

Using Glycerol as Carbon Source for ϵ -Poly-lysine Production by *Streptomyces albulus*

李建德、施英隆

E-mail: 387109@mail.dyu.edu.tw

ABSTRACT

ϵ -Poly-Lysine (ϵ -PL) is a naturally occurring bio-material produced by microbial fermentation. It 's water soluble, biodegradable, edible and nontoxic toward humans and the environment. ϵ -PL shows a wide range of antimicrobial activity, it is stable at high temperatures and safe to eat. It is effective by adding trace amount, and doesn 't affect the taste. Therefore, it 's now widely used as a food preservative. The study first focused on isolation of ϵ -PL production strain *Streptomyces albulus* DYU 1 for using glycerol as carbon source to produce ϵ -PL in two stage culture fermentation. The optimal culture condition was studied, first by using one factor at a time method, followed by using response surface methodology (RSM) in the shaking flask culture. Finally, the use of jar fermentor to study the production of ϵ -PL by batch culture was also described. In the investigation of the effects of carbon and nitrogen source on ϵ -PL production, it was found that $(\text{NH}_4)_2\text{SO}_4$ had significant effect on ϵ -PL production, and glycerol has less effect; in addition, pH also showed significant effect. The optimum culture condition found was glycerol 25 g/L, $(\text{NH}_4)_2\text{SO}_4$ 10 g/L, Citric acid 18 g/L, and L-lysine 1.6 g/L; in this medium, *Streptomyces albulus* DYU 1 produced ϵ -PL 1.2 g/L to 1.6 g/L after incubation at 30 °C, 160 rpm for six days. In the study of optimizing ϵ -PL production by RSM, the results indicated that there is no improvement of ϵ -PL production in the process, the theoretical value predicted from the model was not in accordance with the actual value. Therefore, the optimal condition (glycerol 25 g/L, $(\text{NH}_4)_2\text{SO}_4$ 10 g/L, pH 4.5) found by one factor at time method was used for further investigation in fermenter. In the study of ϵ -PL production in the fermenter, two-stage fermentation was applied. The bacteria was first cultivated in shake flask at pH 6.8, for 36 hours, which were isolated and transferred into medium, and the pH was control at 3.5, 4, and 4.5, respectively. After six day incubation, the results showed that glycerol consumption were 13.553 g/L, 17.379 g/L, and 19.468 g/L, and the ϵ -PL concentration were 1.17 g/L, 2.103 g/L and 1.78 g/L when the pH was at 3.5, 4, and 4.5, respectively. For improving ϵ -PL production, back-feeding of glycerol was applied when the glycerol concentration decreased below 10 g/L. The final ϵ -PL concentration produced increased to 2.805 g/L from 2.103 g/L (1.3 fold increase) after one feeding. Our results have shown that *Streptomyces albulus* DYU 1 was capable of converting glycerol into ϵ -PL, a versatile biopolymer. The conversion of waste glycerol into valuable products will not only enhance the value of biodiesel production, but also solve the environmental problem of waste glycerol.

Keywords : ϵ -Poly-Lysine、two stage culture method、response surface methodology、jar fermentor

Table of Contents

目錄	封面	內頁	簽名頁	中文摘要	iii	ABSTRACT	v	誌謝	vii	目錄	ix	圖目錄	xv	表目錄	xix	第一章 緒論	1	1.1 前言	1	1.2 研究動機與目的	2	第二章 文獻回顧	5	2.1 基本性質及定義	6	2.1.1 生質能(Bioenergy)	6	2.1.2 生質柴油(Biodiesel)	7	2.1.3 甘油化學性質及應用	11	2.1.4 甘油之未來發展	13	2.2 ϵ -聚離胺酸之介紹	16	2.2.1 ϵ -聚離胺酸之生合成	17	2.2.2 ϵ -聚離胺酸的物理化學特性	21	2.2.3 酵素催化 ϵ -聚離胺酸降解	24	2.2.4 ϵ -聚離胺酸抑菌機制	25	2.2.5 ϵ -聚離胺酸之應用	29	2.3 回應曲面法	32	2.3.1 2水準因子實驗設計 (Two-Level Factorial Design)	32	2.3.2 陡升路徑法 (Method of Path of Steepest Ascent, PSA)	33	2.3.3 中心混成實驗設計 (Central Composite Design)	36	2.3.4 數據統計分析 (Regression model analysis)	36	2.4 發酵槽簡介	37	2.4.1 批次發酵	37	2.4.2 饋料發酵	39	第三章 材料與方法	41	3.1 實驗材料及儀器設備	41	3.1.1 實驗藥品	41	3.1.2 實驗儀器	43	3.2 ϵ -聚離胺酸生產菌株培養	44	3.2.1 菌株來源	44	3.2.2 培養基組成	44	3.3 實驗方法	49	3.3.1 菌種之保存	49	3.3.2 菌株活化	49	3.3.3 ϵ -PL生產培養	50	3.3.4 ϵ -聚離胺酸生產菌株篩選	52	3.3.5 環境因子及培養基碳氮源對 ϵ -聚離胺酸生產菌株之影響	52	3.4 以回應曲面法探討生產 ϵ -聚離胺酸之最佳培養基組成	53	3.4.1 回應曲面法因子設定	53	3.4.2 陡升實驗設計(Method of Path of Steepest Ascent)	55	3.4.3 中心混成實驗設計(Central Composition Design)	57	3.4.4 二次迴歸分析(Regression model analysis)	57	3.5 分析方法	58	3.5.1 菌體量之量測	58	3.5.2 生物鹼測試(Dragendorff reagent test)	58	3.5.3 碳源分析	59	3.5.4 ϵ -PL之分析	60	3.6 二階段式培養生產 ϵ -PL	62	3.6.1 改變甘油濃度對 <i>Streptomyces albulus</i> DYU 1在第二階段生產培養基生產 ϵ -PL之影響	62	3.6.2 氮源對 <i>Streptomyces albulus</i> DYU 1在第二階段生產培養基生產 ϵ -PL之影響	63	3.6.3 改變硫酸銨濃度對 <i>Streptomyces albulus</i> DYU 1在第二階段生產培養基生產 ϵ -PL之影響	63	3.6.4 改變檸檬酸濃度對 <i>Streptomyces albulus</i> DYU 1在第二階段生產培養基生產 ϵ -PL之影響	64	3.6.5 額外添加L-lysine或D-lysine及改變濃度對 <i>Streptomyces albulus</i> DYU 1在第二階段生產培養基生產 ϵ -PL之影響	64	3.6.6 前培養時間對 <i>Streptomyces albulus</i> DYU 1在第二階段生產培養基生產 ϵ -PL之影響	65	3.6.7 pH值對 <i>Streptomyces albulus</i> DYU 1在第二階段生產培養基生產 ϵ -PL之影響	65	3.6.8 溫度對 <i>Streptomyces albulus</i> DYU 1在第二階	
----	----	----	-----	------	-----	----------	---	----	-----	----	----	-----	----	-----	-----	--------	---	--------	---	-------------	---	----------	---	-------------	---	----------------------	---	-----------------------	---	-----------------	----	---------------	----	-------------------------	----	----------------------------	----	-------------------------------	----	-------------------------------	----	----------------------------	----	---------------------------	----	-----------	----	--	----	--	----	---	----	--	----	-----------	----	------------	----	------------	----	-----------	----	---------------	----	------------	----	------------	----	----------------------------	----	------------	----	-------------	----	----------	----	-------------	----	------------	----	--------------------------	----	------------------------------	----	--	----	---	----	-----------------	----	---	----	--	----	---	----	----------	----	--------------	----	---------------------------------------	----	------------	----	-------------------------	----	-----------------------------	----	---	----	---	----	--	----	--	----	---	----	--	----	--	----	---	--

段生產培養基生產 -PL之影響 66 3.7 發酵培養 67 3.7.1 發酵槽操作步驟 67 3.7.2 批次發酵培養 68 3.7.3 批次饋料發酵培養 69 第四章 結果與討論 70 4.1 -聚離胺酸生產菌株篩選 70 4.1.1 染劑對Streptomyces albulus DYU 1生產 -聚離胺酸之影響 79 4.1.2 微波時間對 -聚離胺酸生產菌株誘變之影響 81 4.1.3 紫外光對 -聚離胺酸生產菌株誘變之影響 84 4.2 二階段式發酵生產 -PL之探討 88 4.2.1 改變甘油濃度對Streptomyces albulus DYU 1生產 -PL之影響 88 4.2.2 不同氮源對Streptomyces albulus DYU 1生產 -PL之影響 91 4.2.3 改變硫酸銨濃度對Streptomyces albulus DYU 1生產 -PL之影響 94 4.2.4 改變檸檬酸濃度對Streptomyces albulus DYU 1生產 -PL之影響 96 4.2.5 額外添加L-lysine或D-lysine及改變濃度對Streptomyces albulus DYU 1在第二階段生產培養基生產 -PL之影響 99 4.3 環境因子的探討 102 4.3.1 前培養時間對Streptomyces albulus DYU 1在第二階段生產培養基生產 -PL之影響 102 4.3.2 pH值對Streptomyces albulus DYU1在第二階段生產培養基生產 -PL之影響 106 4.3.3 溫度對Streptomyces albulus DYU 1在第二階段生產培養基生產 -PL之影響 109 4.4 以回應曲面法(Response surface methodology, RSM)探討最佳培養基組成 112 4.4.1 一階迴歸分析結果 112 4.4.2 一階回應曲面模式適切性之統計檢驗 114 4.4.3 陡升實驗設計 123 4.4.4 中心混成設計之實驗結果 125 4.5 以發酵槽批次培養Streptomyces albulus DYU 1生產 -聚離胺酸之研究 135 4.5.1 Streptomyces albulus DYU 1在最適培養基及無pH控制之培養 135 4.5.2 控制pH值對Streptomyces albulus DYU 1生產 -PL之影響 139 4.5.3 饋料培養對Streptomyces albulus DYU 1生產 -PL之影響 142 第五章 結論 145 參考文獻 147 圖目錄 Figure 1-1. The frame of experiment. 4 Figure 2-1. The world biomass energy production trend,2002~2008. 6 Figure 2-2. Oils, fats, biodiesel and glycerol. 14 Figure 2-3. Structure of -Poly-lysine. 16 Figure 2-4. Potensial biosynthetic pathway for L-lysine. 19 Figure 2-5. Process flowchart of response surface. 35 Figure 3-1. Scheme of screening strategies of -Poly-lysine microorganisms. 51 Figure 3-2. The standard calibration curve of glycerol concentration. 59 Figure 3-3. The standard calibration curve of -PL concentration. 60 Figure 3-4. Schematic diagram of the fermentor. 68 Figure 4-1. Detection of secreted basic polymer on agar plate embedded with charged dyes. 71 Figure 4-2. (A) (A)-(E) Poly R-478 dye test: -Poly-lysine of experimental production microbes isolates on SG agar plate. 73 Figure 4-2. (B) (F)-(K) Methylene blue dye test: -Poly-lysine of experimental production microbes isolates on SG agar plate. 74 Figure 4-3. (A) (A)-(E) Testing for alkaloids with dragendorff reagent. 76 Figure 4-3. (B) (F)-(K) Testing for alkaloids with dragendorff reagent. 76 Figure 4-4. (A) Special zoon formed by the electrostatic polymer producing strains of Streptomyces albulus DYU 1. 77 Figure 4-4. (B) Special zoon formed by the electrostatic polymer producing strains of Streptomyces albulus IFO 14147. 77 Figure 4-5. The time course of -PL production by Streptomyces albulus DYU 1 in second stage fermentaion medium containing different dye. 80 Figure 4-6. Mortality of Streptomyces albulus DYU 1 by microwave mutation. 82 Figure 4-7. Mortality of Streptomyces albulus DYU 1 by UV treatment. 86 Figure 4-8. The time course of -PL production by Streptomyces albulus DYU 1 in second stage fermentaion medium containing various glycerol concentration. 90 Figure 4-9. The time course of -PL production by Streptomyces albulus DYU 1 in second stage fermentaion medium containing various nitrogen source. 93 Figure 4-10. The time course of -PL production by Streptomyces albulus DYU 1 in second stage fermentaion medium containing various (NH₄)₂SO₄ concentration. 95 Figure 4-11. The time course of -PL production by Streptomyces albulus DYU 1 in second stage fermentaion medium containing various citrate concentration. 98 Figure 4-12. The time course of -PL production by Streptomyces albulus DYU 1 in second stage fermentaion medium containing various L-lysine concentration. 101 Figure 4-13. The time course of biomass by Streptomyces albulus DYU 1 in first stage fermentaion medium. 104 Figure 4-14. Time course of -PL production, biomass, consumption of glycerol and pH values of culture medium in Streptomyces albulus DYU 1 cells growth cultured for a variety of times. 105 Figure 4-15. The time course of -PL production by Streptomyces albulus DYU 1 in second stage fermentaion medium containing various pH. 108 Figure 4-16 The time course of -PL production by Streptomyces albulus DYU 1 in second stage fermentaion medium containing various temperature. 111 Figure 4-17 First-order response surface and contours of the -Poly-lysine production influenced by (NH₄)₂SO₄ and glycerol. 119 Figure 4-18 First-order response surface and contours of the -Poly-lysine production influenced by pH and glycerol. 120 Figure 4-19 First-order response surface and contours of the -Poly-lysine production influenced by pH and (NH₄)₂SO₄. 121 Figure 4-20 Comparison of the observed experimental data with predicted valued by first-order RSM experiment. 122 Figure 4-21 Normal probability plot of residuals for first-order RSM experiment. 122 Figure 4-22 Second-order response surface and contours of the -Poly-lysine production influenced by (NH₄)₂SO₄ and glycerol. 131 Figure 4-23 Second-order response surface and contours of the -Poly-lysine production influenced by pH and glycerol. 132 Figure 4-24 Second-order response surface and contours of the -Poly-lysine production influenced by pH and (NH₄)₂SO₄. 133 Figure 4-25 Comparison of the observed experimental data with predicted valued by second-order RSM experiment. 134 Figure 4-26 Normal probability plot of residuals for second-order RSM experiment. 134 Figure 4-27(A). Time profile of the batch cultivation without control of pH. 137 Figure 4-27(B). Time profile of the batch cultivation without control of pH. 138 Figure 4-28. Time profile of the batch cultivation at different pH values. 141 Figure 4-29. Time profile of -PL production using fed-batch and pH control strategy. 144 表目錄 Table 2-1. The decomposition rate of the different mixing ratio of biodiesel(28 days) (%). 9 Table 2-2. Comparison of biodiesel with fossil diesel emissions. 10 Table 2-3. Current uses of glycerol. 12 Table 2-4. -Poly-lysine producing strain and molecular weight distribution. 23 Table 2-5. Antimicrobial spectrum of -PL. 27 Table 2-6. Antimicrobial spectrum of -PL. 28 Table 3-1. International streptomyces project medium 2. 45 Table 3-2. Synthetic glycerol medium. 46 Table 3-3. Luria-bertani broth medium. 47 Table 3-4. Cell growth culture

medium. 47 Table 3-5. -PL production medium. 48 Table 3-6. Concentration and coded level for 23 factorial design. 54 Table 3-7. Experiment design for path of steepest ascent. 56 Table 4-1. The colony morphology of isolated bacterial strain. 78 Table 4-2. -Poly-lysine production of mutated strain with five passage numbers. 83 Table 4-3. -Poly-lysine production of mutated strain with five passage numbers. 87 Table 4-4 Result of 23 factorial design. 115 Table 4-5 Regression analysis of the derived from the data of 23 factorial design. 116 Table 4-6 ANOVA table of 23 factorial design. 117 Table 4-7 Comparison of the observe experimental data with predicted values by first-order model. 118 Table 4-8 Results of the path of steepest ascent experiments. 124 Table 4-9 Results of central composite design. 127 Table 4-10 Regression analysis of the central composite design experiment. 128 Table 4-11 ANOVA table of the central composite design experiment. 129 Table 4-12 Comparison of the observe experimental data with predicted values by second-order model. 130

REFERENCES

- 1.董懷仁。(2006)。廢食用油與高酸油製造生質柴油之反應參數探討。國立清華大學碩士論文。新竹。
- 2.李俊緯。(2011)。聚乙烯醇-海藻酸鈉包埋固定納豆菌生產菌果聚醣之研究。大葉大學碩士論文。彰化。
- 3.李素月。(2010)。生質柴油發展之概況。大葉大學碩士論文。彰化。
- 4.劉柏毅。(2007)。培養 *Klebsiella* sp.轉化甘油為高附加價值1,3-丙二醇。台灣科技大學碩士論文。台北。
- 5.劉曉歐、李睿穎。(2007)。-聚賴氨酸的發酵生產和應用研究。食品工程。(1): 22-25。
- 6.劉蔚、秦芸樺、周濤。(2007)。-聚賴氨酸生物合成機理的研究進展。食品科學。28(8): 549-553。
- 7.古森本。(2008)。生質能源作物之開發與潛力。植物種苗生技。13: 46-53。
- 8.黃國昌、邊水根。(2006)。-聚賴氨酸研究進展。江西科學。24(6): 538-544。
- 9.黃國昌。(2007)。白色鏈黴菌發酵生產 -聚賴氨酸的工藝研究。南昌大學碩士論文。南昌。
- 10.謝文章。(2008)。國內發展生質柴油潛力評估。國立台北大學碩士論文。台北。
- 11.徐紅華、劉慧。(2000)。聚賴氨酸在牛奶保鮮之應用研究。食品與發酵工業。26 (2): 33-35。
- 12.張東榮、王正剛、毛忠貴。(2005)。聚賴氨酸的研究進展。氨基酸和生物資源。27 (2): 48-51。
- 13.張海濤。(2008)。天然食品防腐劑 -聚賴氨酸的微生物合成及其純化。上海海洋大學碩士學位論文。上海。
- 14.張超、王正剛、段作菅、毛忠貴。(2007)。大劑量紫外光誘變選育 -聚賴氨酸高產菌。生物加工過程。5(3):64-68。
- 15.張宸瑋。(2010)。以納豆菌發酵生產凝乳酵素之研究。大葉大學碩士論文。彰化。
- 16.章瑩。(2009)。聚- -賴氨酸菌株篩選與發酵工藝的研究。湖北工業大學碩士論文。武漢。
- 17.陳登科。(2004)。超臨界二氧化碳萃取蛹蟲草蟲草素之探討。朝陽科技大學碩士論文。台中。
- 18.陳旭升。(2008)。-聚賴氨酸高產菌株選育與發酵過程優化。江南大學碩士論文。無錫。
- 19.陳雄、章瑩、袁金鳳、王實玉、王金華。(2007)。聚- -賴氨酸補料發酵的初步研究。工業微生物。37(4): 20-22。
- 20.陳雄、王金華。(2006)。聚 -賴氨酸的研究進展。湖北工業大學學報。21(6): 58-61。
- 21.陳思潔。(2010)。溫度、壓力對農業廢棄物熱裂解的影響。大葉大學碩士論文。彰化。
- 22.施慶珊、陳儀本、歐陽友生。(2004)。-聚賴氨酸的微生物合成與降解。生物技術。14(6): 77-79。
- 23.施英隆、沈名豪。(2003)。以微生物生產聚離胺酸及其應用。生物資源。生物技術。5: 4-11。
- 24.沈名豪。(2004)。生物合成聚離胺酸之研究。大葉大學碩士論文。彰化。
- 25.任喜東、陳旭升、董難、李樹、李鳳、趙福林、唐蕾、毛忠貴。(2012)。輔助能量物質檸檬酸促成?-聚賴氨酸的合成。食品與發酵工業。38(3)。
- 26.曹安堂、肖素榮。(2008)。-聚賴氨酸結構特性及應用。專論與綜述。(11): 11-14。
- 27.蘇現伐、李怡帆、羅亞紅、孫劍輝、薛萬新。(2010)。UV誘變技術在廢水生物處理中的應用研究進展。工業水處理。30(5): 1-4。
- 28.孫湘婷、李洪亮。(2013)。-聚賴氨酸的發酵培養基優化。贛南醫學院學報。33(1): 8-11。
- 29.楊玉紅。(2007)。-聚賴氨酸產生菌的篩選、鑑定及發酵的研究。瀋陽農業大學博士論文。瀋陽。
- 30.巫國維。(2007)。固體觸媒生產生質柴油之研究。國立清華大學碩士論文。新竹。
- 31.吳昱真。(2004)。聚離胺酸之生產研究。大葉大學專題製作報告。彰化。
- 32.王曼瑩。(2011)。利用篩選之*Bacillus subtilis* DYU6 菌株生產凝乳酵素及其特性之研究。大葉大學碩士論文。彰化。
- 33.Adhikari, S., Fernando, S.D., Filip, T.S.D., Bricka, R.M., Steele, P.H., and Haryanto, A. (2008). Conversion of glycerol to hydrogen via a steam reforming process over nickel catalysts. *Energy Fuel*. 22: 1220-1226.
- 34.Ahmet, N.O., M. Canakci, A. Turkcan, C. Sayin. (2009). Performance and combustion characteristics of a DI diesel engine fueled with waste palm oil and canola oil methyl esters. *Fuel*. 88: 629-636.
- 35.Biodiesel 2020: Global Market Survey, Feedstock Trends and Forecasts,2nd(2008). (<http://www.emerging-markets.com/biodiesel/>)
- 36.Campbell, C.J., and Laherrere, J.H. (1998). The end of cheap oil. *Sci Am*. 3: 78-83.
- 37.Charney, W. (1978). Dihydroxyacetone. US Patent 4,076,589. 1-4.
- 38.Choi, J.S., Joo, D.K., Kim, C. H., Kim, K., Park, J. S. (2000).Synthesis of a Barbell-like triblock copolymer, poly(L-lysine) dendrimer-block-poly(ethylene glycol)-block-poly(L-lysine) dendrimer, and its self-assembly with plasmid DNA. *J. Am. Chem. Soc*. 122: 474-480.
- 39.Choi, Y. H., Liu, F., Park, J.S., Kim, S. W. (1998). Lactose-poly(ethylene glycol)-grafted poly-L-lysine as hepatoma cell-targeted gene carrier. *Bioconjugate Chem*. 9: 708-718.
- 40.Cole, J., Lefler, J., and Chen, R. (2008). Fast Separation of FFA, FAME, and Glycerol for Biodiesel Analysis by Supercritical Fluid Chromatography, Thar Instruments, Inc . 40-41.
- 41.Cooper, C. M., Fernstrom, G. A. and Miller, S. A. (1944). Performance of gas-liquid contactors. *Ind. Eng. Chem*. 36: 504-509.
- 42.Dasari, M. A., Kiatsimkul, P. P., Sutterlin, W. R. and Suppes, G. J. (2005). Low-pressure hydrogenolysis of glycerol to propylene glycol. *Appl Catal., A*. 281(1-2): 225- 231.
- 43.Dharmadi, Y., Murarka, A., Gonzalez, R. (2006). Anaerobic fermentation of glycerol by *Escherichia coli*: a new platform for metabolic engineering. *Biotechnol. Bioeng*. 94: 821-829.
- 44.Freedman, B., Pryde, E. H. and Mount, T. L. (1984). Variables affecting the yield of fatty ester from transesterified vegetable oils. *J. Am. Oil Chem. Soc*. 61: 1638-1643.
- 45.Gallan, M., Bonet, J., Sire, R., Reneaume, J. and Plesu, A.E. (2009). From residual to useful oil: revalorisation of glycerine from the biodiesel synthesis. *Bioresour. Technol*. 100: 3775-3778.
- 46.Hamano, Y., Nicchu, I., Shimizu, Y., et al. (2007). -Poly-L-lysine producer, *Streptomyces albulus*, has feedback-inhibition resistant aspartokinase. *Appl. Microbiol. Biotechnol*. 76 (4): 873-882.
- 47.Hazimah, A. H., Ooi, T. L., Salmiah, A. (2003). Recovery of glycerol and diglycerol from glycerol pitch. *J Oil Palm Res*. 15: 1-5.
- 48.Hill, J., Nelson, E., Tilman, D., Polasky, S. and Tiffany,

D. (2006). Environmental, economic, and energetic costs and benefits of biodiesel and ethanol biofuels. *PNAS*. 103 (30): 11206-11210. 49. Hiraki, J. (1995). Basic and applied studies on ϵ -polylysine. *J. Antibact. Antifungal Agents*. 23: 349-354. 50. Hiraki, J. (2000). ϵ -polylysine, its development and utilization. *Fine chem*. 29: 28-25. 51. Hiraki, J., Ichikawa, T., Ninomiya, S. I., Seki, H., Uohama, K., Seki, H., Kimura, S., Yanagimoto, Y., Barnett, J. W. (2003). Use of ADME studies to confirm the safety of ϵ -polylysine as a preservative in food. *Regulatory Toxicol. Pharm.* 37: 328-340. 52. Hiraki, J., Masakazu, H., Hiroshi, M., Yoshikazu I. (1998). Improved poly-L-lysine production of an S-(2-aminoethyl)-L-cysteine resistane mutant of *Streptomyces albus*. *Seibutsukogaku*. 76: 487-493. 53. Hiraki, J., Masakazu, H., Hiroshi, M., Yoshikazu, I. (1998). Improved poly-L-lysine production of an S-(2-aminoethyl)-L-cysteine resistane mutant of *Streptomyces albus*. *Seibutsukogaku*. 76: 487-493. 54. Hirohara, H., Takehara, M., Saimura, M., et al. (2006). Biosynthesis of poly (ϵ -L-lysine)s in two newly isolated strains of *Streptomyces* sp. *APPI. Microbiol. Biotechnol.* 73(2): 321-331. 55. Hirohara, H., Takehara, M., Saimura, M., Masayuki, A., Miyamoto, M. (2006). Biosynthesis of poly(ϵ -L-lysine)s in two newly isolated strains of *Streptomyces* sp. *Appl Microbiol Biotechnol.* 73: 321-331. 56. Ho, Y. T., Ishizaki, S., Tanaka, M. (2000). Improved emulsifying activity of ϵ -polylysine by conjugation with dextran through the Maillard reaction. *Food Chem*. 68: 449-455. 57. Karha, P., Iwata, T., Hiraki, J., Park, Y. E., Okabe, M. (2001). Enhancement ϵ -Polylysine Production by *Streptomyces albus* Strain 410 Using pH Control. *J Biosci Bioeng*. 91: 190-194. 58. Kido, Y., Hiramoto, S., Murao, M., Horio, Y., Miyazaki, T., Kodama, T., Nakabou, Y. (2003). ϵ -Polylysine inhibits pancreatic lipase activity and suppresses postprandial hypertriglyceridemia in rats. *J Nutr*. 133: 1887-1891. 59. Kito, M., Onji, Y., Yoshida, T., Nagasawa, T. (2002a). Occurrence of ϵ -poly-L-lysine-degrading enzyme in ϵ -poly-L-lysine-tolerant *Sphingobacterium multivorum* OJ10: purification and characterization. *FEMS Microbiol. Lett.* 207: 147-151. 60. Kito, M., Takimoto, R., Yoshida, T., Nagasawa, T. (2002b). Purification and characterization of an ϵ -poly-L-lysine-degrading enzyme from an ϵ -poly-L-lysine-producing strain of *Streptomyces albus*. *Arch. Microbiol.* 178: 325-330. 61. Kunioka, M. (1995). Hydrolytic degradation and biodegradation of hydrogel prepared from microbial poly(ϵ -L-lysine). *Seni gakkaiishi*. 51: 137-142. 62. Kunioka, M., Choi, H. J. (1995). Properties of biodegradable hydrogels prepared by γ -irradiation of microbial (ϵ -L-lysine) aqueous solutions. *J. Appl. Polym. Sci.* 58: 801-806. 63. Kuraray Co. (2001). Water swelling polymer gel and its production method. JP patent 278984. 64. Kushwaha, D. R. S., Mathur, K. E. (1980). Poly(ϵ -L-lysine): Synthesis and Conformation.. *Biopolymers*. 19: 219-229. 65. Lemke, D. (2006). Volumes of versatility. *AURI*. 15(1): 8. (<http://www.auri.org>) 66. Maeda, S., Kunitomo, K. K., Sasaki, C., Kuwae, A., Hanai, K. (2003). Characterization of microbial poly (ϵ -L-lysine) by FT-IR, Raman and solid state ^{13}C NMR spectroscopies., *J Mol Struct*. 655: 149-155. 67. Marchetti, J. M., Miguel, V. U., and Errazu, A. F. (2007). Possible methods for biodiesel production. *Renew. Sust. Energ. Rev.* 11: 1300-1311. 68. Masayuki, S., Munenori, T., Shinya, M., Kazuma, K., Hideo, H. (2008). Biosynthesis of nearly monodispersed poly(ϵ -L-lysine) in *Streptomyces* species. *Biotechnol. Lett.* 30: 377-385. 69. Maureen, D. B., Alexander, I. G., Laurence, T. et al. (2003). In vitro and in vivo gene transfer with poly (amino acid) vesicles [J]. *J Control Release*. 93(2): 193-211. 70. McCoy, M. (2005). An unlikely impact. *Chem Eng News*. 83:24-26. 71. Mickelson, M. N., Werkman, C. H. (1940). Formation of trimethyleneglycol from glycerol by *Aerobacter*. *Enzymologia*. 8: 252-256. 72. Neda, K., Sakurai, T., Stakahashi, M., Shiychi, M., Ohgushi, M. (1999). Two-generation reproduction study with teratology test of ϵ -poly-L-lysine by dietary administration in rats. *Jpn Pharmacol. Ther.* 27: 1139-1159. 73. Nishikawa, M., Ikezaki, A., Takahara, M., Hirohara, H., (2002). Structures of ϵ -polylysine produced by several actinomycetes and their classification on the basis of productivity of ϵ -polylysine. *Biosci. Biotechnol. Biochem.* 2: 54. 74. Nishikawa, M., Ogawa, K. (2002). Distribution of Microbes Producing Antimicrobial ϵ -Poly-L-Lysine Polymers in Soil Microflora Determined by a Novel Method. *Appl. environ. microbiol.* 68: 3575-3581. 75. Novy, S. K., Tsung-Han, T., Gunawan, S., Yi-Hsu, J. (2009). Biodiesel production from rice bran oil and supercritical methanol. *Bioresour. Technol* 100(8): 2399-2403. 76. Perry, R. H., Green, D.W. (1977). Perry ' s Chemical Engineer ' s Handbook. McGraw-Hill: New York. 2-39. 77. Saimura, M., Takehara, M., Mizukami, S., et al. (2008). Biosynthesis of nearly monodispersed poly (ϵ -L-lysine)s in *Streptomyces* species. *Biotechnol. Lett.* 30(3): 377-385. 78. Sheehan, J., Camobreco, V., Duffield, J., Graboski, M., and Shapouri, H. (1998). Life Cycle Inventory of Biodiesel and Petroleum Diesel for Use in an Urban Bus. *NREL*. 1-278. 79. Shen, W. C., Ryser, H. J. P. (1978). Conjugation of poly-L-lysine to albumin and horseradish peroxidase: a novel method of enhancing the cellular uptake of proteins. *Proc. Natl. Acad. Sci. USA*. 75: 1876-1978. 80. Shen, W. C., Ryser, H. J. P. (1981). Poly(L-lysine) has different membrane transport and drug-carrier properties when complexed with heparin. *Proc. Natl. Acad. Sci. USA*. 78(12): 7589-7593. 81. Shih, I. L., Shen, M. H., Van, Y. T. (2006). Microbial synthesis of poly(ϵ -lysine) and its various applications. *Bioresource Technol.* 97(9): 1148-1159. 82. Shih, I. L., Van, Y. T., Shen, M. H. (2004). Biomedical Applications of Chemically and Microbiologically Synthesized Poly(glutamic acid) and Poly(lysine). *Mini-Rev. Med. Chem.* 4: 179-188. 83. Shima, S. Oshima, S. Sakai, H. (1983). Biosynthesis of ϵ -poly-L-lysine by washed mycelium of *Streptomyces albus* No-346. *Nippon Nogeikagaku Kaishi* 57: 221-226 (in Japanese). 84. Shima, S., Fukuhara, Y., Sakai, H. (1982). Inactivation of bacteriophages by ϵ -poly-L-lysine produced by *Streptomyces*. *Agric. Biol. Chem.* 46: 1917-1919. 85. Shima, S., Matsuoka, H., Iwamoto, T., Sakai, H. (1984). Antimicrobial action of ϵ -poly-L-lysine. *J. Antibiot.* 37: 1449-1455. 86. Shima, S., Sakai, H. (1977). Polylysine produced by *Streptomyces*. *Agric. Biol. Chem.* 41: 1807-1809. 87. Shima, S., Sakai, H., (1981a). Poly-L-lysine produced by *Streptomyces*. Part II. Taxonomy and fermentation studies. *Agric Biol Chem.* 45: 2497-2502. 88. Shima, S., Sakai, H., (1981b). Poly-L-lysine produced by *Streptomyces*. Part III. Chemical studies. *Agric Biol Chem.* 45: 2503-2508. 89. Shukla, S. C., Singh, A., Pandey, A. K., Mishra, A. (2012). Review on production and medical applications of ϵ -polylysine. *Biochem. Eng. J.* 65: 70-81. 90. Thompson, J. C., He, B. B. (2006). Characterization of crude glycerol from biodiesel production from multiple feedstocks. *Appl Eng Agric.* 22(2): 261-265. 91. Tristan, M., Pascal, D., Thierry, B., et al. (2004). Efficient gene transfer into human epithelial cell lines using glycosylated cationic carriers and neutral glycosylated co-lipids [J]. *Blood Cell Mol Dis.* 32(2): 271-282. 92. Vasudevan, P. T., Briggs, M. (2008). Biodiesel production – current state of the art and challenges. *J Ind Microbiol Biotechnol.* 35:

421-430. 93.Wang, Z. X., Zhuge, J., Fang, H., Prior, B.A. (2001). Glycerol production by microbial fermentation: A review. *Biotech. Adv.* 19: 201-223. 94.Wirawan, S. S., and Tambunan, A. H. (2006). The Current Status and Prospects of Biodiesel Development in Indonesia: a review. *Third Asia Biomass Workshop*. 1-14. (<http://biomass-asia-workshop.jp/biomassws/03workshop/material/papersoni.pdf>) 95.Xusheng, C., Lei, T., Shu, L., Lijuan, L., Jianhua, Z., Zhonggui, M., (2011). Optimization of medium for enhancement of ϵ -Poly-L-Lysine production by *Streptomyces* sp. M-Z18 with glycerol as carbon source, *Bioresour. Technol.* 102: 1727-1732. 96.Yazdani, S. S., Ramon, G. (2007). Anaerobic fermentation of glycerol: a path to economic viability for the biofuels industry. *Curr. Opin. Biotechnol.* 18: 213-219. 97.Yoshida, T., Nagasawa, T. (2003). ϵ -Poly-L-lysine: microbial production, biodegradation and application potential. *Appl Microbiol Biotechnol.* 62: 21-26.