Optimization of Bacteriocin production, through Biofilm Formation of Lactic acid Bacteria, with repeated batch Fermentation

Faculty of Biotechnology
Assumption University

Mr. Jackie Pom

ID: 5728014

A Thesis Submitted in Partial Fulfillment of the Requirement for the
Degree of Bachelor of Science in Biotechnology
Department of Agro Industry
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ABSTRACT

Six strains of Lactic acid Bacteria were screened for stability of biofilm formation on a designed carrier and, to identify the most stable Bacteriocin producing strain, for up-scale repeated batch fermentation process. Fermentation of 20ml scale, were conducted for, Crystal Violet analysis to identify the two most stable biofilm forming bacteria, *Pediococcus 16AVPd 02, Lactobacillus SD1* with absorbance measures OD600 of (0.108 ± 0.006 and 0.085 ± 0.010) of day 7. Preliminary studies were conducted with fermentation upscaled to 500ml, the productivity and anti-microbial activity of both strains, were analyzed. *Pediococcus 16AVPd 02,* has better productivity and % yield of (65.21 ± 4.84 mmol/l and 4.84 ± 0.37 %) comparing to *Lactobacillus SD1* (33.91 ± 9.58 mmol/l and 3.31 ± 0.13 %). Agar Diffusion assay were conducted to identify the best fermentation duration for further Anti-microbial analysis. Finally, repeated batch fermentation of 3L scale were conducted. Analysis were focused on the third day of each batch, *Pediococcus 16AVPd 02* and *Lactobacillus SD1* has productivity and % yield at (26.60 ± 10.68 mmol/l/hr and 2.37 ± 0.46 %, 29.40 ± 5.8 %, 1.85 ± 0.033). Crude sample shows, the highest anti-microbial activity against the indicator pathogen, with SD1 having higher anti–microbial activity comparing to *Pediococcus 16AVPd 02*, from minimal inhibitory concentration test.

Keywords: Lactic acid Bacteria; Carrier; Bacteriocin; Biofilm; Fermentation; *Pediococcus*; *Lactobacillus*

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I would like to express my gratitude, to Assumption University of Thailand, and the School of Biotechnology and stuff, for the knowledge and experience which I have acquired throughout my years, which without, could not have made this project a reality. Furthermore, I would like to extend my thankfulness and sincerity to all the lecturers, professors and lab technicians which have guided me before and through this project. Asst. Prof. Dr. Tatsaporn Todhanakasem, my project advisor whom without would have not made this whole project possible, I thank you for your time, effort and guidance throughout the lifetime of this project. Finally, I'm thankful and appreciative for my parents, myself and friends, whom direct and indirectly motivated and encouraged me to work hard to achieve the objective of this project.

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INTRODUCTION

We have co-existed with lactic acid producing bacteria for thousands of years, a micro-flora, our probiotics which can be found abundance in our eco-system from plants, food and feed. In fermented food, a delicacy found in many civilizations, is the perfect example of a bio-preservation process, as organic lactic acid produced from LAB (Lactic acid bacteria) metabolic pathway, inhibits the growth of foodborne pathogen, discovery of bioactive peptide known as bacteriocin was observed for its ability to inhibit growth of foodborne pathogen. Many species and strains of Lactic acid bacteria in the wild, are characterized as; gram positive, acid tolerant, aerotolerant, non-motile, non-spore forming and has either bacilli or cocci structure. Lactic acid bacteria are known to secrete Bacteriocin, to suppress the growth of closely related species. In contrast to anti biotics, which are a secondary metabolite and has broad range of inhibition against foodborne pathogen. Bacteriocin has narrow selective groups of foodborne pathogens, mostly relative competitive species to inhibit growth. Since its discovery in 1925 by André Gratia, many bacteriocin has been discovered, currently 'Nisin' is produced commercially. Rising number of antimicrobial resistance microorganism in animal feed; such as *E. coli, Actinobacillus spp.* Those within the group of zoonotic enteropathogens, commensal bacteria and bacterial pathogen of animal, are a raising concern among food and animal feed safety. Being that the species of LAB are food grade, thus allow many possibilities and potential application of being a bio preservative in food and feed industries. There are not many researches, which studies the production of Bacteriocin for industrial scale uses, aside from 'NISIN'. This research is aiming to study the stability of lactic acid bacteria, biofilm formation on the designed carrier, also to develop the bacteriocin production under repeated batch fermentation. To study the effectiveness of the bacteriocin against several foodborne pathogen; *Salmonella typhimurium* ATCC 13311, *Bacillus Cereus* ATCC 11778, *Escherichia coli cereus* ATCC 8739, *Escherichia coli cereus* ETEC 01, *Escherichia coli cereus* ETEC 02, *Salmonella enteritidis* 055 and *Salmonella enteritidis* 010 and to determine the most effective bacteriocin producing lactic acid bacteria strain. Finally, this study is a preliminary study and guide for future researches on industrial production of Bacteriocin from a selective group of LAB strain for feed and food industry.

LITERATURE REVIEW

For thousands of years, agricultural products and food were preserved by fermentation process. Fermentation, a microorganism metabolic process, which enzymatically alters the chemical structure of an organic substance. Along with yeast fermentation which converts sugars to alcohol. Nonetheless, lactic acid fermentation is the most important, the acidification process, inhibits the growth of pathogen microbe [Axelsson, L., 2000] Lactic acid bacteria, can be found in many eco-systems from plants, fermented food, animal, to human some species of the lactic acid bacteria is a microflora. Lactic acid bacteria are, non-spore forming, non-motile, negative catalase and mainly converts sugar to lactic acid. Lactic acid bacteria prefer to grow in anaerobic condition, however, are also aerotolerant [Teneva.A., 2018]

The formation of lactic acid biofilm is a stress responds to survive in harsh environment; this could be due to the lack of nutrient or present of toxic substances. Quorum sensing which is a common mechanism within a mature biofilm colony, were known to regulate the bacteriocin operons [Nes et al. 1996; Kleerezebem et al. 1997]. Biofilm formation begins by synthesizing of EPS (Exopolymeric substance), to adhere to a specific surface. Base of this principle, to study the formation of Lactic acid biofilm formation, the chosen strain, are to be grown in a low concentrated MRS broth. The cell will begin to adhere onto, a carrier, one experiment, studies the formation of biofilm, on a static 96-well polystyrene microtiter dish, which has hydrophilic-treated wells. Incubation time and temperature were set at 35°C, every 30 minutes the supernatant is remove and the biofilm is stained with 0.1% (w/v) crystal violet, washed with water and distained with 95% ethanol. The distained solution was used to measure via spectrophotometer at OD of 595 [Kubota, H., 2008]. Moreover, 'Temperature and pH' can have major effect on the biofilm formation, previous studies have indicated, higher biofilm production in high pH environment (8.5), whereas, at temperature of 32 °C saw the best biofilm production [Hoštacká, A., (2010)].

Cell immobilization are in favor for industrial scale fermentation due, reduction cost of downstream processes as well as better for cell recycling. Secretion of EPS, were enable of cell immobilization due to present of biopolymer which is the initial and permanent adhesion compound.[Suresh Kumar et al., 2007] Not just that, naturally forming biofilm, are economically beneficial as it reduces waste, cost of cell immobilization is cheap, cell population are not easily declined and able to retain cell performance over many generations [Dagher, S.F., 2010]. Stability of bacteriocin production can majorly get affected by the formations of biofilm, being a natural cell immobilizer [Characklis, W. G., 1990], where commercial production of Nisin, indicates better stability of bacteriocin production from *L. lactis* species, as comparing to free floating cells, sees decline of bacteriocin production during fermentation, due to the instability of plasmid which encodes the bacteriocin [Scannell et al., 2000]. Furthermore, production of bacteriocin occurs, during the growth phase of the bacteria, and stops by the end of the exponential phase, or the beginning of the stationary phase. Fermentation processes where Ph are not control at 5.5 – 6.5, see no reduction of bacteriocin titer, as absorption of Bacteriocin, into the cells worsen as pH conditions becomes lower. [Parente.E., 1999]

Lactic Acid bacteria, EPS can be classified into two subclasses, homopolysaccharides and heteropolysaccharides, where heteropolysaccharides are produced from mesophilic and thermophilic class of LAB. Heteropolysaccharides are normally produced by *Pediococcus spp.* and *Streptococcus spp.* The slimy characteristics observed from the secreted EPS, were suspected to be a composition of protein complex, upon further purification saw carbohydrate rich content, thought further study concluded that EPS from LAB, are composed of repeating units of polysaccharides consisting of α- and β-linkages. [De Vuyst, L., 1999]. Furthermore, researches on LAB polysaccharide and production, saw potential application in health and medical industry [Wang et al., 2008]

Lactic acid Bacteriocin, are bio-active antimicrobial peptide, which are ribosomally synthesized and extracellularly secreted, as a primary metabolite during phase 1 of the bacteria growth. Although having the capabilities to inhibit the growth of other microbial, comparing to antibiotics, which are a secondary metabolite, bacteriocin has more specific target ranged, mostly are closely related species, where antibiotics are able to inhibits a broad spectrum of alien microorganisms. Being heat stable, amphiphilic and membrane permeable are the basic characteristics of a Lactic acid bacteriocin. Base on its size and protein complexity, it can be classified into three main class; Class I: The Lantibiotic, Class II: Non-Lantibiotic and Class III: The Bacteriocin. Class I, being the smallest (<5 kDa) can be highly modified, forming closely characterized thioether amino acid lanthiomine and **β**-methyllanthionine. Class II, bacteriocin are slightly bigger (<10 kDa) and requires two peptide components, in order to achieve, anti-microbial activity. Of all three classes, normally consisting of a large molecular weight and size (>30 kDa) are the Class III, bacteriocin, they are heat liable, hence has not been extensively studied, whereas Class II are the most discovered and researched on [Zacharof, M.P., (2012)].

The number of Bacteriocin, has been increasing in number, thus the need for a novel technique to make the screening of Bacteriocin faster and more accurate to detect. Spot on lawn, disc diffusion and agar well diffusion are some of the most popular methods, to screening for the inhibitory effect of the Bacteriocin. Though, the results can be easily visualizing by the formation of clear zone, it can be time consuming, thus rise concerns for the efficiency and accuracy when undergoing screenings for hundredth and thousands of strains. Therefore, Novel method of utilizing PCR and bioinformatics, 'BACTIBASE', to detects for the gene encoding for the synthesis of bacteriocin, is proven to be less time consuming and more accurate for screening for Novel bacteriocin [Zou, J., 2018]. However, the accuracy of screening through Bioinformatics alone, are not enough to produce an accurate result. As presence of Bacteriocin sequencing gene, does not signify production, by the bacteria. With the commercially produced bacteriocin 'Nisin', application of bioluminescent whole cell biosensors was used to accurately screen for Nisin produce LAB strain [O'Bryan, C. A., (2015)].

MATERIALS AND METHODS

1. Carrier Preparation

PLA, Soybean meal and corn flour was dried, in oven to remove moisture at temperature of 60-degree °C. All dried material was mixed thoroughly before extrusion. The mixture ratioed, 4:1 w/w PLA to Soybean meal, plus '10% of ratio', corn flour. The type of extrusion machine used was, twin screw extruder. The operation temperature set were: 150,160,160,165,170,175,175,170. Disc rpm was set to 50 rpm, Torque set to 0.76 Nm and Disc pressure set to: 21 bar.

2. Media

The growth medium for colony plate, was a standard MRS Agar Medium, [Difco™ & BBL™ Manual]. The growth medium for seed 2, was the standard MRS Broth Medium. The Medium used in the 3 L bioreactor and 500 ml working volume flask was half MRS broth which + 2% glucose diluted. Media used in 20 ml working volume flask was half MRS broth. The growth medium for indicator pathogens are Nutrient Broth & Agar.

3. Subculture

I. Lactic Acid Bacteria

Single colony was transferred from MRS plate into 5ml MRS broth. The subculture is incubated at 37°C for 24 hrs. 10% of inoculum is transferred into subculture two. And another 10% of culture is inoculated into the bioreactor.

II. Indicator Pathogen

Single colony was transferred from NA (Nutrient Agar) plate into 5ml NB (Nutrient broth) and was incubated at 37°C for 24 hrs, before being used in Agar diffusion assay and Minimal inhibitory concentration assay.

4. Biofilm Formation on Carrier

Carrier containing culture were sampled on day 0, 1, 3, 5 & 7. And is further dried in the freeze drier under set condition of, pre-freeze, primary and secondary temperature of '-35 °C, '-5°C, 15°C & 35°C'. The dried sample undergo sputter coating process before being observed SEM [Haitao Zhang, revised] (Scanning electron Microscope). With magnification of 5000x, scale bars of 10 micrometer.

5. Quantitative Analysis

I. Determination of biofilm formation, fermentation scale of 20ml working volume.

Over a period of Seven days, *Ent. 16AVEN02, Lactobacillus SD11, L001, SD1, CU20 and Pediococcus 16AVPd 02* lactic acid bacteria strain was cultured, in a medium containing 20 ml of half diluted MRS broth and 10 g of carrier, media was refreshed every day. 0.8 g of the carrier was sampled, on day 0,3,5 & 7 for crystal violet analysis. The carrier was stained in 1% v/v crystal violet solution for 1-minute, excess dye was washed off with distilled water, before undergo oven drying at 70°C, for 30 minutes. 0.5 g of the dried carrier was de-stained in 2 ml of 95% ethanol for 5 minutes, spectrophotometric analysis, with wavelength set to 600 nm, were used to analysis the color intensity of each distained sample. The experiment was done in 2 replications, the result was analyzed using ANOVA with level of significance at $p<0.05$. Mean comparison was performed by Duncan's multiple range test.

II. Preliminary analysis, of Lactic acid productivity and Bacteriocin activity (Agar Diffusion Assay), fermentation scale of 500 ml working volume.

Lactobacillus SD1 and *Pediococcus 16AVPd 02*, were selected for further studies. Both Strains were fermented for a period of five days. pH and aeration were not controlled, with temperature maintained at 37 °C. Sampling were collected in-between 24 hrs interval for five consecutive days. Broth collected, were centrifugated at 4000 rpm for 10 minutes and further undergo filtration. Samples collected, were used for glucose (glucose liquid color test kit), lactic acid concentration (acidimetric titration), and bacteriocin activity (Agar diffusion method) analysis.

Sample were diluted with distilled water to 10^{-1}, before undergoing glucose liquid color macros assay. 0.1M NaOH were used as titrant, with sample volume analyte of 5ml mix with three drops of phenolphthalein, to determine lactic acid concentration. Agar diffusion technique was use [HOOVER, D. G., & HARLANDER, S. K.] to determine the bacteriocin activity of crude samples. *Salmonella typhimurium* ATCC 13311, *Bacillus Cereus* ATCC 11778, *Escherichia coli cereus* ATCC 8739, *Escherichia coli cereus* ETEC 01, *Escherichia coli cereus* ETEC 02, *Salmonella enteritidis* 005, *Salmonella enteritidis* 010 and *Staphylococcus suis* SS – 01 were used as indicator foodborne pathogen. The experiment was conducted for two replications, 0.40 µl of pathogen was mixed into 20 ml NA molten, allowed to solidify before being layered with sterilized filer paper each containing 20 µl of sample suspension. The assay plates were incubated for 24 hrs and measurement of clear zone diameter was recorded.

III. Determination of Lactic acid productivity and Bacteriocin activity (Minimal Inhibition Concentration), repeated batch fermentation scale of 3L working volume.

The fermentation process was conducted for four batches, with fermentation period of three days within each batch. Fermentation media was refreshed, by the end of every batch cycle. Protocol from 500 ml working volume was used, for supernatant collection, as well as glucose and lactic acid concentration analysis.

Bacteriocin activity, was determined through Minimal inhibition concentration analysis. Foodborne Indicator pathogens; *Salmonella typhimurium* ATCC 13311, *Bacillus Cereus* ATCC 11778, *Escherichia coli cereus* ATCC 8739, *Escherichia coli cereus* ETEC 01, *Escherichia coli cereus* ETEC 02, *Salmonella enteritidis* 055 and *Salmonella enteritidis* 010 were, incubated over night with OD 600nm measured ranging between 0.157 – 0.24. Samples used for MCI analysis from each batch includes crude concentration and serial diluted with MRS broth, at 10^{-2}, 10^{-4} and 10^{-6}. 20 µl of each sample were added into 100 µl of indicator pathogen broth. 20 µl of Nutrient Broth was mixed into 100 µl of indicator pathogen as control. Were filled in 96 well plate. Initial OD measure was taken, at 0 hrs of inoculation, 595nm. Final OD measurements was taken after 24 hours of sample incubation. The experiment was done in 2 replications, the result was analyzed using ANOVA with level of significance at $p<0.05$. Mean comparison was performed by Two tailed T-test.

RESULT AND DISSCUSSION

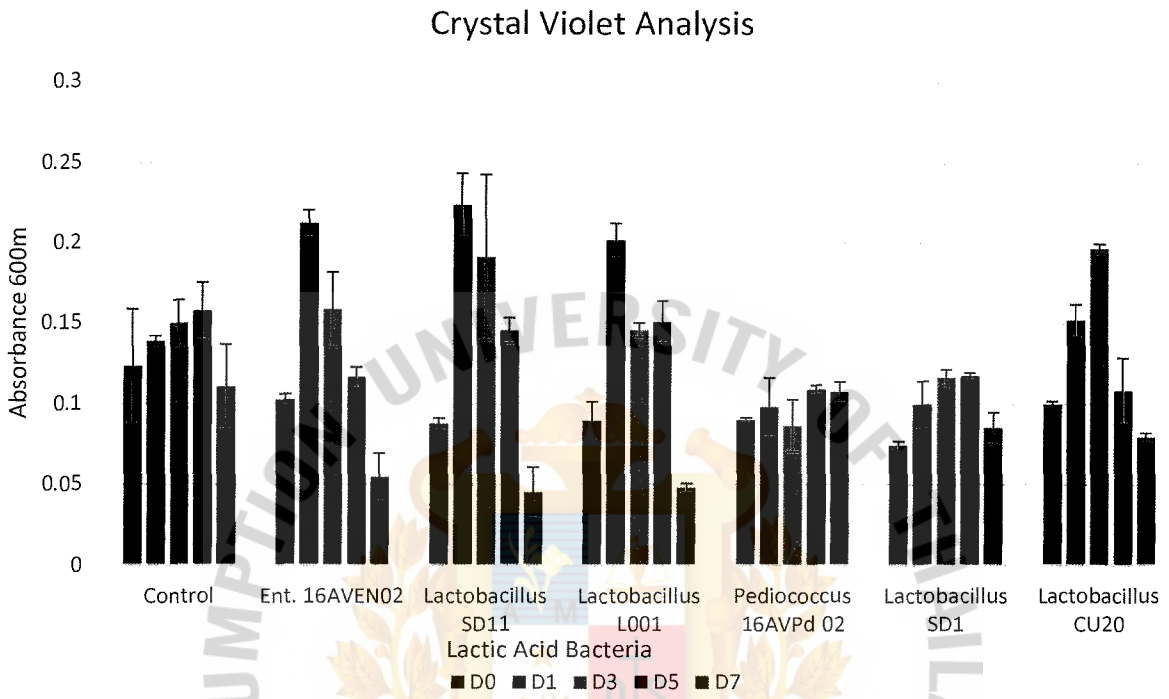
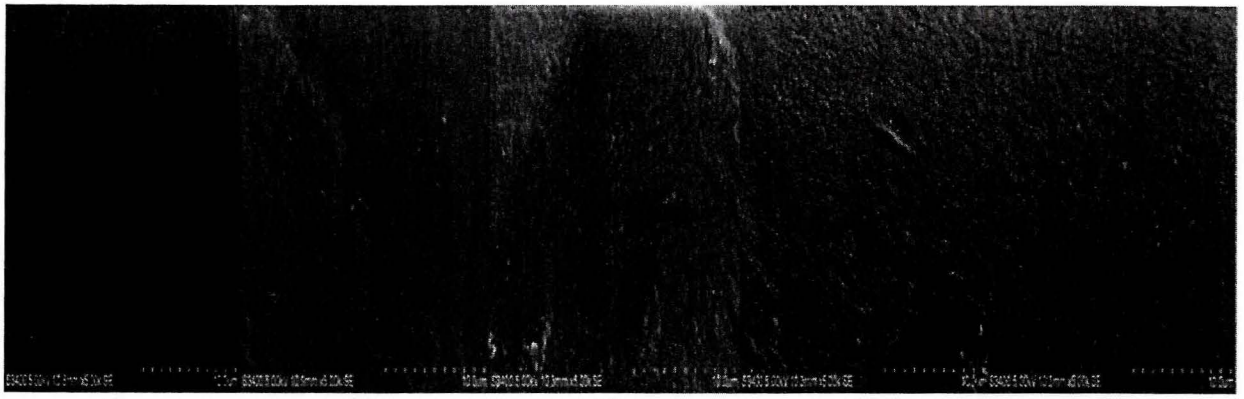


Figure 1: Represents the absorbance efficiency of biofilm formation from the crystal violet analysis.

Highest stability of biofilm formation, were observed from *PD02* (*Pediococcus 16AVPD 02*) and *SD1* (*Lactobacillus SD1*), as shown in Fig.1, sampling of day seven, *PD02* and *SD1* has the highest absorbance of 0.108 and 0.085, which are relatively close to the absorbance of the control at 0.111. In contrast, absorbance of *Ent. 16AVEN02, Lactobacillus SD11, L001 and CU20* were relatively low, at 0.055, 0.045, 0.048 and 0.079. Standard Deviation of biofilm formation between each interval were calculated with *PD02* and *SD1* having the lowest value at 0.008± and 0.019±. Between *Ent. 16AVEN02, Lactobacillus SD11, L001 and Cu20*, standard deviation calculated ranges from 0.073± to 0.047±, suggesting instability, thus satisfy the high fluctuation of biofilm formation between each day as observed. Moreover, indication of biofilm maturation at day one of fermentation were observed for, *Ent. 16AVEN02, Lactobacillus SD11* and *Lactobacillus L001*, having highest crystal violet absorbance measured at 0.212, 0.224 and 0.202, *Lactobacillus CU20*, absorbance peaked at 0.152 by day 2. The major differences of absorbance between sampling, day one and seven of *Ent. 16AVEN02, Lactobacillus SD11* and *L001*, suggest continuous detachment and reduction of biofilm formation efficiency between each sampling period. Whereas biofilm maturation was observed in day 2 of sampling, the detachment observed were significant with absorbance differences of 0.118.

Biofilm formation of *SD1* observed from SEM images in Fig. 2, indicated stage II of biofilm formation, secretion of extracellular polymeric substances onto carrier sample of Day 1, following Day three, five and seven. The biofilm characteristic remained relatively similar, cells appear to grow in multiple layers, protected by a clear sheet of biofilm. Colony attachment of *PD02*, indicated phase I of biofilm formation by Day 1 however, biofilm maturation was observed on day three and five, with thick layers of biofilm sheet, with colony alignments growing in multiple layers. No EPS were observed of results from day seven, however the cell characteristics appeared similarly to sample from day 1, which indicates detachment of biofilm from Day 5 and a phase I of new biofilm formation. Carrier degradation over the seven-day period of fermentation was observed, in Fig. 2, grainy texture as well as development of pours was observed across each day. This effect on the surface could be due to the detachment of soybean meal, from the carrier unit, which are influence by abrasive forces occurring during daily media refreshment activities and water adsorption from media. Moreover, relatively high absorbance of control carrier with OD of 0.123, 0.139, 0.15, 0.158 and 0.111 as shown in Fig.1, could be due to the physical factors and the material composition of the carrier.

Control: D0 – D7

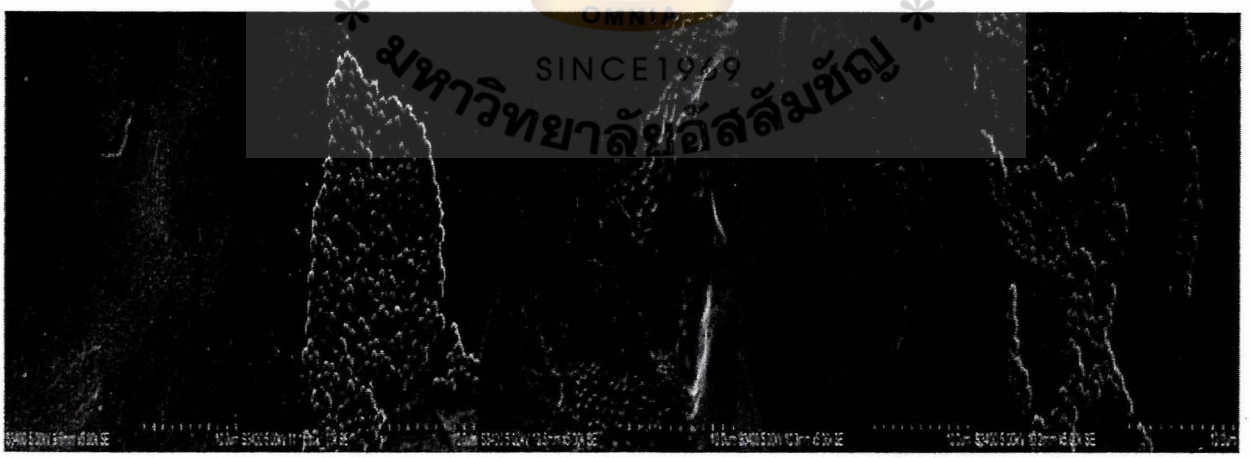


D0 D1 D3 D5 D7

***Lactobacillus SD1*: D0 – D7**

D0 D1 D3 D5 D7

***Pediococcus 16AVPd* 02: D0 – D7**



D0 D1 D3 D5 D7

Figure 2: Represents SEM images of lactic acid biofilm, of *Pediococcus 16AVPD 02 and Lactobacillus SD1* formation on the surface of carrier from D0 – D7 with 5000x magnification.

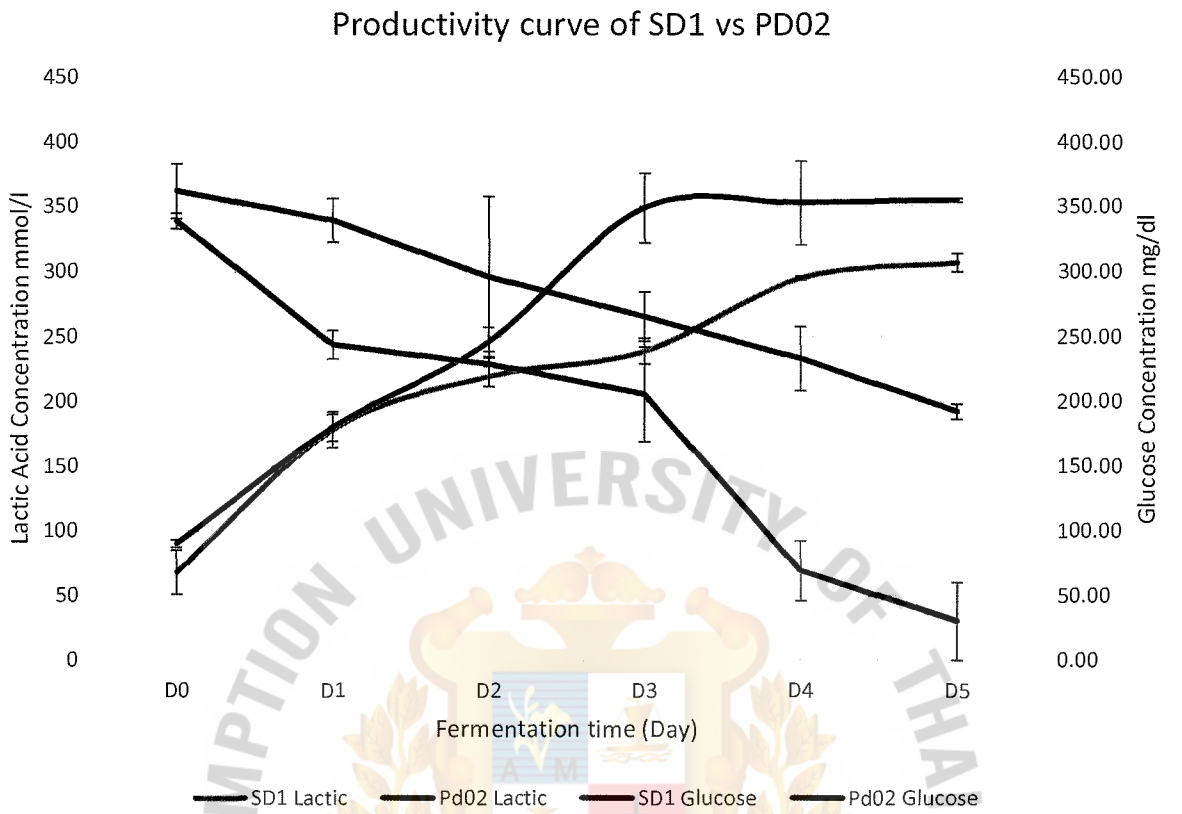


Figure 3: Represents the productivity of Lactic acid and usage of glucose between *Pediococcus 16AVPD 02 and Lactobacillus SD1,* from fermentation date of zero to day 5, 500 ml fermentation scale.

Table 1: Represents the productivity and % yield of lactic acid by *Pediococcus 16AVPD 02 and Lactobacillus SD,* after three days of fermentation in 500 ml working volume scale.

Lactic Acid	**Productivity mmol/l/hr**	**% yield**
PD02	4.85 ± 0.37	65.21
SD1	3.32 ± 0.14	33.91

The percent yield of *PD02* were 31.3 % higher comparing to *SD1* at 65.21% to 33.91%, Table 1. Fig.3, Lactic acid production increased from 90 mmol/l at day zero to 349 mmol/l by day three with productivity of lactic acid of 4.85 ± 0.37, production of lactic acid peaked at 349 mmol/l and negligibly increased to 6 mmol/l by day five, with glucose consumption reduced at a stable rate from 361.18 mg/dl to 192.12 mg/dl by day five. Lactic acid production of *SD1*, peaked at 295 mmol/l by day four of fermentation and increased by 12 mmol/l by the day five, two patterns of exponential phase of lactic acid production were observed, first from day zero to day one at 68 mmol/l to 178 mmol/l and day three to, day four from 295 mmol/l to 307 mmol, overall lactic acid productivity of *SD1* were 3.32 ± 0.14. Rate of substrate consumption at exponential phase dramatically decrease from 338.48 mg/dl to 243.79 mg/dl at day one, two and 205.45 mg/dl to 69.09 mg/dl at day three to four. Substrate consumption were not fully consumed by both LAB with glucose concentration left over at 192.12 mg/dl *PD02* and 30.30 mg/dl *SD1*. Thus, longer

period of fermentation, could resulted in complete consumption of substrate by both LAB. Un-optimized fermentation condition to achieve maximum cell performance, as yield of lactic acid, could inhibit cells productivity by lowering of pH, as pH was not controlled in this process, could be the factor which causes *PD02* and *SD1* to reach maximum lactic concentration ahead of completed consumption of substrates. 75 hours of fermentation were used to calculate for the productivity and percent yield of both strains, hence higher lactic concentration was observed from *PD02* and minimization of fermentation cost, are most feasible for scale fermentation processes.

Sample starting from day three of *SD1* strain, indicated highest bacteriocin activity with , inhibitory clear zones observed against, *Salmonella typhimurium ATCC 13311, Bacillus Cereus ATCC 11778, Escherichia coli cereus ATCC 8739, Escherichia coli cereus ETEC 01, Escherichia coli cereus ETEC 02, Salmonella enteritidis 005, Salmonella enteritidis 010 and Staphylococcus suis SS – 01*. The growth of *Escherichia coli cereus ATCC 8739*, were most susceptible to bacteriocin inhibition from both LAB strains. Bacteriocin produced from *PD02,* were not able to inhibit the growth of *Staphylococcus suis SS – 01*, in addition both LAB strains clear zones from both strains were smallest against *Salmonella typhimurium* ATCC 13311. Lastly, clear zones observed during the Agar diffusion assay were not used to quantify the bacteriocin activity, visual measurement of inhibition zone and diffusion factors of sample into agar medium, could induce high degree of error [M.L Cabo, 1999].

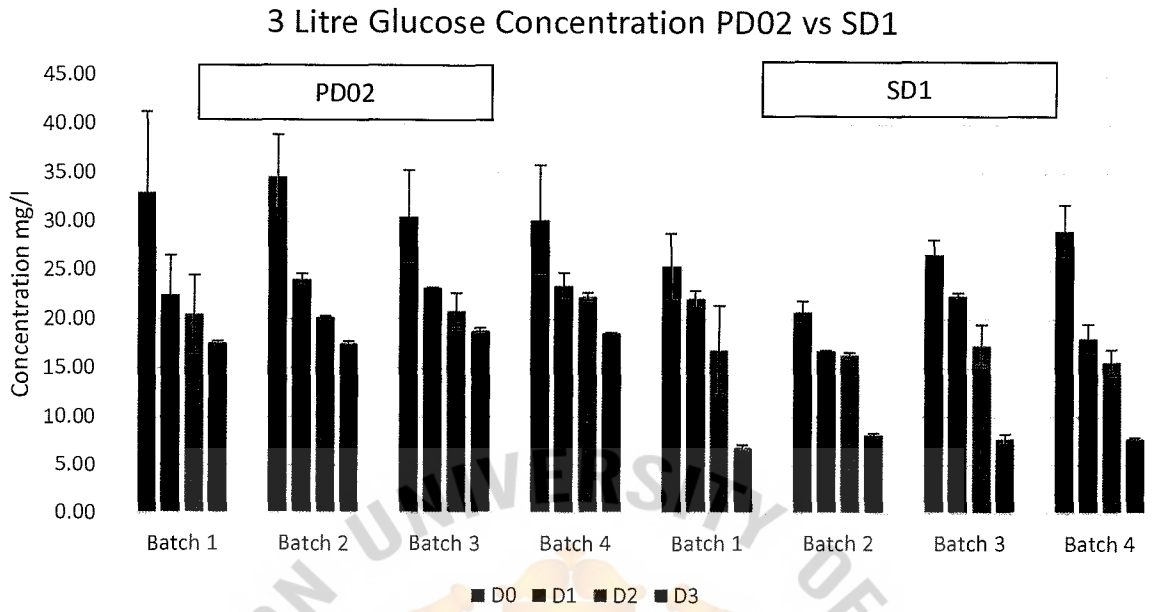


Figure 4: Represents Glucose consumption between *Pediococcus 16AVPD 02 and Lactobacillus SD1,* three days interval and in between each batch.

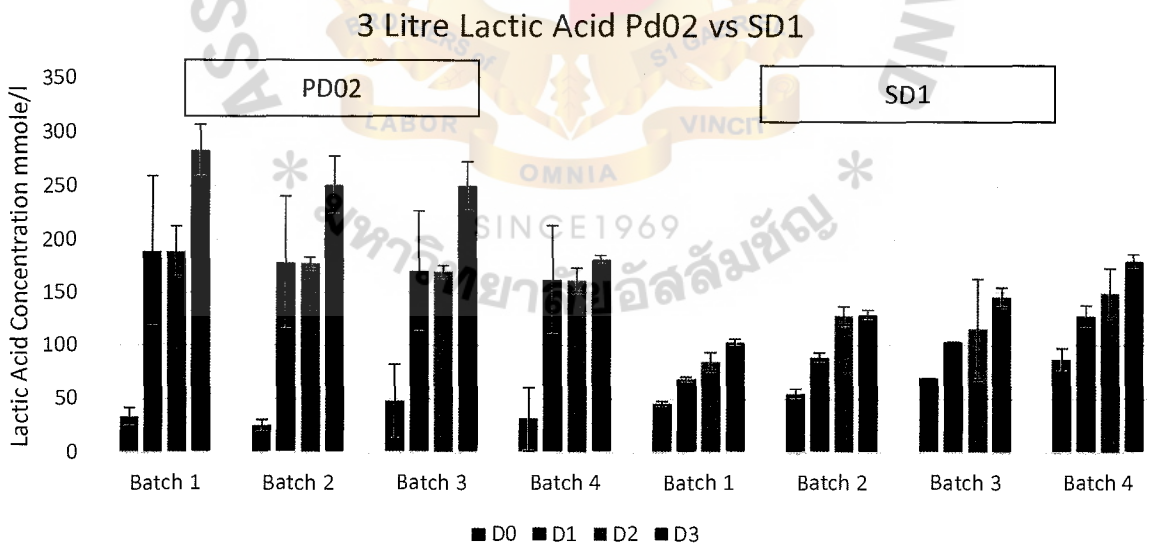


Figure 5: Represents the productivity of Lactic acid between *Pediococcus 16AVPD 02 and Lactobacillus SD1,* three days interval and in between each batch.

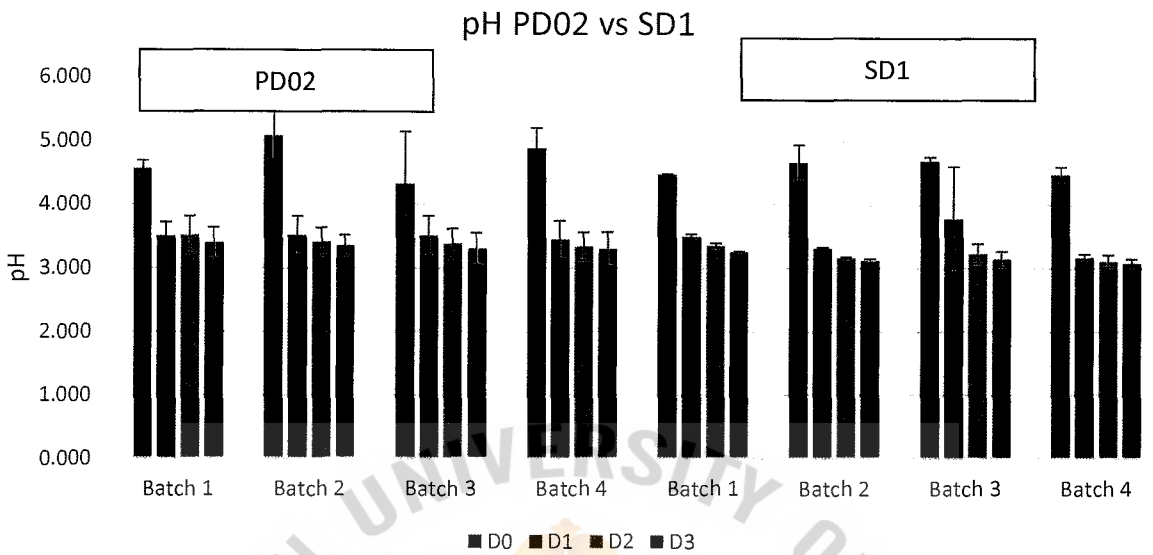


Figure 6: Represent pH condition of *Pediococcus 16AVPD 02 and Lactobacillus SD1* fermentation from day zero to day three and in between each batch.

Table 2: Represents the efficiency of lactic acid productivity of *Pediococcus 16AVPD 02 and Lactobacillus SD,* in 3-liter scale fermentation, sampling of day 3 fermentation of each batches.

Batch	Day 3 Samples	Productivity mmol/l/hr	% yield
1	**PD02**	2.36 ± 1.62	27.09
	SD1	1.37 ± 0.05	17.31
2	**PD02**	2.29 ± 1.09	17.75
	SD1	1.71 ± 0.07	45.06
3	**PD02**	2.55 ± 1.44	34.52
	SD1	1.94 ± 0.12	30.27
4	**PD02**	2.01 ± 0.58	27.04
	SD1	2.39 ± 0.09	24.86
AVG Between Batches	**PD02**	2.37 ± 0.46	26.6
	SD1	1.85± 0.032	29.4

The productivity and percent yield efficiency of *PD02* and *SD1* between each batched, were averaged at 2.37 ± 0.46, 26.6% and 1.85 ± 0.032, 29.4%. Table 2. *PD02* had higher productivity of lactic acid from batch one to batch four; 2.36 ± 1.62, 2.29 ± 1.09, 2.55 ± 1.44 and 2.01 ± 0.58 mmol/l/hr, with *SD1* productivity calculated at 1.37 ± 0.05, 1.71 ± 0.07, 1.94 ± 0.12 and 2.39 ± 0.09. *SD1* productivity of lactic acid increased from batch one to four, and cells productivity of *PD02* remained rather stable between each batch. *PD02* has the highest percentage yield at batch three at 34.52 %, and batch two for *SD1* at 45.06 %, repeated batch fermentation of four cycle the loss of cells performance efficiency was not observed for both *SD1* and *PD02*.

Lactic acid production of SD1 appeared to be continuously improving, Fig. 5. D3 between batch one to batch four saw increased final concentration of lactic acid from, 102.5 mmol/l to 179 mmol/l. With *PD02* decreasing from 283 mmol/l the best production observed in batch one and reduced to 181 mmol/l by batched four. Fig. 4 and 5, observed higher substrate consumption per produced yield for *SD1*, as compared to *PD02* similar observation can be, observed in 500 ml fermentation. Productivity of both strains were observed to be relativity low, with averaging at 26.6% and 29.4%, this could be due to pH inhibition, similar observation for results from 500 ml. As observed in Fig.6, the fermentation pH formed similar pattern, D0 of each batch had high pH to due fresh media recycling averaging at pH of 4.64 between all batches and both samples, though the pH of fresh media was measured at pH 6.9, acid reduction were caused by excess culture left on the carrier and fermenter. Further observation indicated, the pH between D0 to D1 are dramatically reduced and slightly reduced between D1 to D3.

Seven-indicator foodborne pathogen were inhibited by all samples from both LAB strains, in Fig 7 and 8. sample dilution of 10^{-6} was able to inhibit the growth of *E. ETEC – 01* and *02* with absorbance from *SD1* and *PD02* averaged at 0.087, 0.099 and 0.114, 0.136, while control indicator foodborne had absorbances of 0.702 and 0.969. This result suggested, high bacteriocin activity against *Escherichia coli cereus ETEC - 01 and ETEC – 02.* Furthermore, the activity of bacteriocin observed in Fig. 7 to 13, crude sample had the highest bacteriocin activity, crude sample between each batch and from different LAB were not majorly different. Diluted samples of *SD1* at 10^{-2}, 10^{-4} and 10^{-6}, was observed to have higher inhibitory properties against *Salmonella Enteritidis – 005, Salmonella Enteritidis – 010* and *E. coli ATCC 8739*, as compared to the absorbance of samples from *PD02*. Major distinguished of bacteriocin activity were not observed, samples which were very diluted, were observed to higher absorbance value than control Fig 9,11,12 and 13 this may be due the dilution factor used and medium provided additional substrates and nutrient for the foodborne pathogen to utilized.

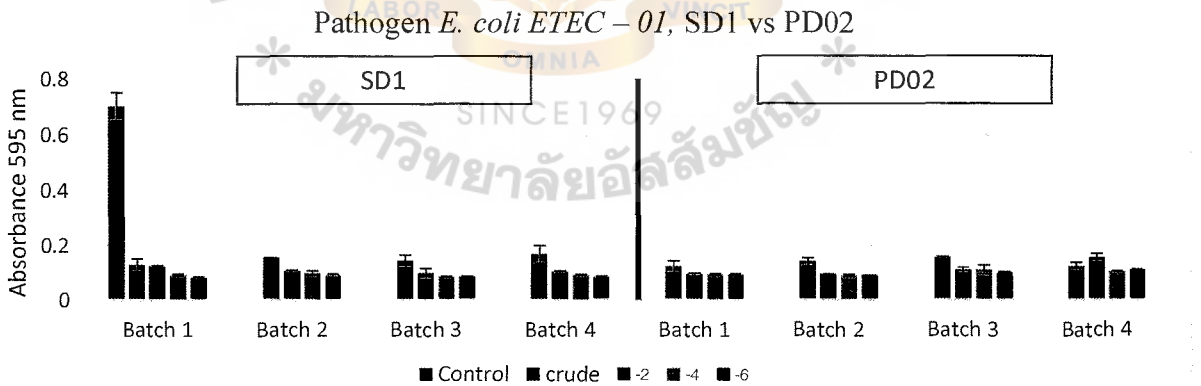


Figure 7: MIC result of sample against *E. ETEC – 01* after 24 hours incubation.

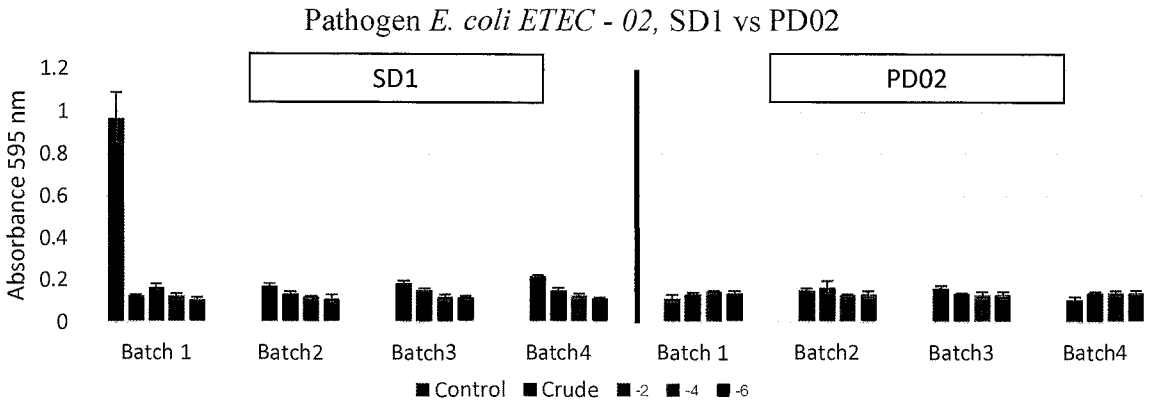


Figure 8: MIC result of sample against *E. ETEC – 02* after 24 hours incubation.

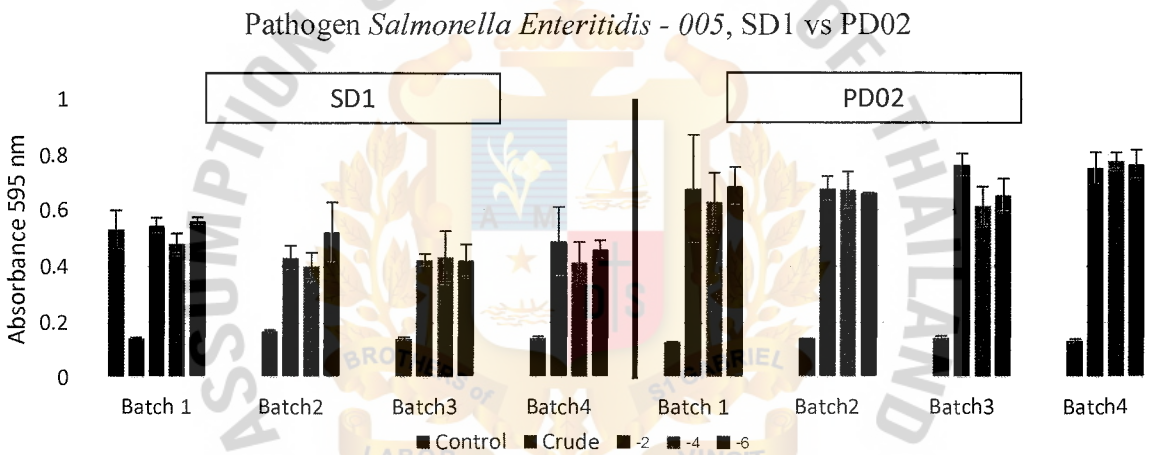


Figure 9: MIC result of sample against *Salmonella Enteritidis - 005* after 24 hours incubation.

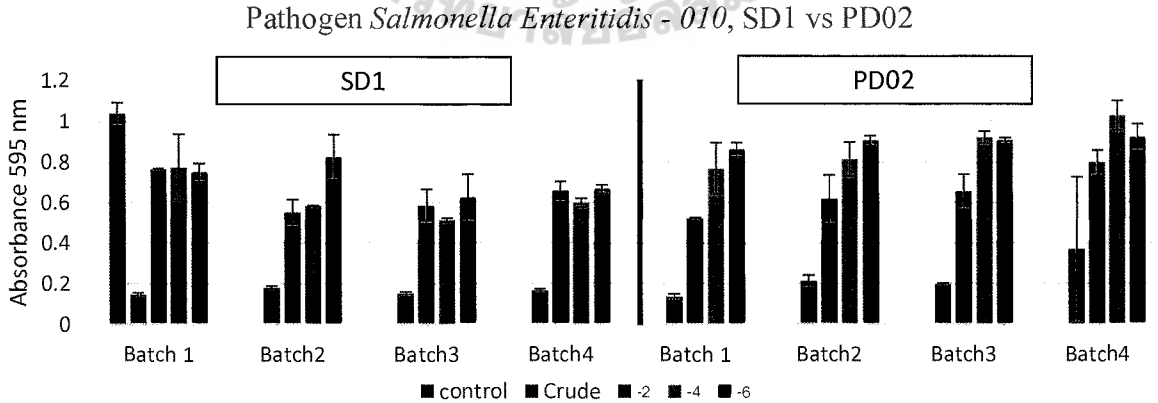


Figure 10: MIC result of sample against *Salmonella Enteritidis - 010* after 24 hours incubation.

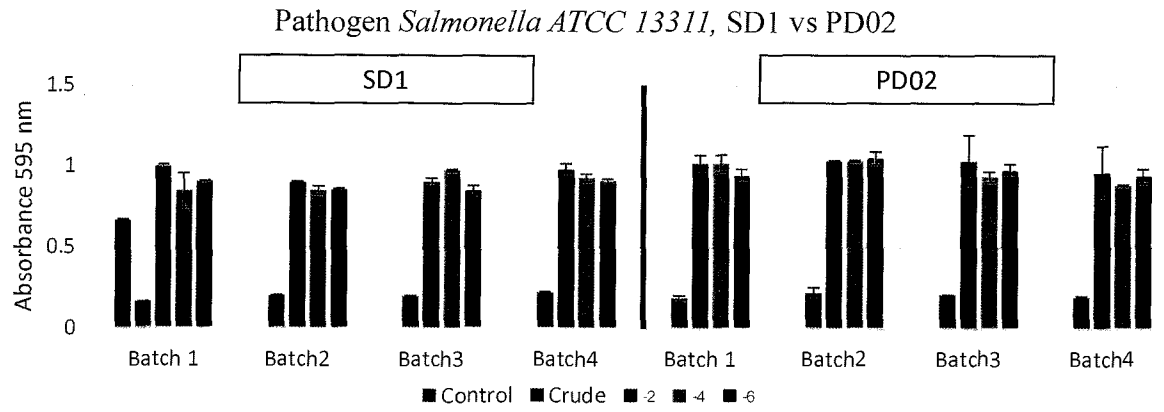


Figure 11: MIC result of sample against *Salmonella ATCC 13311* after 24 hours incubation.

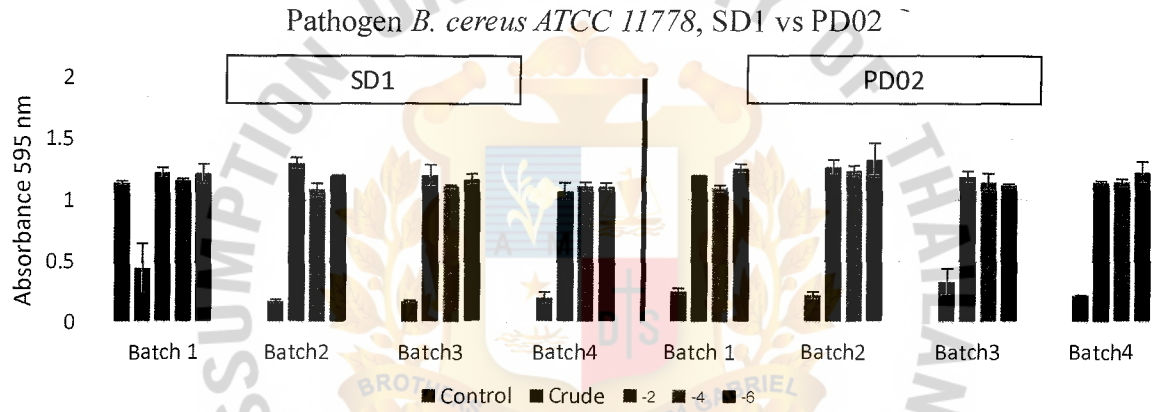


Figure 12: MIC result of sample against *B. cereus ATCC 11778* after 24 hours incubation.

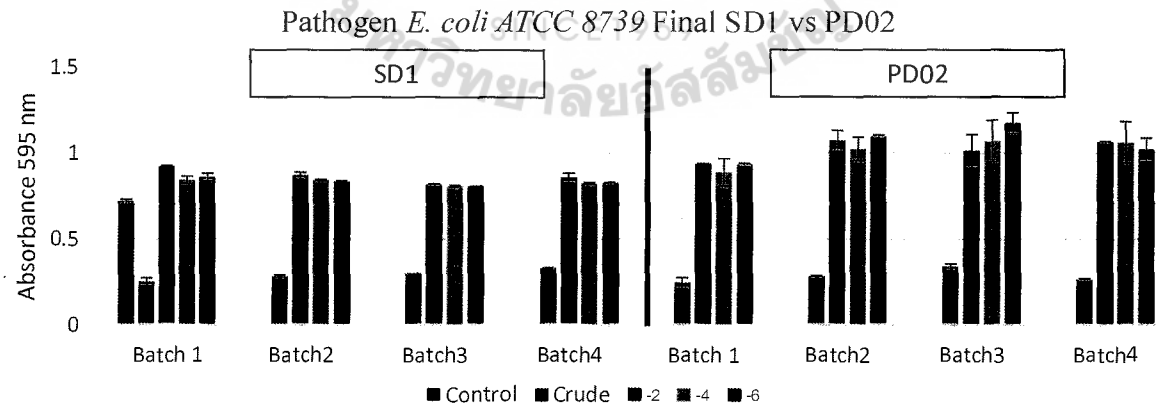


Figure 13: MIC result of sample against *E. coli ATCC 8739* after 24 hours incubation.

CONCLUSION

The objectives of this experiment were partially satisfied, *Lactobacillus SD1* and *Pediococcus 16AVPd 02*, were selected for this research, due to stability of biofilm formation. *SD1* showed earlier formation of biofilm, as well as higher bacteriocin activity observed from *SD1*. *PD02* had better productivity of lactic acid. Further studies are needed, to optimize the fermentation condition, to enhance the antimicrobial activity of *SD1,* as well as number of repeated batches. Depreciation of cells performance were not observed after four batches of fermentation cycle.

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Statistic Program

- Sas Program (9.4) UTR: sas.com/en_us/software/sas9.html

APPENDIX A

Appx. A-1: Raw data (absorbance OD), of biofilm formation, crystal violet analysis from 20 ml fermentation.

1th Trail (0.5g)	D0	D1	D3	D5	D7
Control I	0.148	0.141	0.14	0.145	0.129
Control II	0.098	0.136	0.16	0.17	0.092
***Ent. 16AVEN02*, Rep I**	0.1	0.218	0.142	0.121	0.044
Rep II	0.105	0.206	0.175	0.112	0.065
***Lactobacillus SD11*, Rep I**	0.09	0.21	0.155	0.151	0.056
Rep II	0.085	0.238	0.228	0.14	0.034
***Lactobacillus L001*, Rep I**	0.098	0.194	0.143	0.142	0.05
Rep II	0.081	0.209	0.149	0.16	0.046
***Pediococcus 16AVPD 02*, Rep I**	0.091	0.085	0.098	0.107	0.103
Rep II	0.089	0.111	0.105	0.111	0.112
***Lactobacillus SD1*, Rep I**	0.072	0.089	0.12	0.116	0.092
Rep II	0.076	0.11	0.113	0.119	0.078
***Lactobacillus CU20*, Rep I**	0.101	0.145	0.199	0.123	0.081
Rep II	0.098	0.159	0.195	0.094	0.077

Table A-1i: Absorbance average and SD of crystal violet analysis.

AVG	D0	SD	D1	SD	D3	SD	D5	SD	D7	SD	Batch SD
Control	0.123	0.035	0.139	0.004	0.150	0.014	0.158	0.018	0.111	0.026	0.019
Ent. 16AVEN02	0.103	0.004	0.212	0.008	0.159	0.023	0.117	0.006	0.055	0.015	0.060
Lactobacillus SD11	0.088	0.004	0.224	0.020	0.192	0.052	0.146	0.008	0.045	0.016	0.073
Lactobacillus L001	0.090	0.012	0.202	0.011	0.146	0.004	0.151	0.013	0.048	0.003	0.059
Pediococcus 16AVPd 02	0.090	0.001	0.098	0.018	0.102	0.005	0.109	0.003	0.108	0.006	0.008
Lactobacillus SD1	0.074	0.003	0.100	0.015	0.117	0.005	0.118	0.002	0.085	0.010	0.019
Lactobacillus CU20	0.100	0.002	0.152	0.010	0.197	0.003	0.109	0.021	0.079	0.003	0.047

Appx. A-2: Raw data, of glucose concentration, glucose liquid color assay, fermentation 500 ml.

Glucose Liquid Color	**SD1**	**STD: 100mg/dl, OD: 0.330**	**Unit: ml**	
Time:	**OD: Rep 1**	**OD: Rep 2**	**AVG**	**SD**
D0	1.103	1.131	1.117	0.020
D1	0.83	0.779	0.805	0.036
D2	0.732	0.778	0.755	0.033
D3	0.764	0.593	0.678	0.122
D4	0.174	0.282	0.228	0.076
D5	0.170	0.030	0.100	0.099
		PD02		
Time:	**OD: Rep 1**	**OD: Rep 2**	**AVG**	**SD**
D0	1.244	1.144	1.194	0.071
D1	1.158	1.080	1.119	0.055
D2	1.120	0.832	0.976	0.204
D3	0.920	0.832	0.876	0.062
D4	0.827	0.712	0.770	0.081
D5	0.620	0.648	0.634	0.020

Appx A-2i: Glucose concentration, glucose liquid color assay, fermentation 500 ml.

Glucose concentration 500ml	***SD1***				***PD02***			
	Rep 1 (mg/dl)	**Rep 2 (mg/dl)**	**AVG**	**SD**	**Rep 1 (mg/dl)**	**Rep 2 (mg/dl)**	**AVG**	**SD**
D0	334.24	342.73	338.48	6.00	376.97	346.67	361.82	21.43
D1	251.52	236.06	243.79	10.93	350.91	327.27	339.09	16.71
D2	221.82	235.76	228.79	9.86	339.39	252.12	295.76	61.71
D3	231.52	179.39	205.45	36.86	278.79	252.12	265.45	18.86
D4	52.73	85.45	69.09	23.14	250.61	215.76	233.18	24.64
D5	51.52	9.09	30.30	30.00	187.88	196.36	192.12	6.00

Appx. A-3: Raw data, of lactic acid concentration, acidimetric titration, fermentation 500 ml.

Acid Titration	**SD1**	**Analyst: 5 ml**	**Unit: ml**	
Time:	**Volume: Rep 1**	**Volume: Rep 2**	**AVG**	**SD**
D0	2.8	4	3.400	0.849
D1	8.4	9.4	8.900	0.707
D2	10.7	11.25	10.975	0.389
D3	11.6	12.3	11.950	0.495
D4	14.8	14.7	14.750	0.071
D5	15.6	15.1	15.350	0.354
		PD02		
Time:	**Volume: Rep 1**	**Volume: Rep 2**	**AVG**	**SD**
D0	4.6	4.4	4.500	0.141
D1	9.35	8.6	8.975	0.530
D2	12.7	11.9	12.300	0.566
D3	18.4	16.5	17.450	1.344
D4	18.8	16.5	17.650	1.626
D5	17.7	17.8	17.750	0.071

Appx. A-3i: Lactic acid concentration, acidimetric titration, fermentation 500 ml.

	SD1	**Unit mmol/l**			***PD02***	**Unit mmol/l**		
	Vol i	**Vol ii**	**AVG**	**SD**	**Vol i**	**Vol ii**	**AVG**	**SD**
D0	56	80	68	16.971	92	88	90	2.828
D1	168	188	178	14.142	187	172	179.5	10.607
D2	214	225	219.5	7.778	254	238	246	11.314
D3	232	246	239	9.899	368	330	349	26.870
D4	296	294	295	1.414	376	330	353	32.527
D5	312	302	307	7.071	354	356	355	1.414

Appx. A-4: Raw data, Agar diffusion analysis, clear zone diameter, from 500 ml fermentation.

***SD1*, Replication 1**	**D0**	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**
E.Coli E/TEC - 01	-	-	-	-	0.85	0.7	0.65	0.75
E.Coli E/TEC - 02	-	-	-	0.7	-	-	-	-
S.Suis SS - 01	-	-	-	-	-	-	-	-
S.E 005	0.65	0.9	1	0.95	0.75	0.6	0.6	0.6
S.E 010	0.7	0.65	-	0.9	-	-	-	-
Salmonella ATCC 13311	0	0	0	0	0	0	0	0
B.Cereus ATCC 11778	0.65	0.65	0.7	0.65	0.65	0.6	0.6	0.65
E.Coli ATCC 8739	0.75	0.75	0.8	0.7	1.1	1.05	1	1.1
***SD1*, Replication 2**								
E.Coli E/TEC - 01	-	-	-	-	-	-	-	-
E.Coli E/TEC - 02	0.7	0.75	0.9	1	0.75	0.6	0.75	0.7
S.Suis SS - 01	-	-	0.75	0.8	-	0.7	0.7	0.75
S.E 005	0.65	0.7	1	1.1	0.7	0.8	1	0.7
S.E 010	0.8	0.75	1	1.1	1.1	1.15	1	1.1
Salmonella ATCC 13311	0.8	0.7	0.7	0.9	0.8	0.85	0.9	1.05
B.Cereus ATCC 11778	0.7	0.65	0.75	0.8	0.7	0.7	0.7	0.7
E.Coli ATCC 8739	1	1.3	1.3	1.4	1.4	1.5	1.45	1.3
***Pd02*, Replicate 1**								
E.Coli E/TEC - 01	-	-	-	-	-	-	-	-
E.Coli E/TEC - 02	0.8	0.8	0.65	0.9	-	-	-	-
S.Suis SS - 01	-	-	-	-	-	-	-	-
S.E 005	0.8	0.9	0.95	1.1	0.8	0.9	1	1
S.E 010	1	0.8	0.8	0.85	0.7	-	-	-
Salmonella ATCC 13311	0	0	0	0	0	0	0	0
B.Cereus ATCC 11778	0.6	0.6	0.6	0.65	0.6	0.625	0.65	0.65
E.Coli ATCC 8739	1.2	1.1	1.2	1.15	1.15	1.1	1.05	1.15
***Pd02*, Replication 2**								
E.Coli E/TEC - 01	0.9	0.9	1	-	-	0.95	1	0.8

E.Coli E/TEC - 02	-	-	-	-	-	-	-	-
S.Suis SS - 01	-	-	-	-	-	-	-	-
S.E 005	1	1.1	1.1	1.1	0.75	0.8	0.7	0.6
S.E 010	0.8	0.8	0.8	0.9	0.8	0.7	0.75	0.85
Salmonella ATCC 13311	0.8	0.9	0.85	1	0.8	0.8	0.9	0.9
B.Cereus ATCC 11778	0.65	0.7	0.65	0.65	0.6	0.65	0.65	0.65
E.Coli ATCC 8739	0.9	0.9	0.85	1	0.9	1.1	0.9	1

Appx. A-4i: Average, Agar diffusion analysis, clear zone diameter, from 500 ml fermentation.

AVERAGE:	**Diameter: cm**							
SD1	D0	D1	D2	D3	D4	D5	D6	D7
E.Coli E/TEC - 01	-	-	-	-	0.85	0.7	0.65	0.75
E.Coli E/TEC - 02	0.7	0.75	0.9	0.85	0.75	0.6	0.75	0.7
S.Suis SS - 01	-	-	-	0.75	0.8	-	0.7	0.75
S.E 005	0.65	0.8	1	1.025	0.725	0.7	0.8	0.65
S.E 010	0.75	0.7	1	1	1.1	1.15	1	1.1
Salmonella ATCC 13311	0.4	0.35	0.35	0.45	0.4	0.425	0.45	0.525
B.Cereus ATCC 11778	0.675	0.65	0.725	0.725	0.675	0.65	0.65	0.675
E.Coli ATCC 8739	0.875	1.025	1.05	1.05	1.25	1.275	1.225	1.2
Pd02	D0	D1	D2	D3	D4	D5	D6	D7
E.Coli E/TEC - 01	0.9	0.9	1	-	-	0.95	1	0.8
E.Coli E/TEC - 02	0.8	0.8	0.65	0.9	-	-	-	-
S.Suis SS - 01	-	-	-	-	-	-	-	-
S.E 005	0.9	1	1.025	1.1	0.775	0.85	0.85	0.8
S.E 010	0.9	0.8	0.8	0.875	0.75	0.7	0.75	0.85
Salmonella ATCC 13311	0.4	0.45	0.425	0.5	0.4	0.4	0.45	0.45
B.Cereus ATCC 11778	0.625	0.65	0.625	0.65	0.6	0.6375	0.65	0.65
E.Coli ATCC 8739	1.05	1	1.025	1.075	1.025	1.1	0.975	1.075

Appx. A-5: Raw data, of lactic acid concentration, acidimetric titration, fermentation 3 L.

Acid titration: PD02, replication 1			**Volume of sample used: 5 ml**	
NaOH: 0.1M	**Unit: ml**			
Raw data:	**D0**	**D1**	**D2**	**D3**
Batch 1	1.4, 1.5	8.6, 10.3	12.2, 11.4	13.3, 15
Batch 2	1.3, 1.4	8.7, 9.1	10.3, 10.9	11.3, 11.7
Batch 3	3.5, 3.7	8.7, 8.3	10, 10.4	13.3, 13.5
Batch 4	2.4, 2.8	8.5, 7.7	9.5, 9.8	8.9, 9.2
Average & Standard Deviation				
Batch 1	1.45 ±	9.45 ±	11.8 ±	14.15 ±
Batch 2	1.35 ±	8.9 ±	10.6 ±	11.5 ±
Batch 3	3.6 ±	8.5 ±	10.2 ±	13.4 ±
Batch 4	2.6 ±	8.1 ±	9.65 ±	9.05 ±

Acid titration: SD1 replication 1			**Volume of sample used: 5ml**	
NaOH: 0.1M	**Unit: ml**			
Raw date:	**D0**	**D1**	**D2**	**D3**
Batch 1	1.3, 1.2	3.4, 3.4	4.3, 4.2	4.9, 5.1
Batch 2	0.9, 0.9	4.4, 4.4	5.6, 5.7	6.4, 6.8
Batch 3	1.8, 1.7	5.8, 4.5	7.2, 6.8	7.5, 7.7
Batch 4	2.2, 2.4	6.2, 6.5	7.6, 7.9	8.7, 8.7
Average & Standard Deviation				
Batch 1	1.25 ±	3.4 ±	4.25 ±	5 ±
Batch 2	0.9 ±	4.4 ±	5.65 ±	6.6 ±
Batch 3	1.75 ±	5.15 ±	7 ±	7.6 ±
Batch 4	2.3 ±	6.35 ±	7.75 ±	8.7 ±

Acid titration: PD02, Replication 2			Volume of sample used: 5ml	
NaOH: 0.1M	Unit: ml			
Raw data:	D0	D1	D2	D3
Batch 1	2, 1.9	4.6, 4.3	5.4, 5	5.6, 5.5
Batch 2	1.2, 0.9	4.3, 4.6	5.3, 5.3	5.6, 5.8
Batch 3	1.2, 1.1	4.4, 4.5	5.2, 5.6	5.8, 5.7
Batch 4	1, 1	4.5, 4.4	5.6, 5.3	6, 6
Average & Standard Deviation				
Batch 1	1.95 ±	4.45 ±	5.2 ±	5.55 ±
Batch 2	1.05 ±	4.45 ±	5.3 ±	5.7 ±
Batch 3	1.15 ±	4.45 ±	5.4 ±	5.75 ±
Batch 4	1 ±	4.45 ±	5.45 ±	6 ±

Acid titration: SD1, replication 2			Volume of sample used: 5ml	
NaOH: 0.1M	Unit: ml			
Raw date:	D0	D1	D2	D3
Batch 1	1.4, 1.2	3.6, 3.7	4.5, 3.9	5.6, 4.9
Batch 2	1.4, 1.4	5.7, 5.5	6.6, 6.6	6.3, 6.2
Batch 3	1.9, 1.6	3.3, 2.9	5.2, 5	7, 6.9
Batch 4	1.5, 1.6	5.7, 6.5	6.6, 7.8	9.2,9.2
Average & Standard Deviation				
Batch 1	1.3 ±	3.65 ±	4.2 ±	5.25 ±
Batch 2	1.4 ±	5.6 ±	6.6 ±	6.25 ±
Batch 3	1.75 ±	3.1 ±	5.1 ±	6.95 ±
Batch 4	1.55 ±	6.1 ±	7.2 ±	9.2 ±

Appx. A-5i: Lactic acid concentration, acidimetric titration, fermentation 3 L.

Unit mmol/l		AVG		AVG		AVG		AVG	
		D0	SD	D1	SD	D2	SD	D3	SD
PD02	Batch 1	33	8.485	189	70.711	189	24.042	283	24.042
	Batch 2	25	5.657	178	62.933	178	5.657	251	26.870
	Batch 3	47.5	34.648	170	57.276	170	5.657	250	22.627
	Batch 4	31.25	29.345	162	51.619	162	11.314	181	4.243
SD1	Batch 1	45	2.828	68	2.828	84.5	8.485	102.5	3.536
	Batch 2	54.5	4.950	88	4.950	128	8.485	128.5	4.950
	Batch 3	69	0.000	103	0.000	115.5	47.376	145.5	9.192
	Batch 4	86.5	10.607	127	10.607	149.5	23.335	179	7.071

Appx. A-6: Raw data, of glucose concentration, glucose liquid color assay, fermentation 3 L.

PD02, Replication 1	**Unit: OD**				
Standard OD 500:	**0.31**	**0.307**		**Avg:**	**0.3085**
STD: 100 mg/dl	**OD: 0.3085**				
Raw data:	**Time (Day)**				
Batch No.	**D0**	**D1**	**D2**	**D3**	
Batch 1	0.968, 1.0	0.723, 0.838	0.666, 0.772	0.533, 0.562	
Batch 2	0.956, 1.366	0.686, 0.822	0.620, 0.624	0.535, 0.496	
Batch 3	0.758, 0.912	0.745, 0.685	0.599, 0.608	0.546, 0.580	
Batch 4	0.766, 0.845	0.640, 0.744	0.647, 0.746	0.536, 0.568	
Average & Standard Deviation					
Batch 1	0.984 ± 0.023	0.781 ± 0.081	0.719 ± 0.075	0.548 ± 0.021	
Batch 2	1.161 ± 0.290	0.754 ± 0.096	0.622 ± 0.003	0.516 ± 0.028	
Batch 3	0.835 ± 0.109	0.715 ± 0.042	0.604 ± 0.006	0.563 ± 0.024	
Batch 4	0.806 ± 0.056	0.692 ± 0.074	0.696 ± 0.070	0.552 ± 0.023	
SD1, Replication 1	**Unit: OD**				
Standard OD 500:	**0.31**	**0.307**		**Avg:**	**0.3085**
STD: 100 mg/dl	**OD: 0.3085**				
Raw data:	**Time (Day)**				
Batch No.	**D0**	**D1**	**D2**	**D3**	

Batch 1	0.683, 0.735	0.621, 0.698	0.440, 0.402	0.425, 0.377	
Batch 2	0.550, 0.777	0.466, 0.544	0.476, 0.546	0.462, 0.490	
Batch 3	0.799, 0.909	0.591, 0.616	0.471, 0.509	0.478, 0.380	
Batch 4	0.775, 0.896	0.557, 0.591	0.408, 0.503	0.314, 0.454	
Average & Standard Deviation					
Batch 1	0.709 ± 0.037	0.656 ± 0.054	0.421 ± 0.027	0.401 ± 0.034	
Batch 2	0.664 ± 0.161	0.505 ± 0.055	0.511 ± 0.049	0.458 ± 0.020	
Batch 3	0.854 ± 0.078	0.604 ± 0.018	0.49 ± 0.027	0.429 ± 0.069	
Batch 4	0.836 ± 0.086	0.574 ± 0.024	0.456 ± 0.067	0.384 ± 0.099	
PD02, Replication 2	**Unit: OD**				
Standard OD 500:	**0.31**	**0.307**		**Avg:**	**0.3085**
STD: 100 mg/dl	**OD: 0.3085**				
Raw data:	**Time (Day)**				
Batch No.	**D0**	**D1**	**D2**	**D3**	
Batch 1	0.825, 0.966	0.608, 0.6	0.546, 0.549	0.529, 0.547	
Batch 2	0.867, 1.075	0.753, 0.703	0.616, 0.637	0.540, 0.592	
Batch 3	1.077, 1.013	0.676, 0.756	0.670, 0.695	0.570, 0.624	
Batch 4	1.033, 1.076	0.681, 0.819	0.663, 0.698	0.577, 0.611	
Average & Standard Deviation					
Batch 1	0.896 ± 0.100	0.604 ± 0.006	0.548 ± 0.002	0.538 ± 0.013	
Batch 2	0.971 ± 0.147	0.728 ± 0.035	0.627 ± 0.015	0.566 ± 0.037	
Batch 3	1.045 ± 0.045	0.716 ± 0.057	0.683 ± 0.018	0.597 ± 0.038	
Batch 4	1.055 ± 0.030	0.75 ± 0.098	0.681 ± 0.025	0.594 ± 0.024	
SD1, Replication 2	**Unit: OD**				
Standard OD 500:	**0.31**	**0.307**		**Avg:**	**0.3085**
STD: 100 mg/dl	**OD: 0.3085**				
Raw data:	**Time (Day)**				
Batch No.	**D0**	**D1**	**D2**	**D3**	
Batch 1	0.822, 0.892	0.689, 0.714	0.543, 0.694	0.490, 0.520	
Batch 2	0.637, 0.593	0.570, 0.488	0.480, 0.520	0.459, 0.498	
Batch 3	0.771, 0.800	0.754, 0.800	0.504, 0.660	0.562, 0.630	
Batch 4	0.926, 0.985	0.603, 0.476	0.503, 0.519	0.660, 0.529	
Average & Standard Deviation					
Batch 1	0.857 ± 0.049	0.702 ± 0.018	0.619 ± 0.107	0.505 ± 0.021	
Batch 2	0.615 ± 0.031	0.529 ± 0.058	0.5 ± 0.028	0.479 ± 0.028	
Batch 3	0.786 ± 0.021	0.776 ± 0.033	0.582 ± 0.110	0.596 ± 0.048	

Batch 4	0.9561 ± 0.042	0.540 ± 0.090	0.511 ± 0.011	0.595 ± 0.093	

Appx. A-6i: Glucose concentration, glucose liquid color assay, fermentation 3 L.

Unit mg/l		**AVG**		**AVG**		**AVG**		**AVG**	
PD02		**D0**	**SD**	**D1**	**SD**	**D2**	**SD**	**D3**	**SD**
	Batch 1	32.96	8.29	22.44	4.05	20.53	3.93	17.59	0.18
	Batch 2	34.55	4.35	24.02	0.60	20.24	0.10	17.53	0.21
	Batch 3	30.47	4.81	23.19	0.02	20.84	1.81	18.80	0.32
	Batch 4	30.15	5.71	23.37	1.33	22.32	0.37	18.57	0.03
SD1	**Batch 1**	25.38	3.39	22.06	0.84	16.85	4.53	6.84	0.29
	Batch 2	20.72	1.11	16.76	0.06	16.39	0.25	8.16	0.18
	Batch 3	26.57	1.57	22.37	0.34	17.37	2.11	7.73	0.49
	Batch 4	29.03	2.75	18.05	1.51	15.66	1.27	7.72	0.15

Appx. A-7: Raw data, pH of fermentation 3 L.

PH: PD02, Replication 1				
Raw data:	**D0**	**D1**	**D2**	**D3**
Batch 1	4.65	3.66	3.73	3.57
Batch 2	5.34	3.73	3.57	3.47
Batch 3	4.9	3.73	3.55	3.48
Batch 4	5.11	3.66	3.5	3.49
PH: SD1, Replication 1				
Raw data:	**D0**	**D1**	**D2**	**D3**
Batch 1	4.48	3.53	3.38	3.26
Batch 2	4.86	3.3	3.16	3.1
Batch 3	4.73	3.2	3.12	3.06
Batch 4	4.56	3.13	3.05	3.04
PH: PD02, Replication 2				
Raw data:	**D0**	**D1**	**D2**	**D3**
Batch 1	4.47	3.35	3.32	3.23
Batch 2	4.81	3.31	3.26	3.24

Batch 3	4.79	3.29	3.22	3.13
Batch 4	4.66	3.24	3.19	3.12
PH:SD1, Replication 2				
Raw data:	**D0**	**D1**	**D2**	**D3**
Batch 1	4.46	3.46	3.33	3.25
Batch 2	4.46	3.33	3.17	3.14
Batch 3	4.64	4.36	3.34	3.23
Batch 4	4.38	3.21	3.18	3.13

Appx. A-7i: Combined pH of fermentation 3 L.

		AVG		**AVG**		**AVG**		**AVG**	
pH		**D0**	**SD**	**D1**	**SD**	**D2**	**SD**	**D3**	**SD**
PD02	**Batch 1**	4.56	0.13	3.51	0.22	3.53	0.29	3.40	0.24
	Batch 2	5.08	0.37	3.52	0.30	3.42	0.22	3.36	0.16
	Batch 3	4.32	0.83	3.51	0.31	3.39	0.23	3.31	0.25
	Batch 4	4.89	0.32	3.45	0.30	3.35	0.22	3.31	0.26
SD1	**Batch 1**	4.47	0.01	3.50	0.05	3.36	0.04	3.26	0.01
	Batch 2	4.66	0.28	3.32	0.02	3.17	0.01	3.12	0.03
	Batch 3	4.69	0.06	3.78	0.82	3.23	0.16	3.15	0.12
	Batch 4	4.47	0.13	3.17	0.06	3.12	0.09	3.09	0.06

Appx. A-8: Calculated raw data, for percent yield of *PD02* and *SD2*, of batch 1 to 4.

	PD02		**SD1**	
Percent yield	**AVG**	**SD**	**AVG**	**SD**
Batch 1	27.09	12.72	17.31	1.04
Batch 2	17.75	2.98	45.06	8.53
Batch 3	34.52	28.64	30.27	14.70
Batch 4	27.04	17.78	24.86	4.90

Appx. A-9: Calculated raw data, for productivity of *PD02* and *SD2*, of batch 1 to 4.

Unit mol/l/hr	**PD02**		**SD1**	
Lactic Acid Productivity	**AVG**	**SD**	**AVG**	**SD**
Batch 1	2.63	1.62	1.37	0.05
Batch 2	2.29	1.09	1.71	0.07
Batch 3	2.55	1.44	1.94	0.12

Batch 4	2.01	0.58	2.39	0.09

Appx. A-10: Raw data, absorbance of Microbial inhibition concentration assay, 24 hrs incubation, 595 nm.

						SD1i					
		Unit: OD	Control	Crude	**AVG**	10^{-2}	**AVG**	10^{-4}	**AVG**	10^{-6}	**AVG**
Pathogen:	1.)	**Batch 1**	0.397, 0.467	0.119	0.147 ± 0.039	0.122	0.113	0.08	0.089	0.086	0.471
E. coli ETEC - 01		Rep ii		0.174		0.103	± 0.013	0.097	± 0.012	0.082	± 0.003
		Batch 2		0.171	0.175	0.116	0.124	0.085	0.094	0.099	0.093
		Rep ii		0.179	± 0.006	0.132	± 0.011	0.103	± 0.013	0.087	± 0.008
		Batch 3		0.188	0.179	0.1	0.122	0.092	0.097	0.09	0.089
		Rep ii		0.169	± 0.013	0.143	± 0.030	0.101	± 0.006	0.088	± 0.001
		Batch 4		0.19	0.189	0.104	0.115	0.085	0.090	0.081	0.08
		Rep ii		0.188	± 0.001	0.126	± 0.016	0.094	± 0.006	0.079	± 0.001
			Control	Crude	**AVG**	10^{-2}	**AVG**	10^{-4}	**AVG**	10^{-6}	**AVG**
Pathogen:	2.)	**Batch 1**	1.054, 0.884	0.125	0.129	0.187	0.155	0.124	0.117	0.105	0.092
E, coli ETEC - 02		Rep ii		0.133	± 0.006	0.123	± 0.045	0.11	± 0.010	0.078	± 0.019
		Batch 2		0.191	0.197	0.141	0.154	0.128	0.127	0.129	0.110
		Rep ii		0.203	± 0.008	0.167	± 0.018	0.125	± 0.002	0.09	± 0.028
		Batch 3		0.272		0.175		0.121	0.134	0.115	
		Rep ii		0.219	0.246 ± 0.037	0.206	0.191 ± 0.022	0.146	± 0.018	0.102	0.109 ± 0.009
		Batch 4		0.25	0.229	0.146	0.163	0.119	0.128	0.108	0.103
		Rep ii		0.207	± 0.030	0.18	± 0.024	0.137	± 0.013	0.097	± 0.008
			Control	Crude	**AVG**	10^{-2}	**AVG**	10^{-4}	**AVG**	10^{-6}	**AVG**
Pathogen:	5.)	**Batch 1**	0.58, 0.482	0.136		0.457		0.411		0.356	
Salmonella Enteritidis - 005		Rep ii		0.142	0.139 ± 0.004	0.591	0.524 ± 0.095	0.502	0.457 ± 0.064	0.7	0.528 ± 0.243
		Batch 2		0.172	0.173	0.584	0.537	0.375	0.440	0.312	0.552
		Rep ii		0.173	± 0.001	0.49	± 0.066	0.505	± 0.092	0.792	± 0.339
		Batch 3		0.12		0.442		0.397		0.365	

		Rep ii		0.16	0.140 ± 0.028	0.563	0.503 ± 0.086	0.464	0.431 ± 0.047	0.503	0.434 ± 0.098
		Batch 4		0.118	0.122	0.463	0.492	0.417	0.398	0.333	0.471
		Rep ii		0.126	± 0.006	0.521	± 0.041	0.379	± 0.027	0.609	± 0.195

			Control	Crude	**AVG**	10^{-2}	**AVG**	10^{-4}	**AVG**	10^{-6}	**AVG**
Pathogen:	6.)	**Batch 1**	0.997, 1.077	0.156		0.52		0.499		0.608	
Salmonella Enteritidis - 010		Rep ii		0.126	0.141 ± 0.021	0.706	0.613 ± 0.132	0.86	0.680 ± 0.255	0.732	0.670 ± 0.088
		Batch 2		0.209	0.194	0.608	0.628	0.568	0.600	0.736	0.712
		Rep ii		0.179	± 0.021	0.648	± 0.028	0.631	± 0.045	0.687	± 0.035
		Batch 3		0.176	0.164	0.535	0.658	0.428	0.490	0.554	0.543
		Rep ii		0.151	± 0.018	0.781	± 0.174	0.551	± 0.087	0.532	± 0.016
		Batch 4		0.142	0.148	0.706	0.661	0.65	0.637	0.657	0.627
		Rep ii		0.153	± 0.008	0.615	± 0.064	0.624	± 0.018	0.596	± 0.043
			Control	Crude	**AVG**	10^{-2}	**AVG**	10^{-4}	**AVG**	10^{-6}	**AVG**
Pathogen:	17.)	**Batch 1**	0.659, 0.668	0.159		0.907		0.838		0.843	
Salmonella ATCC 13311		Rep ii		0.154	0.157 ± 0.004	0.92	0.914 ± 0.009	0.821	0.830 ± 0.012	0.865	0.854 ± 0.016
		Batch 2		0.206	0.207	0.899	0.932	0.899	0.858	0.899	0.881
		Rep ii		0.207	± 0.001	0.965	± 0.047	0.817	± 0.058	0.863	± 0.025
		Batch 3		0.202	0.203	1.007	0.988	1.138	1.157	0.928	0.924
		Rep ii		0.204	± 0.001	0.968	± 0.028	1.176	± 0.027	0.92	± 0.006
		Batch 4		0.223	0.216	1.061	1.063	1.005	0.966	0.889	0.923
		Rep ii		0.209	± 0.010	1.064	± 0.002	0.926	± 0.056	0.956	± 0.047
			Control	Crude	**AVG**	10^{-2}	**AVG**	10^{-4}	**AVG**	10^{-6}	**AVG**
Pathogen:	18.)	**Batch 1**	1.128, 1.154	0.972		1.18		1.094		1.131	

B. cereus ATCC 11778		Rep ii		0.551	0.762 ± 0.298	1.3	1.240 ± 0.085	1.151	1.123 ± 0.040	1.365	1.248 ± 0.165
		Batch 2		0.192	0.157	1.279	1.239	1.187	1.128	1.319	1.334
		Rep ii		0.122	± 0.049	1.199	± 0.057	1.069	± 0.083	1.349	± 0.021
		Batch 3		0.152	0.138	1.203	1.195	1.161	1.132	1.111	1.101
		Rep ii		0.123	± 0.021	1.187	± 0.011	1.102	± 0.042	1.09	± 0.015
		Batch 4		0.146	0.130	0.996	1.088	1.145	1.139	1.098	1.076
		Rep ii		0.114	± 0.023	1.179	± 0.129	1.133	± 0.008	1.054	± 0.031
			Control	Crude	**AVG**	10^{-2}	**AVG**	10^{-4}	**AVG**	10^{-6}	**AVG**
Pathogen:	19.)	**Batch 1**	0.728, 0.710	0.194	0.243	0.934	0.906	0.862	0.837	0.883	0.851
E. coli ATCC 8739		Rep ii		0.291	± 0.069	0.878	± 0.040	0.811	± 0.036	0.818	± 0.046
		Batch 2		0.27	0.269	0.934	0.894	0.848	0.836	0.838	0.823
		Rep ii		0.268	± 0.001	0.853	± 0.057	0.823	± 0.018	0.807	± 0.022
		Batch 3		0.327	0.322	0.867	0.860	0.863	0.849	0.845	0.838
		Rep ii		0.317	± 0.007	0.852	± 0.011	0.834	± 0.021	0.831	± 0.010
		Batch 4		0.354	0.351	0.942	0.914	0.874	0.866	0.855	0.858
		Rep ii		0.348	± 0.004	0.886	± 0.040	0.858	± 0.011	0.86	± 0.004

					SD1ii						
			Control	Crude	**AVG**	10^{-2}	**AVG**	10^{-4}	**AVG**	10^{-6}	**AVG**
Pathogen:	1.)	**Batch 1**	- ‖ -	0.117		0.123	0.136	0.092		0.081	
E. coli ETEC - 01		Rep ii		0.108	0.113 ± 0.006	0.149	± 0.018	0.103	0.098 ± 0.008	0.089	0.085 ± 0.006
		Batch 2		0.14		0.09		0.107		0.089	
		Rep ii		0.131	0.136 ± 0.006	0.084	0.087 ± 0.004	0.105	0.106 ± 0.001	0.088	0.089 ± 0.001
		Batch 3		0.122		0.074		0.072		0.081	
		Rep ii		0.124	0.123 ± 0.001	0.076	0.075 ± 0.001	0.082	0.077 ± 0.007	0.085	0.083 ± 0.003
		Batch 4		0.179		0.085		0.094		0.086	
		Rep ii		0.197	0.188 ± 0.013	0.098	0.092 ± 0.009	0.1	0.097 ± 0.004	0.093	0.090 ± 0.005

			Control	Crude	AVG	10^{-2}	AVG	10^{-4}	AVG	10^{-6}	AVG
Pathogen:	2.)	**Batch 1**	- || -	0.112	0.121 ± 0.013	0.158	0.172 ± 0.020	0.115	0.134 ± 0.026	0.123	0.127 ± 0.006
E. coli ETEC - 02		Rep ii		0.13		0.186		0.152		0.131	
		Batch 2		0.125	0.145 ± 0.028	0.117	0.116 ± 0.002	0.117	0.121 ± 0.006	0.115	0.115 ± 0
		Rep ii		0.164		0.114		0.125		0.115	
		Batch 3		0.109	0.124 ± 0.021	0.109	0.115 ± 0.008	0.118	0.115 ± 0.004	0.126	0.128 ± 0.002
		Rep ii		0.139		0.121		0.112		0.129	
		Batch 4		0.189	0.205 ± 0.023	0.133	0.135 ± 0.003	0.128	0.128 ± 0.001	0.133	0.130 ± 0.005
		Rep ii		0.221		0.137		0.127		0.126	
			Control	Crude	AVG	10^{-2}	AVG	10^{-4}	AVG	10^{-6}	AVG
Pathogen:	5.)	**Batch 1**	- || -	0.138		0.467		0.42		0.433	
Salmonella Enteritidis - 005		Rep ii		0.152	0.145 ± 0.010	0.658	0.563 ± 0.135	0.586	0.503 ± 0.117	0.747	0.590 ± 0.222
		Batch 2		0.152	0.160 ± 0.011	0.318	0.321 ± 0.004	0.345	0.362 ± 0.023	0.357	0.489 ± 0.187
		Rep ii		0.168		0.324		0.378		0.621	
		Batch 3		0.128	0.141 ± 0.018	0.305	0.342 ± 0.052	0.312	0.438 ± 0.178	0.277	0.404 ± 0.180
		Rep ii		0.153		0.378		0.564		0.531	
		Batch 4		0.151	0.163 ± 0.017	0.33	0.483 ± 0.216	0.342	0.433 ± 0.128	0.343	0.447 ± 0.147
		Rep ii		0.175		0.635		0.523		0.551	
			Control	Crude	AVG	10^{-2}	AVG	10^{-4}	AVG	10^{-6}	AVG
Pathogen:	6.)	**Batch 1**	- || -	0.122		0.997		0.885		0.841	
Salmonella Enteritidis - 010		Rep ii		0.174	0.148 ± 0.037	0.824	0.911 ± 0.122	0.85	0.868 ± 0.025	0.808	0.825 ± 0.023
		Batch 2		0.164	0.161 ± 0.005	0.384	0.470 ± 0.122	0.535	0.568 ± 0.047	0.802	0.937 ± 0.191
		Rep ii		0.157		0.556		0.601		1.072	
		Batch 3		0.133	0.133 ± 0.001	0.469	0.508 ± 0.054	0.489	0.541 ± 0.073	0.575	0.702 ± 0.180
		Rep ii		0.132		0.546		0.592		0.829	
		Batch 4		0.199	0.183 ± 0.023	0.744	0.649 ± 0.134	0.527	0.562 ± 0.049	0.698	0.708 ± 0.014
		Rep ii		0.166		0.554		0.597		0.718	
			Control	Crude	AVG	10^{-2}	AVG	10^{-4}	AVG	10^{-6}	AVG
Pathogen:	17.)	**Batch 1**	- || -	0.166		1.097	1.078	0.982	0.867	0.95	0.963

Salmonella ATCC 13311		Rep ii		0.173	0.170 ± 0.005	1.058	± 0.028	0.751	± 0.163	0.976	± 0.018
		Batch 2		0.199		0.89	0.865	0.778	0.846	0.858	0.837
		Rep ii		0.194	1.97 ± 0.004	0.839	± 0.036	0.913	± 0.095	0.815	± 0.030
		Batch 3		0.183	0.188	0.778	0.821	0.783	0.796	0.739	0.773
		Rep ii		0.193	± 0.007	0.863	± 0.060	0.808	± 0.018	0.806	± 0.047
		Batch 4		0.233	0.219	0.856	0.896	0.880	0.896	0.875	0.897
		Rep ii		0.205	± 0.020	0.935	± 0.056	0.912	± 0.023	0.918	± 0.030
			Control	Crude	**AVG**	10^{-2}	**AVG**	10^{-4}	**AVG**	10^{-6}	**AVG**
Pathogen:	18.)	**Batch 1**	- || -	0.123		1.182		1.245		1.385	
B. cereus ATCC 11778		Rep ii		0.137	0.130 ± 0.010	1.218	1.20 ± 0.025	1.163	1.204 ± 0.058	1.004	1.195 ± 0.269
		Batch 2		0.21	0.194	1.446		1.064		1.064	
		Rep ii		0.177	± 0.023	1.269	1.358 ± 0.125	1.044	1.054 ± 0.014	1.096	1.080 ± 0.023
		Batch 3		0.183	0.213	1.109	1.207	1.085	1.106	1.178	1.234
		Rep ii		0.242	± 0.042	1.304	± 0.138	1.127	± 0.030	1.289	± 0.078
		Batch 4		0.205	0.271	1.071	1.053	1.059	1.095	1.194	1.141
		Rep ii		0.337	± 0.093	1.034	± 0.026	1.131	± 0.051	1.088	± 0.075
			Control	Crude	**AVG**	10^{-2}	**AVG**	10^{-4}	**AVG**	10^{-6}	**AVG**
Pathogen:	19.)	**Batch 1**	- || -	0.231	0.257	0.965	0.932	0.847	0.852	0.881	0.871
E. coli ATCC 8739		Rep ii		0.282	± 0.036	0.899	± 0.047	0.857	± 0.007	0.861	± 0.014
		Batch 2		0.276	0.290	0.861	0.842	0.864	0.855	0.87	0.851
		Rep ii		0.303	± 0.019	0.822	± 0.028	0.845	± 0.013	0.832	± 0.027
		Batch 3		0.267	0.274	0.77	0.770	0.781	0.772	0.779	0.774
		Rep ii		0.28	± 0.009	0.769	± 0.001	0.762	± 0.013	0.768	± 0.008
		Batch 4		0.298	0.306	0.794	0.795	0.768	0.782	0.798	0.797
		Rep ii		0.313	± 0.011	0.796	± 0.001	0.795	± 0.019	0.796	± 0.001

Pd02i											
			Control	Crude	**AVG**	10^{-2}	**AVG**	10^{-4}	**AVG**	10^{-6}	**AVG**
Pathogen:	1.)	**Batch 1**	- || -	0.102		0.073		0.074		0.079	

E. coli ETEC - 01		Rep ii		0.102	0.102 ± 0	0.084	0.079 ± 0.008	0.081	0.078 ± 0.005	0.085	0.082 ± 0.004
		Batch 2		0.126	0.128 ± 0.003	0.085	0.092 ± 0.009	0.081	0.088 ± 0.009	0.09	0.086 ± 0.006
		Rep ii		0.13		0.098		0.094		0.081	
		Batch 3		0.115	0.128 ± 0.018	0.137	0.131 ± 0.009	0.099	0.102 ± 0.004	0.101	0.103 ± 0.002
		Rep ii		0.141		0.124		0.105		0.104	
		Batch 4		0.063	0.086 ± 0.033	0.108	0.108 ± 0	0.102	0.101 ± 0.001	0.099	0.099 ± 0
		Rep ii		0.109		0.108		0.1		0.099	
			Control	Crude	**AVG**	10^{-2}	**AVG**	10^{-4}	**AVG**	10^{-6}	**AVG**
Pathogen: *E. coli ETEC - 02*	2.)	**Batch 1**	- || -	0.119	0.095 ± 0.034	0.116	0.120 ± 0.006	0.114	0.115 ± 0.001	0.113	0.125 ± 0.017
		Rep ii		0.071		0.124		0.115		0.137	
		Batch 2		0.152	0.127 ± 0.036	0.122	0.148 ± 0.036	0.132	0.138 ± 0.008	0.128	0.145 ± 0.023
		Rep ii		0.101		0.173		0.144		0.161	
		Batch 3		0.127	0.127 ± 0	0.122	0.135 ± 0.018	0.123	0.125 ± 0.002	0.118	0.123 ± 0.006
		Rep ii		0.127		0.148		0.126		0.127	
		Batch 4		0.071	0.072 ± 0.001	0.123	0.142 ± 0.027	0.125	0.139 ± 0.019	0.13	0.136 ± 0.008
		Rep ii		0.072		0.161		0.152		0.141	
			Control	Crude	**AVG**	10^{-2}	**AVG**	10^{-4}	**AVG**	10^{-6}	**AVG**
Pathogen: *Salmonella Enteritidis - 005*	5.)	**Batch 1**	- || -	0.105	0.110 ± 0.006	0.286	0.486 ± 0.283	0.304	0.418 ± 0.161	0.467	0.596 ± 0.182
		Rep ii		0.114		0.686		0.531		0.724	
		Batch 2		0.123	0.123 ± 0.001	0.431	0.507 ± 0.107	0.462	0.545 ± 0.117	0.526	0.588 ± 0.088
		Rep ii		0.122		0.582		0.627		0.65	
		Batch 3		0.127	0.126 ± 0.002	0.645	0.718 ± 0.103	0.529	0.664 ± 0.191	0.484	0.636 ± 0.214
		Rep ii		0.124		0.79		0.799		0.787	
		Batch 4		0.118	0.105 ± 0.018	0.686	0.776 ± 0.127	0.772	0.804 ± 0.045	0.827	0.822 ± 0.008
		Rep ii		0.092		0.866		0.835		0.816	
			Control	Crude	**AVG**	10^{-2}	**AVG**	10^{-4}	**AVG**	10^{-6}	**AVG**
Pathogen: *Salmonella Enteritidis - 010*	6.)	**Batch 1**	- || -	0.078	0.090 ± 0.017	0.372	0.462 ± 0.127	0.575	0.589 ± 0.019	0.771	0.848 ± 0.108
		Rep ii		0.102		0.552		0.602		0.924	
		Batch 2		0.169		0.69		0.716		0.716	

		Rep ii		0.166	0.168 ± 0.002	0.727	0.709 ± 0.026	0.727	0.722 ± 0.008	0.77	0.743 ± 0.038
		Batch 3		0.137	0.127	0.801	0.717	0.792		0.7	0.721
		Rep ii		0.117	± 0.014	0.632	± 0.120	1.048	0.920 ± 0.181	0.741	± 0.029
		Batch 4		0.147	0.132	0.401	0.509	0.802		0.795	0.913
		Rep ii		0.117	± 0.021	0.617	± 0.153	0.997	0.900 ± 0.138	1.03	± 0.166
			Control	Crude	**AVG**	10^{-2}	**AVG**	10^{-4}	**AVG**	10^{-6}	**AVG**
Pathogen:	17.)	**Batch 1**	- || -	0.163		0.899		0.899		0.847	
Salmonella ATCC 13311		Rep ii		0.157	0.160 ± 0.004	0.953	0.926 ± 0.038	0.921	0.910 ± 0.016	0.941	0.894 ± 0.066
		Batch 2		0.184	0.188	0.959	1.031	1.172		1.099	1.080
		Rep ii		0.191	± 0.005	1.102	± 0.101	1.074	1.123 ± 0.069	1.061	± 0.027
		Batch 3		0.177	0.172	1.212	1.048	0.864		0.926	0.937
		Rep ii		0.167	± 0.007	0.883	± 0.233	0.972	0.918 ± 0.076	0.947	± 0.015
		Batch 4		0.176	0.164	1.25	1.084	0.892		1.015	0.958
		Rep ii		0.152	± 0.017	0.918	± 0.235	0.827	0.860 ± 0.046	0.901	± 0.081
			Control	Crude	**AVG**	10^{-2}	**AVG**	10^{-4}	**AVG**	10^{-6}	**AVG**
Pathogen:	18.)	**Batch 1**	- || -	0.195		1.105		1.073		1.314	
B. cereus ATCC 11778		Rep ii		0.14	0.168 ± 0.039	1.074	1.090 ± 0.022	1.139	1.106 ± 0.047	1.163	1.239 ± 0.107
		Batch 2		0.117	0.166	1.142	1.211	1.147		1.48	1.302
		Rep ii		0.215	± 0.069	1.28	± 0.098	1.175	1.161 ± 0.020	1.124	± 0.252
		Batch 3		0.451	0.337	1.258	1.210	1.221		1.23	1.220
		Rep ii		0.223	± 0.161	1.162	± 0.068	1.034	1.128 ± 0.132	1.209	± 0.015
		Batch 4		0.165	0.184	1.137	1.192	1.077		1.285	1.300
		Rep ii		0.202	± 0.026	1.247	± 0.078	1.011	1.044 ± 0.047	1.314	± 0.021
			Control	Crude	**AVG**	10^{-2}	**AVG**	10^{-4}	**AVG**	10^{-6}	**AVG**
Pathogen:	19.)	**Batch 1**	- || -	0.22		0.807		0.801		0.817	
E. coli ATCC 8739		Rep ii		0.217	0.219 ± 0.002	0.816	0.812 ± 0.006	0.802	0.802 ± 0.001	0.814	0.816 ± 0.002
		Batch 2		0.255	0.259	1.092	1.186	1.174		1.157	1.208
		Rep ii		0.263	± 0.006	1.279	± 0.132	1.184	1.179 ± 0.007	1.258	± 0.071

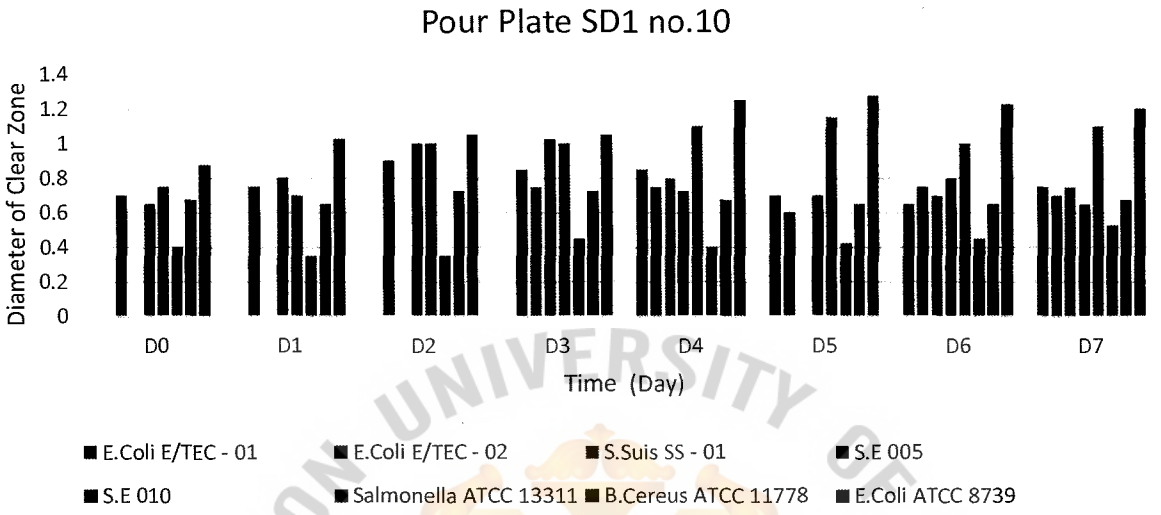
		Batch 3		0.267	0.282 ± 0.021	0.917	1.040 ± 0.173	1.022	1.053 ± 0.044	0.983	1.075 ± 0.129
		Rep ii		0.296		1.162		1.084		1.166	
		Batch 4		0.235	0.224 ± 0.016	1.068	1.126 ± 0.082	0.896	1.036 ± 0.198	0.903	1.005 ± 0.144
		Rep ii		0.213		1.184		1.176		1.107	

							Pd02ii				
			Control	Crude	**AVG**	10^{-2}	**AVG**	10^{-4}	**AVG**	10^{-6}	**AVG**
Pathogen:	1.)	**Batch 1**	- ‖ -	0.123	0.145 ± 0.030	0.109	0.110 ± 0.001	0.111	0.112 ± 0.001	0.106	0.106 ± 0.001
E. coli ETEC - 01		Rep ii		0.166		0.111		0.113		0.105	
		Batch 2		0.138	0.155 ± 0.023	0.091	0.096 ± 0.007	0.091	0.094 ± 0.004	0.091	0.096 ± 0.007
		Rep ii		0.171		0.101		0.096		0.101	
		Batch 3		0.191	0.182 ± 0.013	0.071	0.089 ± 0.025	0.14	0.121 ± 0.028	0.096	0.013 ± 0.006
		Rep ii		0.173		0.106		0.101		0.104	
		Batch 4		0.169	0.161 ± 0.011	0.115	0.100 ± 0.021	0.107	0.110 ± 0.004	0.126	0.021 ± 0.002
		Rep ii		0.153		0.085		0.112		0.123	
			Control	Crude	**AVG**	10^{-2}	**AVG**	10^{-4}	**AVG**	10^{-6}	**AVG**
Pathogen:	2.)	**Batch 1**	- ‖ -	0.124	0.129 ± 0.006	0.135	0.147 ± 0.017	0.183	0.181 ± 0.004	0.157	0.161 ± 0.005
E. coli ETEC - 02		Rep ii		0.133		0.159		0.178		0.164	
		Batch 2		0.158	0.174 ± 0.022	0.237	0.180 ± 0.081	0.121	0.125 ± 0.006	0.121	0.123 ± 0.002
		Rep ii		0.189		0.123		0.129		0.124	
		Batch 3		0.182	0.196 ± 0.019	0.126	0.138 ± 0.016	0.125	0.140 ± 0.021	0.121	0.140 ± 0.026
		Rep ii		0.209		0.149		0.155		0.158	
		Batch 4		0.129	0.143 ± 0.019	0.133	0.138 ± 0.018	0.16	0.140 ± 0.032	0.109	0.140 ± 0.025
		Rep ii		0.156		0.107		0.115		0.144	
			Control	Crude	**AVG**	10^{-2}	**AVG**	10^{-4}	**AVG**	10^{-6}	**AVG**
Pathogen:	5.)	**Batch 1**	- ‖ -	0.145		0.865		0.851		0.722	
Salmonella Enteritidis - 005		Rep ii		0.15	0.148 ± 0.004	0.873	0.869 ± 0.006	0.836	0.844 ± 0.011	0.839	0.781 ± 0.083
		Batch 2		0.159	0.160 ± 0.001	0.818	0.848 ± 0.042	0.788	0.806 ± 0.025	0.677	0.740 ± 0.088
		Rep ii		0.161		0.877		0.824		0.802	
		Batch 3		0.17		0.778		0.772		0.581	

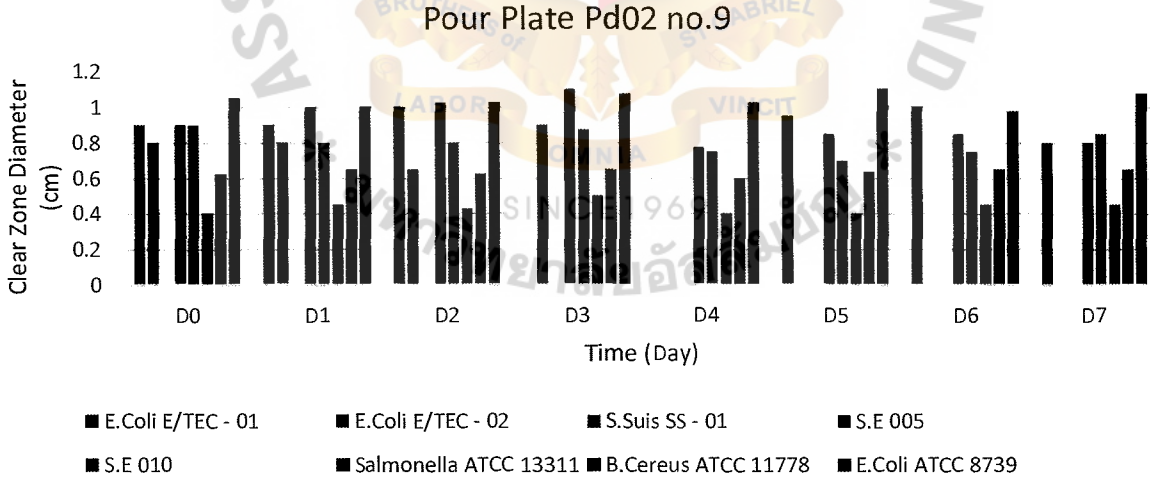
		Rep ii		0.15	0.160 ± 0.014	0.838	0.808 ± 0.042	0.36	0.566 ± 0.291	0.762	0.672 ± 0.128
		Batch 4		0.154	0.159	0.76	0.729	0.749		0.766	0.707
		Rep ii		0.163	± 0.006	0.697	± 0.045	0.75	0.750 ± 0.001	0.647	± 0.084
			Control	Crude	**AVG**	10^{-2}	**AVG**	10^{-4}	**AVG**	10^{-6}	**AVG**
Pathogen:	6.)	**Batch 1**	- || -	0.206		0.476		0.808		0.769	
Salmonella Enteritidis - 010		Rep ii		0.15	0.178 ± 0.040	0.672	0.574 ± 0.139	1.089	0.949 ± 0.199	0.989	0.879 ± 0.156
		Batch 2		0.284	0.252	0.383	0.522	0.817		1.068	1.065
		Rep ii		0.219	± 0.046	0.661	± 0.197	0.996	0.907 ± 0.127	1.062	± 0.004
		Batch 3		0.259	0.263	0.419	0.590	1.021		1.083	1.089
		Rep ii		0.266	± 0.005	0.76	± 0.241	0.823	0.922 ± 0.140	1.095	± 0.008
		Batch 4		0.232	0.606	1.042	1.088	1.177		0.745	0.928
		Rep ii		0.979	± 0.528	1.133	± 0.064	1.129	1.153 ± 0.034	1.11	± 0.258
			Control	Crude	**AVG**	10^{-2}	**AVG**	10^{-4}	**AVG**	10^{-6}	**AVG**
Pathogen:	17.)	**Batch 1**	- || -	0.228		1.192		1.195		1.085	
Salmonella ATCC 13311		Rep ii		0.187	0.208 ± 0.029	1.028	1.110 ± 0.116	1.067	1.131 ± 0.091	0.911	0.998 ± 0.123
		Batch 2		0.283	0.246	1.114	1.039	1.001		1.087	1.028
		Rep ii		0.209	± 0.052	0.964	± 0.106	0.892	0.947 ± 0.077	0.969	± 0.083
		Batch 3		0.246	0.242	1.025	1.023	0.981		1.071	1.022
		Rep ii		0.237	± 0.006	1.021	± 0.003	0.938	0.960 ± 0.030	0.973	± 0.069
		Batch 4		0.219	0.222	0.847	0.848	0.875		0.945	0.932
		Rep ii		0.225	± 0.004	0.849	± 0.001	0.95	0.913 ± 0.053	0.919	± 0.018
			Control	Crude	**AVG**	10^{-2}	**AVG**	10^{-4}	**AVG**	10^{-6}	**AVG**
Pathogen:	18.)	**Batch 1**	- || -	0.281		1.329		1.153		1.316	
B. cereus ATCC 11778		Rep ii		0.397	0.339 ± 0.082	1.294	1.312 ± 0.025	1.047	1.1 ± 0.075	1.239	1.278 ± 0.054
		Batch 2		0.254	0.271	1.332	1.324	1.375		1.309	1.356
		Rep ii		0.288	± 0.024	1.315	± 0.012	1.272	1.356 ± 0.073	1.402	± 0.066
		Batch 3		0.333	0.329	1.069	1.164	1.186		1.001	
		Rep ii		0.325	± 0.006	1.264	± 0.138	1.141	1.164 ± 0.032	1.0418	

											1.021 ± 0.029
		Batch 4		0.27	0.258 ± 0.017	1.052	1.243 ± 0.052	1.25	1.243 ± 0.011	1.252	1.150 ± 0.145
		Rep ii		0.246		1.126		1.235		1.047	
			Control	Crude	**AVG**	10^{-2}	**AVG**	10^{-4}	**AVG**	10^{-6}	**AVG**
Pathogen:	19.)	**Batch 1**	- ‖ -	0.235	0.268 ± 0.047	1.066	1.064 ± 0.003	0.9	0.980 ± 0.113	1.061	1.050 ± 0.016
E. coli ATCC 8739		Rep ii		0.301		1.062		1.06		1.039	
		Batch 2		0.282	0.296 ± 0.019	0.992	0.958 ± 0.048	0.796	0.869 ± 0.103	0.941	0.982 ± 0.058
		Rep ii		0.309		0.924		0.942		1.023	
		Batch 3		0.427	0.393 ± 0.048	0.957	0.984 ± 0.038	0.938	1.090 ± 0.214	1.304	1.274 ± 0.043
		Rep ii		0.359		1.011		1.241		1.243	
		Batch 4		0.298	0.300 ± 0.003	1.049	0.999 ± 0.071	1.102	1.082 ± 0.028	0.996	1.031 ± 0.049
		Rep ii		0.302		0.948		1.062		1.066	

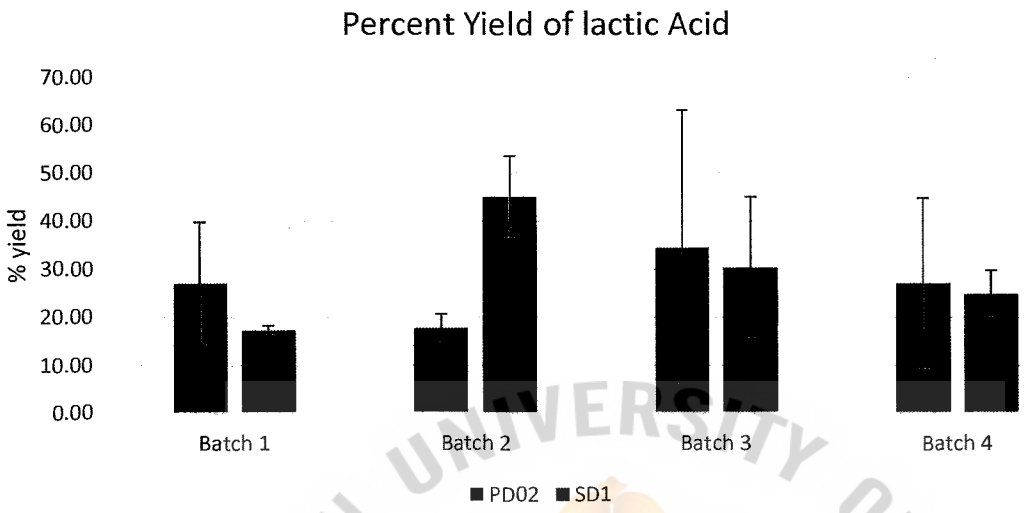
APPENDIX B



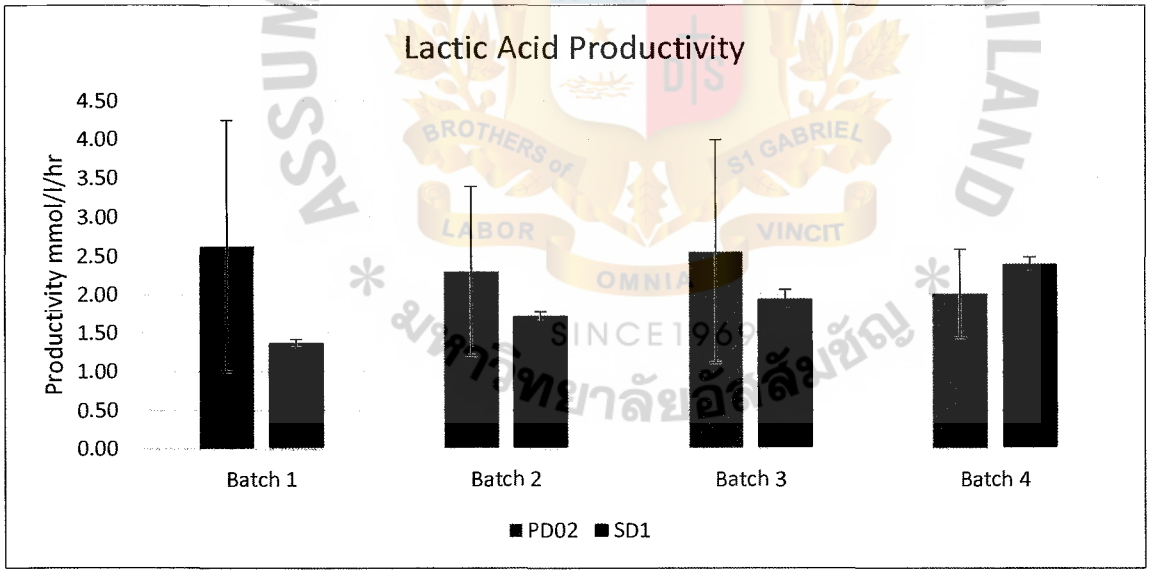
Appx. B-1: Inhibitory clear zone, agar diffusion assay of, *SD1* against *Salmonella typhimurium* ATCC 13311, *Bacillus Cereus* ATCC 11778, *Escherichia coli cereus* ATCC 8739, *Escherichia coli cereus* ETEC 01, *Escherichia coli cereus* ETEC 02, *Salmonella enteritidis* 005, *Salmonella enteritidis* 010 and *Staphylococcus suis* SS – 01 from fermentation of Day 0 to Day 7.



Appx. B-2: Inhibitory clear zone, agar diffusion assay of, *PD02* against *Salmonella typhimurium* ATCC 13311, *Bacillus Cereus* ATCC 11778, *Escherichia coli cereus* ATCC 8739, *Escherichia coli cereus* ETEC 01, *Escherichia coli cereus* ETEC 02, *Salmonella enteritidis* 005, *Salmonella enteritidis* 010 and *Staphylococcus suis* SS – 01 from fermentation of Day 0 to Day 7.



Appx. B-3: Represent the percent yield of *PD02* and *SD1*, of batch 1 to batch 4 from, 3liter fermentation.



Appx. B-4: Represent the lactic acid productivity of *PD02* and *SD1*, of batch 1 to batch 4 from, 3liter fermentation.

APPENDIX C

1.) Formula used to calculate the glucose concentration, from glucose liquid color assay.

$$\textbf{Glucose Concentration} = 100 * \frac{\textbf{Absorbance sample}}{\textbf{Absorbance Standard}} = [\frac{mg}{dl}]$$

2.) Formula used to calculate concentration of lactic acid from acidimetric titration.

N1V1 = N2V2

N1 = Normality of NaOH
V1 = Volume of NaOH
N2 = Normality of Lactic acid bacteria (x)
V2 = Volume of analyte used

3.) Glucose conversion from mg/dl to mmol/l, to calculate from productivity and yield.

Glucose conversion:

$$\frac{mg}{dl} * 0.0555 = mmol/l$$

4.) Formula used to calculate lactic acid percent yield.

Lactic acid % Yield:

$$\frac{\textbf{Final lactic acid concentration}}{\textbf{Glucose Consumption}} * 100\%$$

5.) Formula used to calculate lactic acid productivity.

Productivity of Lactic Acid:

$$\frac{\textbf{Lactic acid concentration } (\frac{mmol}{l})}{\textbf{Fermentation Time (hrs)}} = \frac{mmol}{l}/hr$$