp}{dt}(mM \quad / L / h) = \left(\frac{(Na\ ) * FR\ * M\ .W\ .}{V} \right) \qquad \text{(Equation 1)}$$

where Na is the moles of the alkali solution used, FR is the feeding rate of the alkali solution in the culture, M.W. is the molecular weight of lactic acid and V is the overall volume of the culture. (1)

3. Results and Discussion

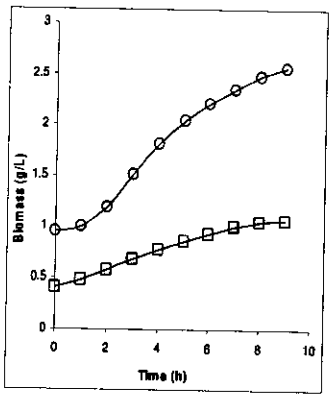
3.1 The effect of standard and optimum medium on Lactobacilli growth

Thus, to achieve the optimum maximum growth rate and of the bacteria and enhance their productivity, the bacilli were inoculated in a medium of liquid form containing all the optimised parameters. (2% glucose, 1.5% yeast extract, 0.5% potassium hydrogen phosphate, 1% tri-sodium citrate, 1%sodium acetate, lactose 2%, Tween 80 0.1%).

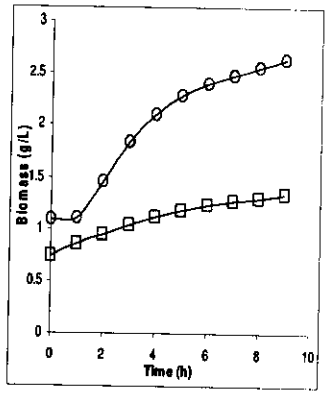
There has been an effort to use the same parameters in the optimised medium for the inoculation and intensive propagation of all the chosen *Lactobacilli.*, so to form a common economic simplified medium which will ameliorate distinctively the growth rates and the cellular yields of the bacteria.

The maximum growth rate of *L.casei* on the optimised medium was $0.24\,h^{-1}$ and the doubling time reduced to 2.87 h. The final biomass concentration was 2.43 g/l. When compared to the basal medium where maximum growth rate was 0.16 h^{-1} a significant increase in the maximum growth rate was achieved. Similarly the final cell concentration of the fermentation has been raised from 1.19 g/l to 2.43 g/l. The maximum growth rate of *L.plantarum* on the optimised medium was $0.30\,h^{-1}$ and the doubling time reduced to 2.30 h. The final biomass concentration was 2.61 g/l. When compared to the basal medium where maximum growth rate was 0.13 h^{-1} a significant high increase in the maximum growth rate was achieved. Similarly the final cell concentration of the fermentation has been raised from 1.32 g/l to 2.63 g/l. The maximum growth rate of *L.lactis* on the optimised medium was $0.22\,h^{-1}$ and the doubling time reduced to 3.13 h. The final biomass concentration was 1.81 g/l. When compared to the basal medium where maximum growth rate was 0.07 h^{-1} a significant high increase in the maximum growth rate was achieved. Similarly the final cell concentration of the fermentation has been raised from 0.69 to 1.81. The maximum growth rate of *L.delbruckiii* on the optimised medium was $0.32\,h^{-1}$ and the doubling time reduced to 2.13 h. The final biomass concentration was 3.00 g/l. When compared to the basal medium where maximum growth rate was 0.20 h^{-1} a significant increase in the maximum growth rate was achieved. Similarly the final cell concentration of the fermentation has been raised from 1.80 g/l to 3.00 g/l.

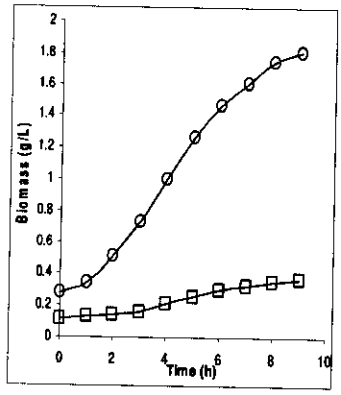
The optimised medium will be used as a medium for further investigation. (Figures 1. 2. 3.4)



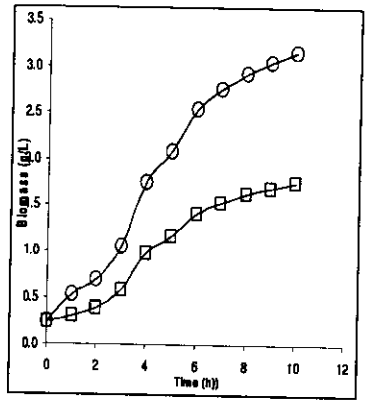
Fg.1. Growth of *L.casei* on basal and optimised (○) media



Fg.2. Growth of *Lplantarum* on basal (□) and optimised (○) media



Fg.3Growth of *Llactis* on basal (□) and optimised (○) media



Fg.4. Growth of *L.delbruckii* on basal (□) and optimised (○) media

3.2 Growth of Lactobacilli on a STR

In order to obtain a better maximum growth rate and higher growth yields and improved productivity a pH controlled STR system was developed. As to investigate the influence of pH over growth in terms of growth rate, doubling time and product and biomass yields the system was operated with a continuous pH control maintenance system. The influence of pH was tested in a range of highly acidic (4) to neutral (7) pH. All the process was performed in batch mode. The optimised medium was used.(3)

The results of the experiments are shown in Figures 5, 6, 7 and 8. and Table 1 There is a strong correlation between the pH and the growth of the bacilli. The maximum growth rate was enhanced when the culture was controlled at pH 5.5, 6.5 and 7. Maintenance of pH on a steady state throughout the 10 h fermentation process was combined with the use of the optimised liquid medium gave highest biomass yields and maximum growth rates as compare to the uncontrolled pH growth systems. It can be also observed that on acidic pH values of 4 and 4.5, the growth of the bacilli is strongly inhibited. The amount of lactic acid produced by the bacilli was identified as being equal to the amount of NaOH used for pH maintenance. Over the 10 h fermentation the pH 5, 5.5 and 6 the bacilli were still growing as they had slower maximum growth rates and long lag periods prior to growth.

Samples were measured on an hourly basis and they were analysed for biomass, pH and in some occasions the glucose and the end product were also analysed. The effect of reduced pH is strong where no growth was observed at pH 4 and pH 4.5.The optimum pH was 6.5 in the conditions studied for *Lactobacillus casei NCIMB 11970 Lactobacillus plantarum NCIMB 8014, Lactobacillus lactis NCIMB 8586.* Though for *Lactobacillus delbruckii subsp.bulgaricus NCIMB 11778* pH 5.5 was set as the optimum pH condition.

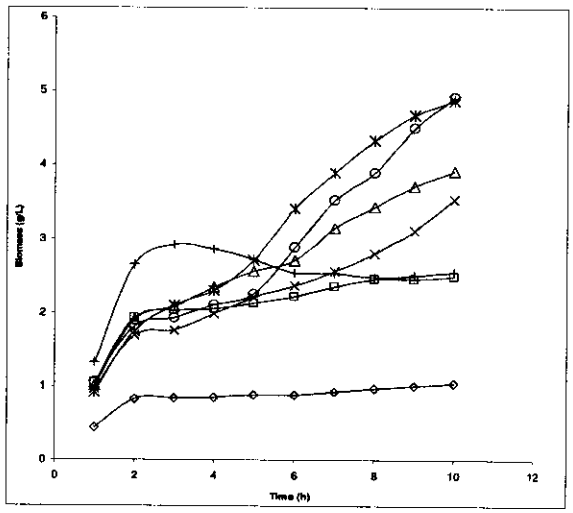


Figure 7 Growth of L.lactis on Different pH range in a 2L STR, Growth(◊) on pH 4, Growth (□) on pH 4.5, Growth (Δ) on pH 5 Growth (x) on pH 5.5, Growth (*) on pH 6, Growth (○) on pH 6.5, Growth on pH 7(+)

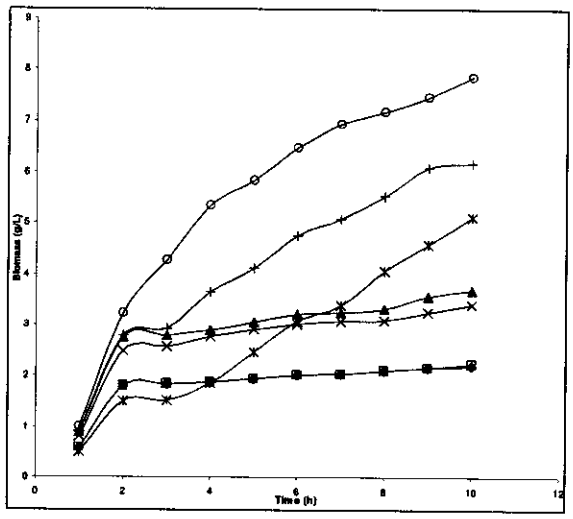


Figure 8 Growth of L.ldelbruckii on Different pH range in a 2L STR Growth(+) on pH 4, Growth (□) on pH 4.5, Growth (⌂) on pH 5, Growth (Δ) on pH 5.5, Growth (*) on pH 6, Growth (○) on pH 6.5 Growth on pH 7(◊)

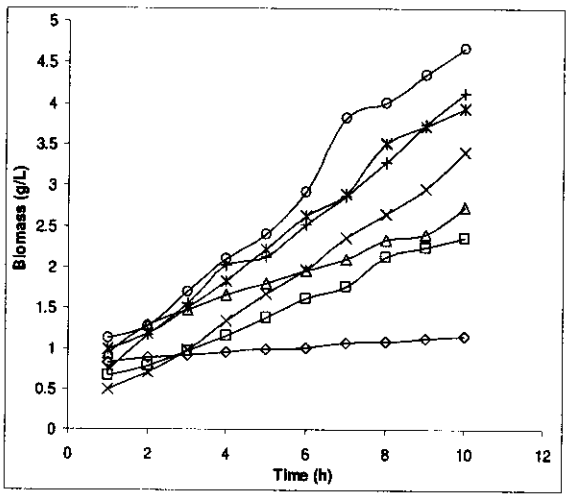


Figure 7 Growth of L.lactis on Different pH range in a 2L STR, Growth(◊) on pH 4, Growth (□) on pH 4.5, Growth (Δ) on pH 5 Growth (x) on pH 5.5, Growth (*) on pH 6, Growth (○) on pH 6.5, Growth on pH 7(+)

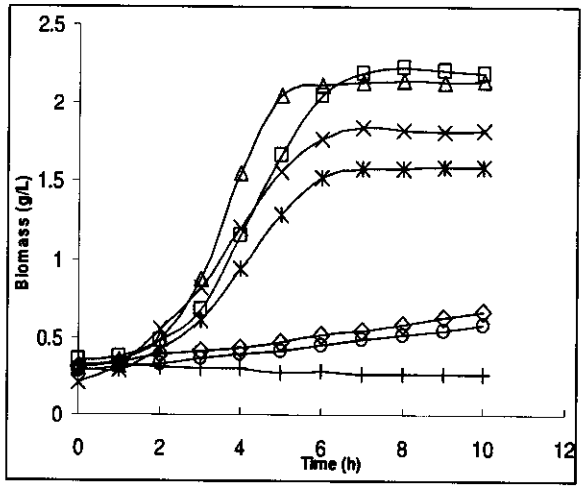


Figure 8 Growth of L.ldelbruckii on Different pH range in a 2L STR Growth(+) on pH 4, Growth (□) on pH 4.5, Growth (⌂) on pH 5, Growth (Δ) on pH 5.5, Growth (*) on pH 6, Growth (○) on pH 6.5 Growth on pH 7(◊)

Selected Strains	pH	*Rate of Lactic acid produced (mM/L/h)*	*Total amount of Lactic acid produced (mM)*	Selected Strains	pH	*Rate of Lactic acid produced (mM/L/h)*	*Total amount of Lactic acid produced (mM)*
L.casei	4	9.94	50	*L.plantarum*	4	1.09	2
	4.5	8.45	30		4.5	1.24	4
	5	125.2	500		5	5.73	23
	5.5	181.32	650		5.5	8.89	48.25
	6.0	158.31	715		6.0	136.2	407
	6.5	239.98	900		6.5	161.5	613
	7	2.82	4		7	133.52	556
L.lactis	4	4.9	23	*L.delbruckii*	4	8.34	45
	4.5	2.8	8		4.5	17.59	70
	5	7.2	37		5	98.78	465
	5.5	127.99	487		5.5	115.36	514
	6	165.33	543		6.0	100.33	498
	6.5	183.23	563		6.5	74	185
	7	140.71	474.4		7	10.09	38

Table 1: Lactic acid production on different pH conditions on an STR

4. Conclusions

In this work, a new growth strategy to enhance lactic acid production and the growth of *Lactobacilli* was studied. Significant changes were notified when the optimized medium was used on the growth of *Lactobacilli*. Optimized pH conditions also reinforced the cellular growth and the productivity of lactic acid. Further research should be performed though to develop extraction techniques for lactic acid and test further the lactic acid productivity and the nutrient media.

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เอกสารอ้างอิง 5.1.2

The importance of *Lactobacilli* in contemporary food and pharmaceutical industry
A review article

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Abstract

Fermentation technology has been a widely researched and exploited field of the science of biotechnology. Through out the recent years the vast majority of microbial groups have been tested for the production of beneficial compounds especially for the replacement of products produced by petrol such as lactic acid. A bacterial group that heavily attracts attention due to its products are Lactic Acid Bacteria (LAB) and especially Lactobacilli. Lactobacilli are widely used in the food and pharmaceutical industry nowadays. These microorganisms have several distinguishing features based on their main ability to ferment carbohydrates such as the production of acids, enzymes and natural antimicrobial substances called bacteriocins. They are mainly used as natural acidifiers for the inoculation of bulk quantities of milk and vegetables in order to produce a variety of fermented products. As such, large quantities of their biomass and the end products of their metabolism are necessary. In this article some of the most important uses of Lactobacilli in the industry will be reviewed. Emphasis will be given in the production of lactic acid, β-galactosidase and lantibiotics through the usage of modern fermentation technology.

Keywords: LAB, fermentation technology, food industry, β-galactosidase, lactic acid,

1. Lactic Acid Bacteria and their Industrial Importance

Lactobacilli are Gram positive (+) bacteria, shaped as rods which belong to the group of LAB. They are natural habitants, rapidly colonising mammalian mucosal membranes such as oral cavity, intestine and vagina In general; they are found where rich carbohydrate sources are available such as plants and materials of plant origin for example sewage and fermenting or spoiled food. (Bernardeau *et al*, 2006) These bacteria, in the form of starter cultures are essential for many industrial processes in the food industry, mainly for the fabrication dairy and meat products, fermentation of plants and vegetables, brewing and wine making. (Cutting, Carr, & Whiting, 1975) *Lactobacilli* may affect the quality, flavour, odour and texture of the final product in either a favourable or a detrimental way. (Cutting, Carr, & Whiting, 1975) The genera important members of this group are *Lactobacillus, Leuconostoc, Pediococcus and Streptococcus*. These organisms are heterotrophic and generally have complex nutritional requirements due to lacking of many biosynthetic capabilities. Consequently, most species have multiple requirements for amino acids and vitamins. *Lactobacilli* are also considered to be probiotic bacteria. Probiotics are live microorganisms that exhibit beneficial effects on the host's health beyond inherent basic nutrition. (Rose, 1978) *Lactobacilli* fill in the major criteria that a microorganism should meet to be considered as a probiotic. They are surviving in a low pH environment, they are capable or surviving contact with digestive fluids and adhering to intestinal epithelial cells, they are non pathogenic to the host, they can work in multiple hosts, they have the ability of host multiplication and can easily colonise the gastrointestinal tract either permanently or temporarily and beneficially to the host and they survive in feedstuffs.Due4 to all the above abilities they are used in the production and packaging of foods (Bernardeau *et al*, 2006) *Lactobacilli* have proven to be effective against intestinal inflammation, maintenance of remission in Chron's disease, treatment of infections during pregnancy, prevention of urinary tract infections (Ahrne *et al.*, 2005) *Lactobacilli* distinctive ability is to produce lactic acid from carbohydrate sources, especially from lactose and glucose and many of them have been found to produce antimicrobial activity possessing molecules called bacteriocins. These compounds have gained major industrial interests due to their potential application to be used as natural preservatives (Rose, 1982, Board, 1983)

1.1 Application of *Lactobacilli* in the Contemporary Food industry Starter Cultures of Lactic Acid Bacteria (LAB)

Nowadays, LAB are constantly used in the food process industry in the form of starter cultures. Starter cultures, are carefully selected and propagated cultures of known strains of bacteria or yeasts in order to produce the suitable type of fermentation (homolactic, heterolactic, citrate etc.) .The starter cultures either consist of one pure strain of bacteria or yeasts or of a combination of strains of different microbial species.(Ross *et al.*, 2005)As previously referred, due to their distinctive ability to produce organic acids such as lactic acid and acetic acid from carbohydrates *Lactobacilli* are widely applied in the food industry. These two organic acids suppress pH below the growth range causing metabolic inhibition of most pathogenic bacteria. This means that these two organic acids are among the most widely employed preservatives, used also as antimicrobial compounds. (Gruger& Gruger, 1989)

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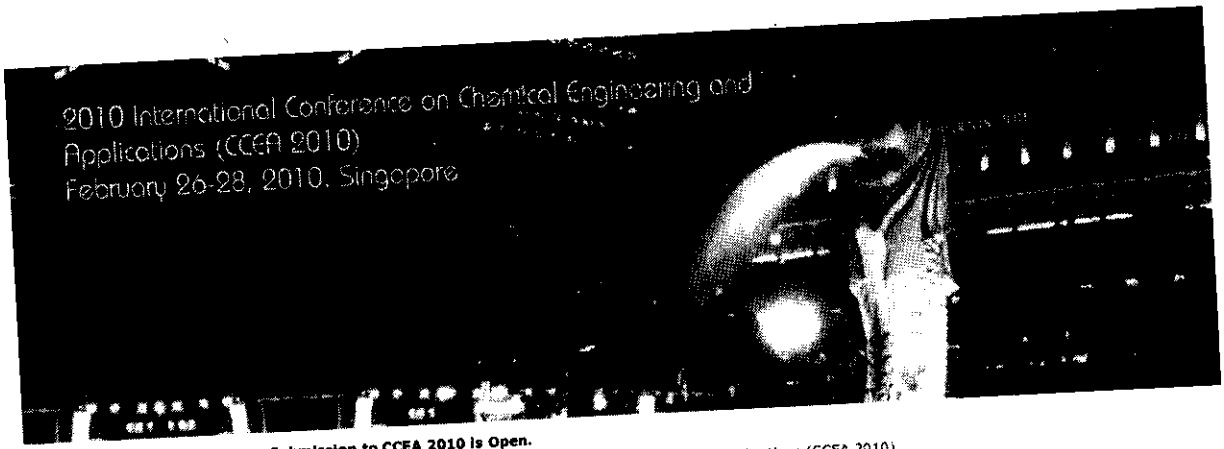
ตอบรับข้อ 5.1.3



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The importance of *Lactobacilli* in contemporary food and pharmaceutical industry A review article

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1.2 Application of *Lactobacilli* in Dairy Industry

Lactic Acid Bacteria (LAB) especially *Lactobacilli* are responsible for the formation of the microflora of most dairy products especially of cheese and fermented milk. *Lactobacilli* are important for flavour, colour and texture of dairy products through acidification due to lactic acid and of the metabolism of milk proteins. The most commonly used species in dairy products are *L.casei, L.helveticus, L.rhamnosus, L.lactis, L.curvatus and L.plantarum.* (Jack *et al.*, 1995) Furthermore, *Lactobacilli* are incorporated into yogurt, cheese and fermented milk as probiotics due to their beneficial effect especially on acute and chronic inflammations of the gastrointestinal tract. (Bernardeau *et al.*, 2006) In addition, due to the production of bacteriocins *Lactobacilli* also help on the preservation of dairy products. (Chen &Hoover, 2003)

1.3 Application of *Lactobacilli* on Wine Industry

Lactobacilli are also applied in wine industry both for grape and fruit wines, such as cider. The organic acids existing in wine which are mainly malic and tartaric acid can be easily metabolised by *Lactobacilli.* (Board, 1983) Malic acid is converted to lactic acid and carbon dioxide, this phenomenon is called malolactic fermentation which is extensively used for fruit wines maturation. (Liu *et al.*, 2003) If though tartaric acid is decomposed into pyruvic and citric acid complete spoilage of the selected food product occurs. So the appropriate choice of the fermenting *Lactobacilli* is necessary. Usually decomposition of tartare is observed *by Lactobacillus plantarum* and *Lactobacillus brevis* (Rose, 1982)

1.4 Application of *Lactobacilli* on non-beverage food products of plant origin

Lactobacilli are applied in the fermentation of sauerkraut that is the product of fresh cabbage. The starter culture for sauerkraut production is the normal flora of cabbage, in addition with *L.plantarum* and an amount of NaCl so to avoid the growth of pathogenic bacteria. (Miller & Litsky, 1976) Another fermented product where Lactobacilli are involved is pickles. Pickles are the fermented products of cucumbers. The desirable effect is again achieved by the propagation of an L.plantarum starter culture. *L.plantarum* is also involved in the fermentation of olives which follows a similar pattern with the fermentation of pickles and sauerkraut, the only difference being that it is slower and involves a lye treatment. (Bernardeau *et al.*, 2006)

2. Application of *Lactobacilli* in the Contemporary Pharmaceutical industry

2.1. The Production of Enzymes

Lactobacilli are well known for their role in the preparation of fermented dairy product, including yoghurt, cheese, butter, buttermilk and kefir and they are the important living bacteria in connection with lactose hydrolyzed in the present of β-galactosidase. B-galactosidase enzyme from lactic acid bacteria have been attractively attended because this bacteria group normally considered as safe so the enzyme derived from them might be used with no need of extensive purification and there are little or no adverse effects on fermented dairy products. Various strains of lactic acid bacteria have been recently researched for the enzyme for example;

Strains	Researchers	Comments
Lactobacillus acidophilus	Lin *et al.*, 1991; Noh and Gilliland, 1993; Gupta *et al.*, 1994; 1995; Montes *et al.*, 1995; Wang and Sakakibara, 1997	
Lactobacillus delbruckii subsp.bullgaricus	Ohmiya *et al.*, 1977; Wang and Sakakibara, 1997	highest enzyme activity was about 1.5 U/cm^3
Streptococcus Thermophilus	Greenberg and Mahoney, 1982	
Lactococcus lactis subsp.cremoris	Shah and Jelen, 1991	
Lactobacillus kefiranofaciens	Itoh *et al.*, 1992	
Lactobacillus helveticus	Wang and Sakakibara, 1997	
Lactobacillus brevis	Montanari *et al.*, 2000	release the enzyme imme after the end of cell multip and is connected to cell a and breakage of the cell wall
Lactobacillus plantarum	Fernandez *et al.*, 1999; Montanari *et al.*, 2000	
Lactobacillus crispatus	Kim and Rajagopal, 2000	

2.2 The Production of Lactic acid

2.2.1 Carbohydrate Metabolism by *Lactobacilli*

The purpose of *Lactobacilli* fermenting carbohydrate is primarily to achieve energy in the form of ATP capturing the primary sugar existing in the milk which is lactose. Fermentation can be described as a genre of anaerobic respiration where oxygen is **not** used as a final electron acceptor. In fermentation an organic molecule, in most cases a chemical intermediary accepts the electrons. In the case of *Lactobacilli* pyruvate is used as an electron receptor and nitrogen as an electron acceptor, being able to accept the electrons and the proton from NADH. This process is done via NAD which exists in a very small rate within the cell cytoplasm and has to be constantly regenerated so that glycolysis can continue. (Alcamo, 1997).Many circles of oxidation are required to give rapid metabolism of sugars. (Paul-Ross et al., 2002)Fermentation process can be inhibited by the Pasteur Effect which means the inhibition of glycolysis by the presence of oxygen. (Atlas & Bartha, 1993)Lactose or any other disaccharide in order to be catabolised has to be transferred into the internal of the cell. Lactobacilli use the Phosphoenolpyruvate: carbohydrate phosphotransferace system (PTS system) which is located in the cellular membrane of the bacilli. (Stanier &Gunsulus, 1961; Gerhard, 1979)Then, the primary step is the conversion of lactose into galactose and glucose. This is done by the enzyme β-galactosidase which belongs to the family of oxidases and cleaves off the β-oxygen bonded attachments to galactose. (Fytou-Pallikari, 1997). In L.lactis β-galactosidase is not strongly bonded with the cell wall but it floats freely within the cell. *Lactobacilli* catabolise glucose to pyruvate acid by the Embden-Meyerhof (EMP) glycolytic pathway and galactose by the Leloir pathway. The pathways are connected via phosphate-6-glucose which is the final end product in the Leloir pathway and through this form can enter the glycolysis pathway and be further converted to pyruvate acid. (Davidson &Sittman, 1999)

2.2.2 Conversion of Pyruvate to Lactic Acid

Pyruvate acid is the anion form of pyruvic acid (CH_3COCOO^-).Is an alpha-keto acid of the keto-acid group and under the presence of the enzyme lactate dehydrogenase (LDH) and the coenzyme NAD. Pyruvate is the major end product of glycolysis. In the case of L.lactis pyruvate is converted to $C_3H_6O_3$ via lactate dehydrogenase isoform (L-lactate: NAD oxidoreductase). Lactate dehydrogenase oxidises the C=O and the CH-OH (carbinol) part of pyruvic acid. The $C_3H_6O_3$ produced is of L-stereo isomeric form. (Board, 1983)

2.2.3 Industrial Importance of Lactic Acid

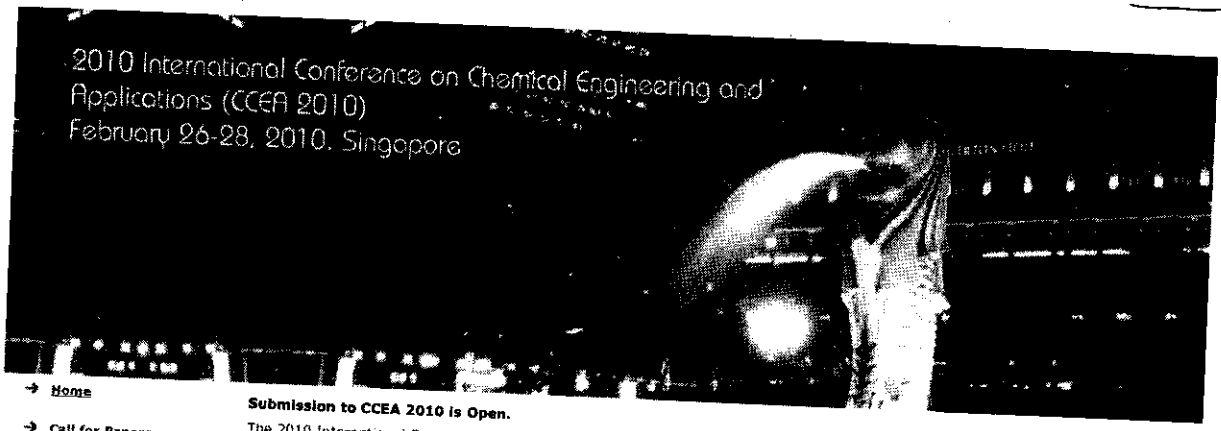
Lactic acid or 2-hydroxypropanoic acid ($C_3H_6O_3$, $CH_3CHOHCOOH$) is an important chemical substance widely used in food industry and in pharmaceutical and cosmetics industry. (Wasewar *et al.*, 2003).Lactic acid is a carboxylic acid with a hydroxyl group and it is considered to be an Alpha hydroxyl acid. (AHA). It has two optically isomer forms L-(+) - lactic acid and D-(-) – lactic acid and is a chiral acid (Fytou-Pallikari, 1997).The reaction of production of L- lactic acid which is the most important form biologically, is catalysed by the enzyme lactate dehydrogenase (LDH) and its isoenzymes. It has a melting point of 53 °C, though the racemic form (D/L) has a boiling point of 122°C at 12 mm Hg.

Lactic acid can be produced into large amounts, biotechnologically, through fermentation process performed by bacteria such *Lactobacilli*. Usually, the product of fermentation is a racemic mixture conglomerate mixture of D (-) - and L (+) – isomers but there are also strains which produce optically pure forms of one of the stereoisomer's. (Martau *et al.*, 2003) Another modern application is the use of lactic acid as a monomer participating in the synthesis of biodegradable homopolymers and co-polymers, such as polylactide (Choi & Hong, 1999). For the synthesis though of such fine polymers highly purified forms of lactic acid are demanded. Most of these polymers are used in the pharmaceutics industry especially for artificial prosthesis and controlled drug delivery.Traditional recovery methods for fermentation products (crystallization, extraction with solvent, filtration, carbon treatment evaporation) have high operational cost, so other methods such as distillation and distillation simultaneously with reaction are proposed due to low cost. (Choi & Hong, 1999)

2.3 The Production of Bacteriocins

As it has been known, a great number of Gram positive (+) bacteria and Gram negative (-) bacteria produce during their growth, substances of protein structure (either proteins or polypeptides) possessing antimicrobial activities, called bacteriocins. (Beasly & Saris, 2004) Although bacteriocins could be categorised as antibiotics, they are not. The major difference between bacteriocins and antibiotics is that bacteriocins restrict their activity to strains of species related to the producing species and particularly to strains of the same species. Antibiotics on the other hand have a wider activity spectrum and even if their activity is restricted this does not show any preferential effect on closely related strains. (Reeves, 1972) In addition, bacteriocins are ribosomally synthesised and produced during the primary phase of growth, though antibiotics are usually secondary metabolites. (Beasly & Saris, 2004) Bacteriocins usually have low molecular weight (rarely over 10 kDa); they undergo posttranslational modification and can be easily degraded by proteolytic enzymes especially by the proteases of the mammalian gastrointestinal tract, which makes them safe for human consumption. Bacteriocins are in general cationic, amphipathic molecules as they contain an excess of lysyl and arginyl residues. (Rodriguez *et al.*, 2003) They are usually unstructured when they are incorporated in aqueous solutions but when exposed to structure promoting solvents such as

ตอบรับบทความ 5.1.3



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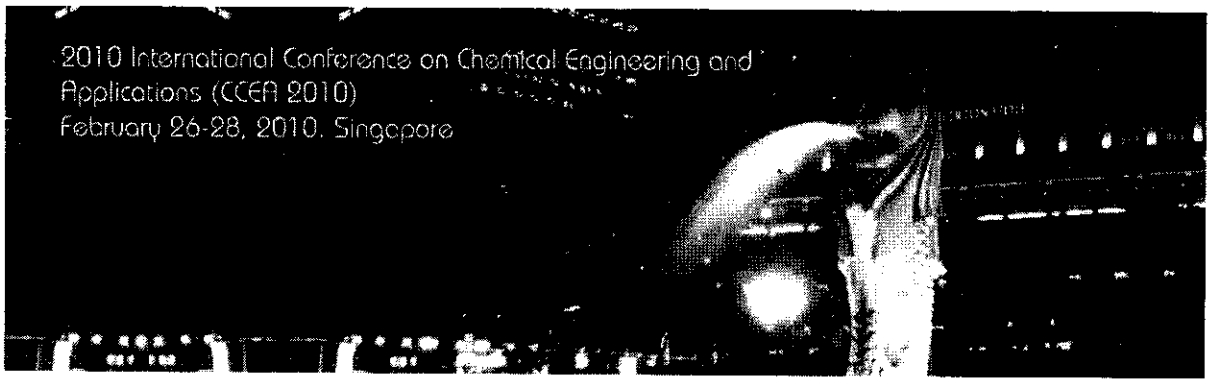

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ประกาศคณะวิศวกรรมศาสตร์

ฉบับที่ 36/2549

เรื่อง หลักเกณฑ์การนำเสนอ/เผยแพร่ผลงานวิจัย/วิชาการ ระดับนานาชาติ (ฉบับที่ 2 พ.ศ.2549)

คณะวิศวกรรมศาสตร์ มหาวิทยาลัยอุบลราชธานี

เพื่อให้การพิจารณาการนำเสนอ/เผยแพร่ผลงานวิจัย/วิชาการ ระดับนานาชาติ เป็นไปด้วยความเรียบร้อย และถูกต้องตามวัตถุประสงค์ ฉะนั้น อาศัยอำนาจตามความในมาตรา 27 แห่งพระราชบัญญัติ มหาวิทยาลัยอุบลราชธานี พ.ศ.2533 และโดยมติที่ประชุมคณะกรรมการประจำคณะวิศวกรรมศาสตร์ ครั้งที่ 6/2549 เมื่อวันที่ 19 กรกฎาคม 2549 คณะวิศวกรรมศาสตร์จึงออกประกาศในการกำหนดหลักเกณฑ์การนำเสนอ/เผยแพร่ผลงานวิจัย/วิชาการ ระดับนานาชาติ ดังนี้

1. ให้ยกเลิกประกาศคณะวิศวกรรมศาสตร์ ฉบับที่ 50/2547 เรื่องหลักเกณฑ์การนำเสนอ/เผยแพร่ผลงานวิจัย/วิชาการ ระดับนานาชาติ และใช้ประกาศนี้แทน

2. ลักษณะของโครงการในการขอรับการสนับสนุน มีดังนี้

ผู้ขอรับทุนสนับสนุนต้องขอรับการสนับสนุนจากเงินรายได้มหาวิทยาลัยด้วยโดยคณะจะสนับสนุนเฉพาะค่าใช้จ่ายในส่วนที่เกินจากการสนับสนุนของเงินรายได้มหาวิทยาลัยเท่านั้น ตามที่จ่ายจริงแต่ไม่เกิน 40,000 บาท/โครงการ ในกรณีที่มหาวิทยาลัยได้มีหนังสือแจ้งว่าเงิน สนับสนุนของมหาวิทยาลัยในปีงบประมาณดังกล่าวหมดลง หรือ ผลงานที่ขอรับการสนับสนุนไม่เข้าเกณฑ์การขอรับการสนับสนุนจากมหาวิทยาลัยนั้น ให้ขอรับการสนับสนุนโดยตรงที่คณะ คณะจะสนับสนุนค่าใช้จ่ายตามที่จ่ายจริงแต่ไม่เกิน 40,000 บาท/โครงการ

3. ลักษณะของผลงานที่นำเสนอ/เผยแพร่

ลักษณะของผลงานวิจัยหรือผลงานทางวิชาการที่นำเสนอหรือเผยแพร่ในการประชุมวิชาการระดับนานาชาติ ต้องได้รับการตีพิมพ์ผลงานในรายงานการประชุมวิชาการระดับนานาชาตินั้นด้วย ทั้งนี้ต้องได้รับความเห็นชอบจากคณะกรรมการบริหารงานวิจัยและบริการวิชาการ คณะวิศวกรรมศาสตร์(ERB)

4. คุณสมบัติผู้ที่จะขอรับการสนับสนุนค่าใช้จ่ายสำหรับ การนำเสนอ/เผยแพร่ผลงานทางงานวิจัย/วิชาการ มีดังนี้

4.1 เป็นบุคลากรสังกัดคณะวิศวกรรมศาสตร์

4.2 ไม่อยู่ในระหว่างการลาศึกษาต่อ

/4.3 ไม่เคย...

4.3 ไม่เคยปฏิบัติตนที่ขัดต่อระเบียบของมหาวิทยาลัยอุบลราชธานี

4.4 ไม่เคยได้รับการสนับสนุนค่าใช้จ่ายในการนำเสนอหรือเผยแพร่ผลงานวิจัยหรือผลงานในระดับนานาชาติ จากคณะวิศวกรรมศาสตร์ ในปีงบประมาณเดียวกันมาก่อน

4.5 ผู้ขอรับการสนับสนุนต้องชี้แจงรายละเอียดของโครงการต่อคณะกรรมการบริหารงานวิจัยและบริการวิชาการ คณะวิศวกรรมศาสตร์ (ERB)

5. การขอรับการสนับสนุน มีแนวทางปฏิบัติดังนี้

5.1 ยื่นรายละเอียดเอกสาร/หลักฐาน ได้แก่

5.1.1 หลักฐานตอบรับบทความหรือผลงาน	จำนวน 1 ชุด
5.1.2 บทความหรือผลงานทางวิชาการที่จะนำไปเสนอต่อที่ประชุม	จำนวน 1 ชุด
5.1.3 หลักฐานการจัดประชุม	จำนวน 1 ชุด
5.1.4 สำเนาใบสมัครการเข้าร่วมประชุม	จำนวน 1 ชุด
5.1.5 ตารางกำหนดการการเข้าร่วมประชุม (ถ้ามี)	จำนวน 1 ชุด

5.2 ผู้ขอรับการสนับสนุนต้องยื่นเอกสารตามรายละเอียดข้างต้น พร้อมแนบ บันทึกข้อความที่ลงนามโดยหัวหน้าภาควิชาหรือหัวหน้าหน่วยงาน

5.3 ผู้ได้รับการสนับสนุน ต้องส่งสำเนาบทความหรือเอกสารที่นำไปเสนอที่ประชุมวิชาการ พร้อมหลักฐานการใช้จ่ายเงินภายใน 1 เดือน หลังจากวันจัดประชุมทางวิชาการเสร็จสิ้น

5.4 ผู้ที่สนใจจะขอรับการสนับสนุน สามารถที่จะแจ้งความจำนงได้ตลอดปีงบประมาณ หรือช่วงเวลาที่มีความเหมาะสมของกิจกรรมดังกล่าว

5.5 การพิจารณาข้อเสนอโครงการให้คณะกรรมการบริหารงานวิจัยและบริการวิชาการ คณะวิศวกรรมศาสตร์(ERB) เป็นผู้พิจารณาเพื่อเสนอขอความเห็นชอบจากคณบดี

ประกาศ ณ วันที่ 31 เดือน กรกฎาคม พ.ศ. 2549

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