# Screening of Lactic Acid Bacteria Isolated from Piglet Faeces for Antimicrobial Activity
# การคัดเลือกแบคทีเรียแลคติกจากมูลลูกสุกร ที่มีฤทธิ์ต้านเชื้อจุลินทรีย์

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## Abstract

Lactic acid bacteria (LAB) were isolated from 136 samples of pig faeces. The number of LAB varied from 8.03 to 8.97 log cfu/g faeces. A total of 317 lactic acid bacteria were isolated and tested for antimicrobial activities against 8 pathogenic bacterial strains using agar well diffusion technique. The inhibitory activity of most LAB was due to the production of organic acids except one isolate, WX153, which was found to produce bacteriocin-like substance. Strain WX153 was identified by using API50 CHL kit and 16S rDNA sequencing as *Lactococcus lactis* subsp. lactis. The production of antimicrobial substance by *L. lactis* subsp. *lactis* WX153 in MRS medium followed a growth-associated pattern. This study allowed the preliminary selection of LAB isolates with antimicrobial activity to be used as potential probiotic in pig. In addition, inhibitory compound produced by *L. lactis* subsp. *lactis* WX153 may have potential applications as bio-preservative in food industry.

**Keywords:** antimicrobial activity, *Lactococcus lactis* subsp. *lactis*, piglet faeces

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# บทคัดย่อ

ทำการแยกแบคทีเรียแลคติกจากตัวอย่างมูลสุกร 136 ตัวอย่าง พบแบคทีเรียแลคติกอยู่ในช่วง 8.03- 8.97 log cfu/กรัมของมูลสุกร จากการนำแบคทีเรียแลคติกที่แยกได้จำนวน 317 ไอโซเลท มาทดสอบความสามารถในการยับยั้งเชื้อก่อโรค 8 ชนิด โดยวิธี agar well diffusion พบว่าการยับยั้งเชื้อก่อโรคส่วนใหญ่เกิดจากฤทธิ์ของกรดอินทรีย์ที่แบคทีเรียแลคติกผลิตขึ้น ยกเว้นเชื้อ 1 ไอโซเลท คือ WX153 ที่สามารถสร้างสารยับยั้งคล้ายแบคเทอริโอซิน การพิสูจน์เอกลักษณ์ของเชื้อไอโซเลท WX153 โดยใช้ API 50CHL kit และวิเคราะห์ลำดับนิวคลีโอไทด์ของ 16S rDNA พบว่าเป็นเชื้อ *Lactococcus lactis* subsp. *lactis* โดย *L. lactis* subsp. *lactis* WX153 จะผลิตสารยับยั้งไปพร้อม ๆ กับการเจริญในอาหาร MRS ผลจากงานวิจัยนี้ทำให้สามารถคัดเลือกแบคทีเรียแลคติกที่มีฤทธิ์ในการยับยั้งเชื้อก่อโรค และมีศักยภาพในการนำมาใช้เป็นโพรไบโอติกในสุกร นอกจากนี้สารยับยั้งที่ผลิตโดยเชื้อ *L. lactis* subsp. *lactis* WX153 อาจนำไปประยุกต์ใช้เพื่อเป็น bio-preservative ในอุตสาหกรรมอาหารได้


**คำสำคัญ:** กิจกรรมการยับยั้ง, *Lactococcus lactis* subsp. *lactis*, มูลลูกสุกร

## Introduction

Gastrointestinal tract (GIT) diseases in suckling and weaned pigs are regarded as a major factor influencing the mortality of pigs (Itoi et al., 2008). Pathogens such as *Escherichia coli*, *Salmonella* sp., *Isospora suis*, *Eimeria debliecki*, *E. scabra*, *E. perminuta*, *E. porci* and rotavirus are involved in GIT diseases in pig. Antibiotics such as tetracycline, penicillin G, bacitracin, oxytetracycline and chloramphenicol have been used as therapeutic drug for prevention or treatment of intestinal infections caused by pathogenic bacteria and also as antimicrobial growth promoters. However, development and spreading of antibiotic resistant bacteria to environment and human is evidenced after prolong treatment of antibiotics (Teuber et al., 1999; Wegener, 2003). Moreover, the presence of residual antibiotics in meat and meat products may cause direct and indirect negative effects on human health (Hughes and Heritage, 2009). Regarding safety issues, the EU has banned the use of antibiotics as growth promoter in animal feed since January, 2006. Several feed additives such as amino acids, enzymes, prebiotics, probiotics and organic acids have been used to replace antibiotics.

Lactic acid bacteria (LAB) such as *Lactobacillus reuteri* (Serna and Rodríguez, 2006), *L. amylovorus, L. plantarum, L. johnsonii* (Du Toit et al., 2003) and *L. sobrius* (Konstantinov et al., 2006) are normally found as micro-flora in the GIT of pigs. Some of LAB has been reported to produce antimicrobial agents including organic acids, hydrogen peroxide, diacetyl and bacteriocins (Du Toit et al., 2003). Based on the proteinatious structure, bacteriocins produced by lactic acid bacteria are considered safe antimicrobial agents (Joerger, 2003). Even though a number of studies have reported the antagonistic activities of LAB isolated from pig GIT against many enteric pathogens such as *Salmonella* sp. (Casey et al., 2004), *Escherichia coli* and *Clostridium perfringen* (De Angelis et al., 2006), a few strains have been described to produce bacteriocins (Du Toit et al., 2003).

The aim of this study was to isolate and screen for lactic acid bacteria with antimicrobial activity from faeces of both suckling and weaned pigs collected from different farms in Nakorn-prathom

province, Thailand. Antimicrobial activities of the isolates were tested against several enteric pathogens. In this report, a strain of *Lactococcus lactis* subsp. *lactis* was found to produce bacteriocin-like substance.

## Materials and Methods

### *Isolation of lactic acid bacteria*

Faeces of piglet age between 3-12 weeks were collected from three pig farms located in Nakorn-prathom province. One gram of faeces sample was homogenized in a stomacher lab blender (Interscience, St. Nom, France) for 1 min in $CO_2$-flushed plastic bags with 9 ml of anaerobic solution. The suspension was tenfold serially diluted and appropriate dilutions were plated onto MRS (de Man Rogosa Sharp) agar supplemented with 0.05% L-cysteine-hydrochloride (MRSC) and 0.5 % calcium carbonate ($CaCO_3$). Plates were incubated anaerobically at 37°C for 48 h in anaerobic jars using Anaerocult A (Merck, Germany). After incubation, colonies producing a clear zone and with different morphology were picked up and purified by streaking on the same medium. Lactic acid bacteria then were selected based on Gram-staining and catalase reaction test. Only Gram-positive, non-spore forming and catalase-negative bacterial isolates were selected and stored at -80°C in 10% (w/v) skim milk for further investigation.

### *Bacterial strains and culture conditions*

Eight pathogenic indicator strains used in this study were obtained from Bangkok MIRCEN, Thailand Institute of Scientific and Technological Research (TISTR) and the Department of Medical Science, Ministry of Public Health (DMST), Bangkok, Thailand. The strains included *Campylobacter jejuni* DMST 15190, *Escherichia coli* O157:H7 DMST 12743, *Pseudomonas aeruginosa* DMST 15501, *Salmonella* Typhimurium TISTR 292, *Enterococcus faecalis* TISTR579, *Listeria monocytogenes* DMST 4553, *Staphylococcus aureus* TISTR 118 and *Streptococcus suis* DMST 18783. All strains were cultured in TSB (Tryptic Soy Broth), with the exception of *C. jejuni* and *S. suis* which were cultured in Blood Agar medium, and incubated at 37°C. All cultures were maintained as frozen stocks at -80°C in TSB medium with 30% glycerol.

### *Screening of lactic acid bacteria producing antimicrobial compounds*

An agar well diffusion method described by Barefoot and Klaenhammer (1983) was used to screen for LAB producing antimicrobial compounds against eight indicator strains. LAB were grown in MRS broth at 37 °C for 24 h and harvesting the cells by centrifugation at 4,000 g for 5 min. The supernatants were used to test for antimicrobial activity. In order to exclude the possibility of inhibition caused by organic acids and hydrogen peroxide, the supernatants were adjusted to pH 6.5-7.0 with 5 N NaOH and treated with catalase enzyme (Herreros et al., 2005), respectively. The treated supernatants then were filter-sterilized by a 0.45 μm membrane filter before antimicrobial assay. For indicator strains preparation, log phase cell cultures of each indicator were diluted with sterile 0.85% NaCl to about ~$10^8$ cfu/ml (0.5 McFarland) and swab on the surface of appropriate media for each strain. Wells of 7.0 mm diameter were bored with sterile cork borer and 80 μl of culture supernatants of LAB were dispensed into each well. The diameters of inhibition zones around each well were measured after incubation at 37 °C for 24 h. Lactic acid (2% v/v) was used as a positive control. All the experiments were carried out in duplicate.

***Identification of isolated LAB strain by API 50 CHL kit and partial 16s rRNA gene sequence analysis***

Sugar fermentation of the selected strain was performed using the API 50 CHL kit (Biomerieux, Nuertingen, Germany) according to the manufacturer's instructions. Identification was also confirmed by partial sequencing of the 16S rDNA using 27f primer. A sequence of 16S rDNA was amplified using two primers 27f and 1525r (Lane, 1991). The PCR reaction mixture (50 µl) contained 100-200 ng of genomic DNA, 10 pmole of each primer, 1.5 U Taq DNA polymerase, 0.2 mM of each dNTP, 1.5 mM of $MgCl_2$, 1X PCR buffer and Milli Q water. PCR reaction was performed using the GeneAmp PCR system 2400 (PE Applied Biosystem). The amplification conditions were as follows: initial denaturation at 94 °C for 5 min; 30 cycles of denaturation for 1 min at 94 °C; annealing for 1 min at 54 °C; elongation at 72 °C for 2 min and final extension at 94 °C for 7 min. The PCR products were electrophoresed in 0.8 % agarose gel. The 16S rDNA-amplified products were purified using a Gel/PCR purification kit (Geneaid, USA), according to the manufacturer's instructions. DNA was sequenced using an ABI Big Dye Terminator Cycle Sequencing Ready Reaction Mix kit on the Perkin Elmer Model 9400 thermal cycler. The sequencing reaction products were analyzed in an automated 310 DNA sequencer (Applied Biosystem/Perkin-Elmer).

***Growth and antimicrobial substance production of the LAB isolate***

An overnight culture of selected LAB was inoculated into 250 ml Erlenmeyer flasks containing 100 ml MRS broth and incubated at 37 °C. Samples were taken every 3 h over a period of 24 h to measure optical density (600 nm), pH and antimicrobial activity (AU/ml). Antimicrobial activity was quantified by a modified critical dilution method (De Vuyst et al., 1996) using *S. aureus* TISTR 118 as the indicator organism. Briefly, cell-free supernatants were neutralized (pH 7.0), filter sterilized and twofold serially diluted in fresh MRS broth. The inhibitory activity of each dilution was determined by agar well diffusion method as described above. The activity was expressed in arbitrary unit per milliliter (AU/ml). One arbitrary unit was defined as the reciprocal of the highest dilution showing a zone of inhibition on the indicator lawn.

## Results and discussion

***Isolation and screening of LAB with antimicrobial activity***

A total of 317 LAB were isolated from 136 samples of pig faeces obtained from piglet age between 3-12 weeks. The number of LAB from each farm varied from approximately 8.0 to 9.0 log cfu/g faeces. The populations of LAB were quite similar either among the three pig farms or the piglets of different ages (3-12 weeks). It has been reported the change in diversity and population of lactic acid bacteria in GIT of piglet during suckling and weaning periods (Janczyk et al., 2007). A number of lactobacillus including *L. sobrius, L. salivarius, L.crispatus, L. delbrueckii* subsp. *bulgaricus, L. reuteri* and *L. johnsonii* were found in ileal digesta of piglets. *L. sobrius* and *L. reuteri* were also reported to be predominant species in the gut of piglets before and post weaning (Konstantinov et al., 2006). These reports demonstrated the abundant of LAB in piglet GIT. Hence, pig faeces are considered to be the major source of candidate for antimicrobial compound-producing LAB.

The cell-free supernatants of 317 LAB isolates were examined for their antimicrobial activities against eight indicator strains by agar well diffusion technique and 2% lactic acid (v/v) was included in the experiment as a positive control. The presence

of inhibition zones around the wells is shown in Figure 1. It was found that all eight indicator strains were sensitive to 2% lactic acid, however, with different degrees of sensitivity. The results of the antimicrobial activity assays of 317 LAB isolates showed that all isolates exhibited inhibitory activity against at least one indicator strain and 171 isolates which account for 54% of the total isolates could inhibit at least three indicator strains (data not shown). Only 15 isolates (4.7%) were able to inhibit all eight indicator strains (Table 1). The maximum zone of inhibition of 17-18 mm was observed in strains PS1112, PL1171, PL1177, WX112 and WX143, however, against different indicator strains.

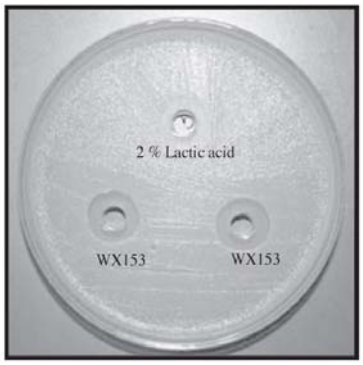


**Figure 1**. Antimicrobial activity assay of cell-free supernatants of lactic acid bacteria isolated from piglet faeces against *S. aureus* TISTR 118 as detected by the agar well diffusion method on TSA agar plate and 2 % lactic acid was used as a control.

**Table 1.** Antimicrobial activity of fifteen lactic acid bacteria isolated from piglet faeces against eight indicator strains detected by the agar well diffusion method.

| LAB isolates | Diameter of inhibition zone (mm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Indicator strains | | | | | | | |
| | *E. faecalis* TISTR579 | *S.* Typhimurium TISTR 292 | *S. aureus* TISTR 118 | *L. monocytogenes* DMST 4553 | *E. coli* O157:H7 DMST 12743 | *P. aeroginosa* DMST 15501 | *C. jejuni* DMST 15190 | *S. suis* DMST 18783 |
| PS1112 | 9 | 2 | 10 | 10 | 9 | 10 | 11 | 17 |
| PL1171 | 11 | 11 | 10 | 10 | 11 | 10 | 18 | 14 |
| PL1177 | 11 | 12 | 11 | 12 | 10 | 9 | 17 | 16 |
| SS213 | 10 | 11 | 9 | 12 | 11 | 12 | 9 | 10 |
| WM126 | 10 | 12 | 11 | 12 | 11 | 12 | 12 | 9 |
| WM127 | 9 | 12 | 11 | 13 | 11 | 11 | 12 | 9 |
| WM146 | 10 | 12 | 10 | 11 | 13 | 11 | 11 | 9 |
| WM226 | 9 | 10 | 11 | 11 | 11 | 13 | 10 | 10 |
| WM236 | 11 | 13 | 10 | 13 | 13 | 11 | 10 | 10 |
| WM237 | 13 | 13 | 10 | 13 | 12 | 12 | 10 | 10 |
| WM246 | 10 | 11 | 10 | 13 | 10 | 10 | 10 | 10 |
| WM249 | 10 | 10 | 10 | 12 | 10 | 10 | 10 | 10 |
| WX112 | 10 | 10 | 11 | 17 | 13 | 14 | 13 | 10 |
| PXL83 | 9 | 11 | 10 | 13 | 11 | 13 | 13 | 9 |
| WX143 | 9 | 9 | 10 | 17 | 11 | 11 | 11 | 9 |

The inhibitory effect of cell-free supernatants of 317 LAB isolates against each indicator strain varied between 4.41 to 34.70% (Figure 2). Gram-positive indicators, S *aureus*, *E. faecalis*, *L. monocytogenes* and *S. suis* were inhibited by 4.41%, 11.04 %, 19.87% and 21.45% of total LAB isolates, respectively. The frequency of growth inhibition to Gram-negative indicators, *S.* Typhimurium, *P. aeruginosa*, *E. coli* O157:H7 and *C. jejuni* were 15.14 %, 26.18 %, 26.81% and 34.70 %, respectively

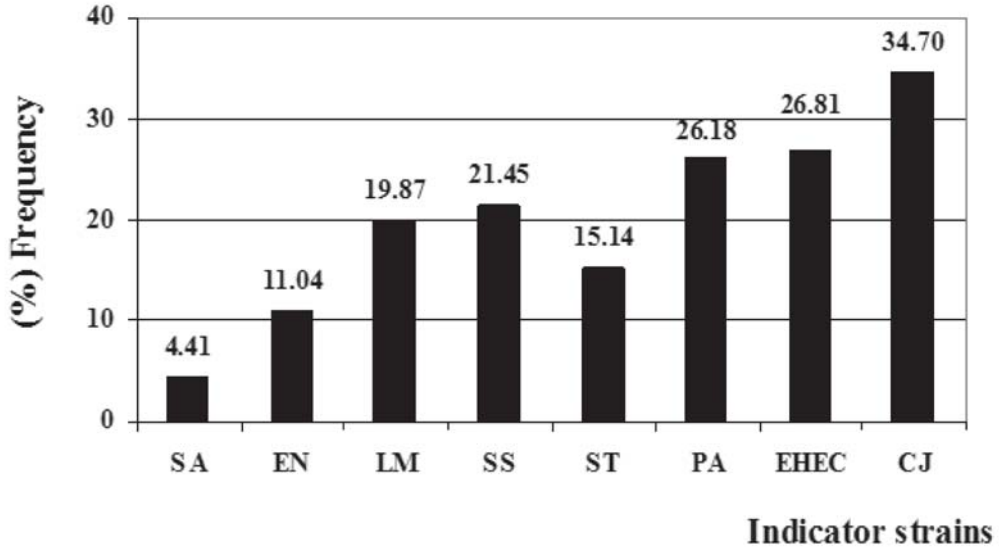


**Figure 2.** Frequency of inhibition by 317 lactic acid bacteria isolated from piglet faeces against eight indicator strains *S. aureus* (SA), *E. faecalis* (EN), *L. monocytogenes* (LM), *S. suis* (SS), *S.* Typhimurium (ST), *P. aeruginosa* (PA), *E. coli* O157:H7 (EHEC) and *C. jejuni* (CJ).

(Figure 2). From this result, it is likely that Gram-positive bacteria were less sensitive than Gram-negative bacteria, except *S.* Typhimurium. *S. aureus* was the least sensitive indicator strain while *C. jejuni* was the most sensitive indicator. *C. jejuni* is a micro-aerophilic bacterium and sensitive to oxygen (Kelly, 2001), this might be the reason why this indicator strain is more sensitive to inhibition than other indicator strains.

Since 2% lactic acid exhibited inhibitory activity against all indicator strains, the inhibitory activity of cell-free supernatants from LAB isolates might be also due to the production of lactic acid. The presence of lactic acid in the medium can cause cell death by lowering pH. The penetration of undissociated form of lactic acid into cytoplasm caused a reduction in the intracellular pH, as a consequence, the proton gradient is disrupted (Thomas et al., 2000).

Besides lactic acid, LAB also produce other compounds with antimicrobial activity such as other organic acids, hydrogen peroxide, diacetyl, bacteriocin, reuterin and reuterocyclin (De Vuyst and Leroy, 2007). Two LAB, *Enterococcus faecalis* and *Lactobacillus reuteri* have been reported to produce antibacterial compounds, bacteriocin and reuterin respectively, against a food-borne pathogen *C. jejuni* (Nazef et al., 2008). The inhibition of *S. aureus*, a strain that cause acute metritis of dairy cows, by *L.*

*gasseri* CRL1421 isolated from the vagina of cattle was due to the combination effect of hydrogen peroxide and lactic acid (Otero and Nader-Macas, 2006). Twenty six lactic acid bacteria isolated from pig faeces and caeca were found to have anti-*Salmonella* activity, however, the inhibitory compounds were not further identified (Casey et al., 2004).

To test if the inhibitory activity is caused by lactic acid, the cell-free supernatants of LAB isolates were neutralized prior antimicrobial activity assay. The results showed that only one LAB isolate, WX153, which was later identified as *Lactococcus lactis* subsp. *lactis* retained its inhibitory activity against *S. aureus* (18 mm inhibition zone), *S. suis* (9 mm) and *C. jejuni* (9 mm). *S. aureus* was the most sensitive strain among the three indicators and then was later used as a target micro-organism for further study of antimicrobial substance production. Initially, before neutralization, cell-free supernatant of strain WX153 exhibited inhibitory activity against 6 indicator strains including *S. aureus*, *S. suis*, *P. aeruginosa*, *L. monocytogenes* and *C. jejuni*. *S. aureus* was the most sensitive as the maximum inhibition zone of 20 mm was observed while zone of inhibition of only 11-12 mm were recorded for other 5 indicator strains.

The lost of inhibitory activity of most LAB isolates after neutralization of cell-free supernatants suggested that the inhibition was mainly due to organic acids, except for strain WX153. Elimination of hydrogen peroxide in cell-free supernatant of strain WX153 with catalase did not destroy its antimicrobial activity against *S. aureus* (Figure 3). Therefore, the inhibition was also not due to hydrogen peroxide. It is likely that antimicrobial compound produced by *L. lactis* subsp. *lactis* WX153 might be bacteriocin-like compound. Further purification and characterization of the compound are currently under way. Several works demonstrated the ability of *L. lactic* subsp. *lactis* to produce varieties of bacteriocins active against *S. aureus*, *L. monocytogenes* (Benkerroum et al., 2000; Moreno et al., 2000). One of the best known bacteriocin produced by some strains of *L. lactis* subsp. *lactis* is nisin (Cai et al., 1997; Choi et al., 2000). Nisin was approved by FAO/WHO to be used as a preservative in food industry in 1969 (Thomas et al., 2000). Other bacteriocins produced by *L. lactis* subsp. *lactis* such as bacteriocin HV219 (Todorov et al., 2006) and lactococcin (Dufour et al., 1991) have also been reported.

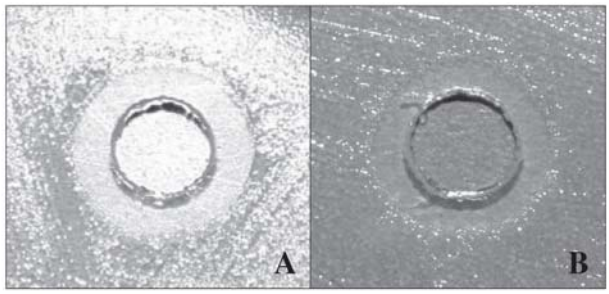


**Figure 3.** Antimicrobial activity of neutralized cell-free supernatant (CFNS) (A) and catalase treated CFNS (B) from isolate WX153 against *S. aureus* TISTR 118 as detected by the agar well diffusion method.

***Identification of strain WX153***

LAB strain WX153, a Gram-positive cocci, was identified as *Lactococcus lactis* subsp. *lactis* based on carbohydrate fermentation pattern using API 50 CHL test kit, with similarity index of 99.9% (Table 2). Identification of the strain WX153 by partial 16S rDNA sequence analysis confirmed the result obtained using biochemical test. The sequence shared 98% homology to that of *L. lactis* subsp. *lactis* (accession no. EU872263.1). Therefore the LAB strain WX153 was assigned as *L. lactis* subsp. *lactis* WX153.

It has been reported that identification of some LAB based on carbohydrate fermentation patterns is unreliable and several molecular typing methods such as sequence analysis of either partial or complete sequence of 16S rRNA gene, species-specific PCR amplification and 16S-23S spacer sequence analysis have been proposed to give more accurate identification (Ben Amor et al., 2007; Yin and Zheng, 2005). In the case of Lactococci, misidentification with the genus Enterococcus was also reported using biochemical test, especially if the bacteria were isolated from non-dairy source (Svec and Sedlacek, 2008). However, in this work, both methods gave the same identification results. *L. lactis* group are known to associate with milk and milk products. However, *L. lactis* subsp. *lactis* was also found in other sources such as surface water (Svec and Sedlacek, 2008), leaves of sugar cane plants (Serna and Rodríguez, 2006), minimally processed fresh fruit and vegetables (Kelly et al., 1998), humans (Elliott and Facklam, 1996) and intestinal tract of coastal fish (Itoi et al., 2008). *L. lactis* subsp. *cremoris* was isolated from human faeces after administration of fermented milk containing this strain (Maruo et al., 2006). This result indicated the possibility to detect *L. lactis* subsp. *lactis* in piglet faeces, as the piglet was fed with fermented milk (personal communication) and it may be able to survive passage through the GIT.

**Table 2.** Carbohydrate fermentation of *Lactococcus lactis* subsp. *lactis* WX153.

| Fermented acid from :- | Result | Fermented acid from :- | Result | Fermented acid from :- | Result |
|---|---|---|---|---|---|
| Control | - | Inositol | - | Melezitose | - |
| Glycerol | - | Mannitol | + | Raffinose | - |
| Erythritol | - | Sorbitol | - | Starch | + |
| D-Arabinose | - | α Methyl-D-mannoside | - | Glycogene | - |
| L-Arabinose | + | α Methyl-D-glucoside | - | Xylitol | - |
| Ribose | + | N Acetyl glucosamine | + | β Gentiobiose | + |
| D-Xylose | + | Amygdaline | + | D-Turanose | - |
| L-Xylose | - | Arbutine | + | D-Lyxose | - |
| Adonitol | - | Esculine | + | D-Tagatose | - |
| β Methyl-xyloside | - | Salicine | + | D-Fucose | - |
| Galactose | + | Cellobiose | + | L-Fucose | - |
| D-Glucose | + | Maltose | + | D-Arabitol | - |
| D-Fructose | + | Lactose | - | L-Arabitol | - |
| D-Mannose | + | Melibiose | - | Gluconate | - |
| L-Sorbose | - | Sucrose | + | 2 Keto-gluconate | - |
| Rhamnose | - | Trehalose | + | 5 Keto-gluconate | - |
| Dulcitol | - | Inuline | - | | |

+, the color of the fermentation liquid is yellow

-, the color of the fermentation liquid is purple

***Growth and antimicrobial substance production***

Growth and antimicrobial substance production of *L. lactis* subsp. *lactis* WX153 in MRS broth at 37 $^{o}$C is presented in Figure 4. The production of antimicrobial substance was associated with growth. Exponential growth phase was observed after 3 h of incubation and entered early stationary phase after 6 h. The maximum optical density ($600_{nm}$) of 1.75 was observed at 15 h and kept constant until the end of incubation (24 h). Antimicrobial substance was detected early at 3 h of incubation and the levels increased in the exponential and stationary phase (400 AU/ml). The maximum activity of 800 AU/ml was detected in late stationary phase (21-24 h) where the pH drops from an initial of 6.2 to 4.6. In this experiment, organic acids were removed before the antimicrobial activity assay, therefore the decrease in pH of culture broth did not affect the antimicrobial activity detected. However, the accumulation of organic acids in culture broth may affect both cell growth and antimicrobial substance production (Leroy and De Vuyst, 2001).

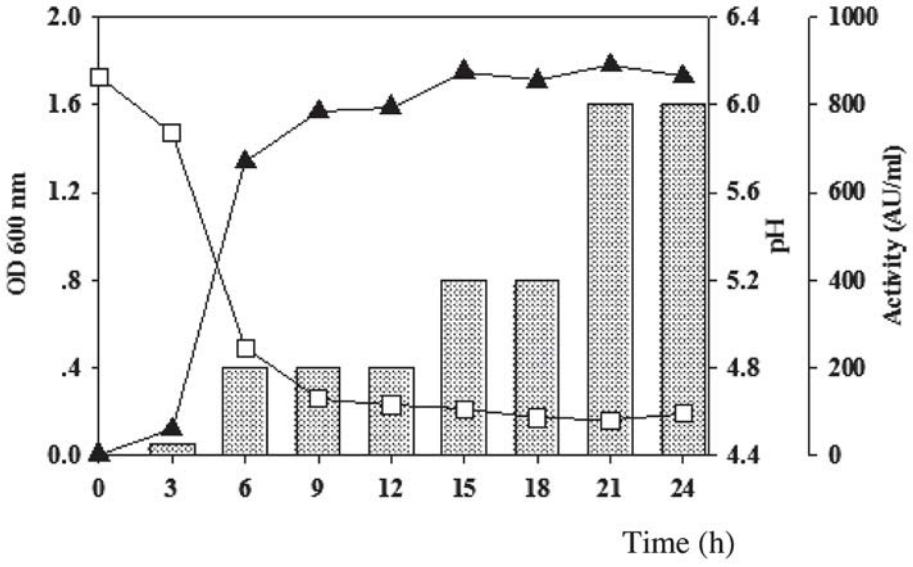


**Figure 4.** Cell growth (▲, OD 600 nm), antimicrobial activity (bars, AU/ml) and pH (□) of *L. lactis* subsp. *lactis* WX153 grown in MRS broth and incubated at 37 °C.

The detection of antimicrobial activity in early exponential phase of growth was also reported for several bacteriocins from LAB. However, bacteriocin production decreased or stopped after the end of growth phase mainly due to the inactivation by protease and the adsorption of bacteriocin to the producer cells (De Vuyst et al., 1996; Mataragas et al., 2003). *L. lactis* subsp. *lactis* WX153 also produced inhibitory compound early, however, antimicrobial activity remained steady until late stationary phase. This result indicated the stability of the antimicrobial compound produced by *L. lactis* subsp. *lactis* WX153.

In conclusion, piglet faeces are abundant source of LAB candidate for both probiotic and antimicrobial producing strains. Most of LAB isolates exhibited antimicrobial activity against several pathogens including *S. aureus, E. faecalis, L. monocytogenes, S. suis, S.*Typhimurium, *P. aeruginosa, E. coli* O157:H7 and *C. jejuni.* Of 317 isolates, 15 isolates exhibited antimicrobial activity against all eight pathogens tested. The antagonistic activity of most LAB isolates was mainly due to the production of organic acids, except for one strain *L. lactis* subsp. *lactis* WX153 which was found to produce bacteriocin-like substance. Based on its broad spectrum of antimicrobial activity and the ability to produce bacteriocin-like compounds, *L. lactis* Sub sp. *lactis* WX153 could be a good candidate for potential application as probiotics in pig and also as natural food preservatives.

## Acknowledgements

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