In Vitro Evaluation of Probiotic Potential of Novel Isolates of *Lactobacillus* from Native Pig Feces

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Abstract

The native pigs are a potential source of lactic acid bacteria (LAB) with probiotic properties since animal feed diversity plays a significant role in the intestinal tract's bacterial population. Usually, intestinal bacteria's activity promotes digestion, strengthens the immune system, and reduces deleterious microorganisms. These bacterial populations in the intestinal tract contain a probiotic group, of which the most qualified bacteria constitute LAB. This study proposes screening LAB and testing probiotics' properties from 25 feces of individual healthy native pigs. From the 139 isolates selected from feces samples, the antimicrobial activity inhibits pathogenic bacteria such as Enterohemorrhagic *Escherichia coli* (EHEC) isolated strain SC451-1, Enteropathogenic *Escherichia coli* (EPEC) isolated strain SC451-2, *Staphylococcus aureus* ATCC25923, *Klebsiella pneumoniae* ATCC700603, *Pseudomonas aeruginosa* ATCC27853, and *Salmonella* Typhimurium isolated strain SC2451-3, entirely tolerant at 1.0 % bile salt. Moreover, some of them could survive at a low pH of three and have a high hydrophobicity potential. This study, shown four isolates of the LAB high probiotic properties, and sequencing analysis indicated that four isolates were *L. plantarum*, *L salivarius*, *L. paracasei*, and *L. paraplantarum*. These properties allow the LAB to be considered a hopeful probiotic candidate for a feed additive of pig.

Keywords: Probiotics Properties, *Lactobacillus* spp, Pig feces

Introduction

In pig farming, antimicrobial are often supplements in feed to promote growth and prevent pathogenic bacteria infection. However, using antibiotics for an extended time accelerates bacterial resistance, spreading to farm environments and human food contamination (Lunden, Autio, & Korkeala, 2002; Zhao et al., 2018). Beside antimicrobial resistance, antimicrobial residue in pork and pork products also effects human health problem. (Yamaguchi et al., 2015). An alternative approach to reducing antibiotics in farms is the use of probiotic microorganisms (Patterson & Burkholder, 2003). Most of the probiotic groups constitute lactic acid bacteria (LAB). The LAB is beneficial microbes that help to improve the benefit microbial flora in the intestinal, promotes the growth of animals, inhibits pathogenic microbes in the digestive system, and strengthens immune system function in animals (Patterson & Burkholder, 2003; Galdeano, de LeBlanc, Vinderola, Bonet, & Perdigon, 2007; van der Aar, Molist, & van der Klis, 2017). The LAB with good properties commonly used as probiotics in animals such as *Lactobacillus* sp., *Bifidobacterium* sp., *Enterococcus faecalis*, *E. faecium*, *Leuconostoc mesenteroides*, *Pediococcus acidilactici*, *Streptococcus thermophilus*, and *Leuconostoc mesenteroidis* (Carlson & Slavin, 2016). Although probiotics are not a new option for pig production, most commercially available probiotics for pigs are unknown to the species' source,

and they may not isolate from the pigs. The use of bacteria isolated from one animal species to use in another may decrease probiotics' efficiency. Data confirms that probiotic bacteria isolated from one animal species were best able to colonize within the homologous animal intestinal tract. Moreover, it shows the best performance stimulating growth within the homologous host that choosing to use probiotics isolated from the same will effectively colonize the homologous species. (Campana, van Hemert, & Baffone, 2017). Isolation of strains from normal flora in the gastrointestinal tract of the native pig naturally released without ever receiving antimicrobial food. Animal food has an important influence on the diversity of the intestinal bacteria population. Thus, this research aims to isolate and test lactic acid bacteria properties from the of native pigs manure suitable for probiotics.

Methods and Materials

Sample Collection and Isolation of LAB

The LAB was isolated from the feces of native pigs in Phatthalung and Nakorn Sri Thammarat Province. Samples were taken anaerobically to the laboratory for microbiological tests. Serial dilutions of feces samples in 0.85% normal saline solution were spread on De Man, Rogosa and Sharpe (MRS) agar (Himedia: India) supplement 0.01% bromocresol purple, and plates were incubated at 37 °C for 72 h. under anaerobic. Then select the colony with a yellow zone in the media and stick on MRS agar that added 0.01% bromocresol purple to get pure bacteria. The characterization of isolated strains tested the gram staining and catalase enzyme formation.

Antimicrobial Activity of LAB

Inhibition of pathogenic bacteria was tested by the well diffusion method. The LAB was cultured in MRS agar at 37 °C overnight. Pathogenic bacteria in this study were Enterohemorrhagic *Escherichia coli* (EHEC) isolated strain SC2451-1, Enteropathogenic *Escherichia coli* (EPEC) isolated strain SC2451-2, *Klebsiella pneumoniae* ATCC700603, *Pseudomonas aeruginosa* ATCC27853, *Salmonella* Typhimurium isolated strain SC2451-3, and *Staphylococcus aureus* ATCC25923, which bacterial fresh to be adjusted to 0.5 McFarland (10^8 CFU mL^{-1}) with a densitometer (Biosan: England) and these bacteria were spread on Mueller-Hinton agar (Himedia: India). Well were drilled on the plate using cork borer (6 mm), and then each well was added 100 µL of cell free of the LAB supernatant. The well plates were incubated overnight at 37 °C. The inhibition zone around the wells was measured by vernier calipers.

Acid Tolerance and Bile Tolerance

Preparation of inoculum for acid and bile salt tolerance assay was performed as described previously (Ehrmann, Kurzak, Bauer, & Vogel, 2002). The LAB was incubated anaerobically in MRS broth at 37 °C for 24 h and then bacterial cells were adjusted to 10^8 CFU mL^{-1}. For acid tolerance assay, transfer 100 µL of isolated strains were transferred to 900 µL MRS broth with pH 3.0 (1 M HCl), incubated at 37 °C for 0 and 3 h. After that 100 µL of each test was spread onto MRS agar and incubated at 37 °C in 24 h. The survival rate in a pH 3.0 was calculated by the following equation: percent survival rate = (N1/N0) × 100 (Feng, Wang, Zhou, Yang, & Zhao, 2016). For bile salts tolerance assay, transfer 100 µL volume cells to MRS broth 900 µL containing 1.0 % bile, incubated at 37 °C for 0 and 3 hours counting the number of survivors by diluting with a 0.85% normal saline and spreading the germs in MRS agar incubated at 37 °C

for 24-48 hours. The survival rate in a 1 % bile salt solution was performed as described in acid tolerance above.

Cell Surface Hydrophobicity

Bacterial adhesion was determined to appraise LAB adherence potential to hydrocarbons, which referred to the adherence to the gut epithelial cells. The bacterial was allowed to grow in MRS broth for 24 h and centrifuged. Pellets were washed twice with phosphate buffered saline. The pellets are re-suspended in a PBS buffer, vortex, and absorb adsorption 0.7 - 0.9 at 600 nm. The LAB cell suspension (3.0 mL) was mixed with 1 ml of hydrocarbon (xylene) for 5 min and incubated at 37 °C for one h for separation. The aqueous 1 ml of suspensions mixed was gradually removed, and absorbance was measured at 610 nm (A1). Percent hydrophobicity was measured by a decrease in absorbance and calculated using the following formula Percent hydrophobicity = (1 − A1/A0) × 100 (Collado, Meriluoto, & Salminen, 2008).

Antibiotic Susceptibility

Antimicrobial susceptibility assay was performed by disc diffusion method on MRS agar (Prabhurajeshwar & Chandrakanth, 2017). All selected isolates were tested with vancomycin (30 μg), streptomycin (10 μg), ciprofloxacin (5 μg), erythromycin (15 μg), ampicillin (10 μg), chloramphenicol (30 μg) and norfloxacin (10 μg). After overnight incubation at 37 °C, the inhibition diameter (mm) around each disk was measured.

The *E. coli* ATCC259, *S. aureus* ATCC25923, and *P. aeruginosa* ATCC27853 were used as a reference control. Susceptibility testing was performed according to recommendations of Clinical and Laboratory Standards Institute (CLSI, 2020).

16S rDNA Sequencing

Amplification of the 16S rDNA gene was performed using universal primers (bact-0341 5' - CCTACGGGNGGCWGCAG-3' and R: bact-0785 5' GACTACHVGGGTATCTAATCC-3') (Klindworth et al., 2013). The 50 μL reaction volume was performed under the following conditions: initial denaturation at 94 °C for 10 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 59 °C for 1 min, extension at 72 °C for 1 min, and a final extension step at 72 °C for 10 min. The PCR products were visualized through 1.2 % (w/v) agarose gel electrophoresis. Before sending for nucleotide analysis, the PCR products were purified using a PCR purification kit (PureDireX™ :Tiwan). The PCR products were sequenced by the Macrogen DNA Sequencing Service (Korea). The multiple sequence alignment of 16S rRNA genes was used CLUSTAL W, and a phylogenetic tree of 16S rRNA genes was recreated using the maximum likelihood method implemented in the MEGAX software; bootstrap values were calculated with 1,000 bootstrapping.

Results

Characterization and Identification of Isolates

A total of 139 isolates bacteria were identified from 25 individual native pig feces from Phatthalung and Nakorn Sri Thammarat Province. All of them were gram positive, rod shaped, and catalase negative bacteria. Based on morphological assays, the authors assumed that these isolates are possibly *Lactobacillus* spp.

Antimicrobial Activity

A total of 139 isolates were tested for antimicrobial activity with common pathogenic bacteria in the gastrointestinal tract. The results showed all the ten selected isolates were able to inhibit the growth of the target pathogens of more than five pathogens strain (Table1). The most isolated selected strains were able to inhibit the growth of EHEC SC2451-1, EPEC SC2451-2, *S. aureus* ATCC 25923, *K. pneumoniae* ATCC700603, *P. aeruginosa* ATCC27853, and *S.* Typhimurium SC2451-3 include BC 1/3.4, BC 1/5.5, BC 4/4.2, BC 4/4.2 BC 4/5.2, BS 1/3.4, and LS 6/8.14. However, some isolates unable to inhibit the growth of *K. pneumoniae* ATCC700603 were LS 6/8.2 and *S.* Typhimurium SC2451-3 were BS 3/3.2 and BS 3/3.3.

Table1 The inhibitory effect of selected isolates strains against pathogenic microorganisms

Isolates No.	Inhibition zone (mm)					
	EHEC SC2451-1	EPEC SC2451-2	*S. aureus* ATCC25923	*K. pneumoniae* ATCC700603	*P. aeruginosa* ATCC27853	*S.* Typhimurium SC2451-3
BC1/3.4	25	17	18	18	19	22
BC1/5.5	26	16	18	18	22	18
BC4/4.2	13	15	10	16	12	14
BC4/5.2	14	14	15	16	15	14
BC4/5.3	14	16	21	20	14	12
BS1/3.4	24	21	28	15	22	18
BS3/3.2	14	14	10	19	21	-*
BS3/3.3	14	15	16	22	19	-
LS6/8.2	10	13	20	-	10	15
LS6/8.14	20	15	17	14	22	16

*- Diameter of well = 6 mm.

Acid and Bile Tolerance

The survival in acid media (pH 3.0) was used to assess the acid tolerance profile of *Lactobacillus* spp. We found that the BC 4/5.3 and BS6.8/2 isolates were highly capable of surviving in acid media from 10 isolates candidates (Fig. 1A). The tolerance effect of bile salts of 1.0 % of isolated bacteria showed nine well-grown isolates, and there was only one isolate resistant to bile salt was BC 4/4.2 (Figure1B).

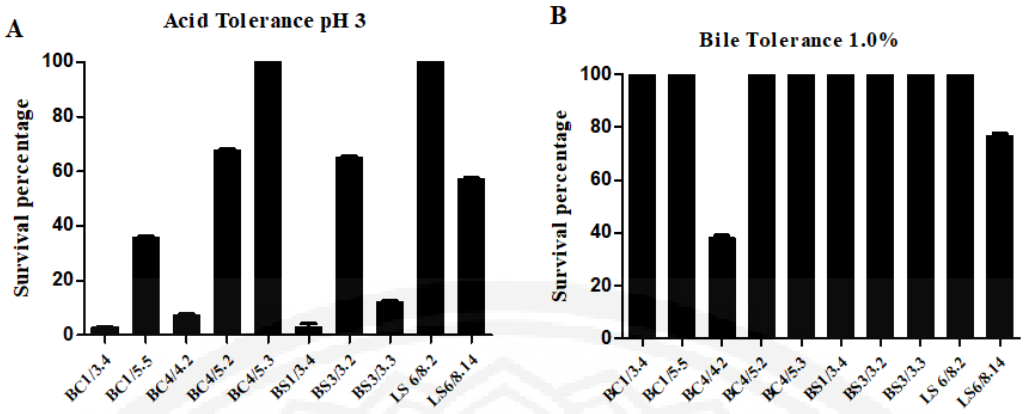

Figure 1 Acid and bile tolerance, The survival of isolates after 3 h. at pH 3 concentration (A) and the survival of isolates after 3 h. at 1.0 % bile concentration (B)

Cell Surface Hydrophobicity

The hydrophobicity rate of the *Lactobacillus* indicated the adherence ability to the intestinal mucosa epithelium. This study found a high hydrophobicity rate was observed for isolates BC 4/4.2 and LS 6/8.14 with 88.38 3.4 and 82.70 3.05, respectively. The moderate hydrophobicity rate includes isolates BS 3/3.3 BC1/3.4 and BS 3/3.2 with 68.49 3.4, 64.04 2.9, and 51.02 2.8, respectively (Figure 2).

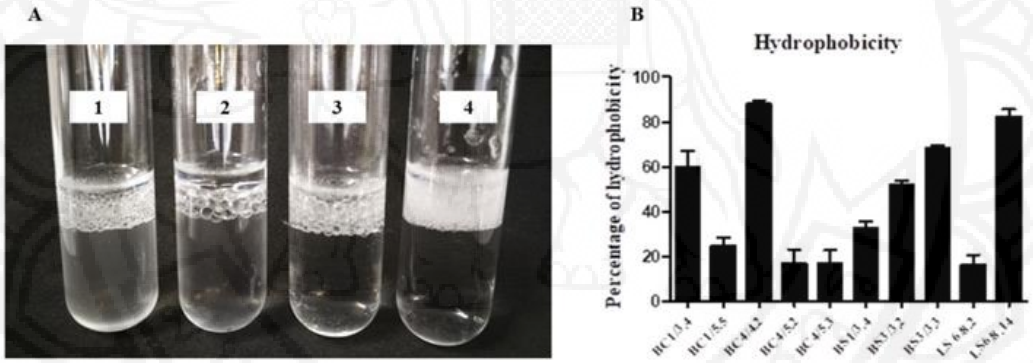

Figure 2 Hydrophobicity of selected isolates. Level of hydrophobicity of bacterial surface to bind to hydrocarbon were shown 1: Negative, 2: ++, 3: ++++, 4: Positive (A). Percentage of hydrophobicity of selected isolates surface to indicated the ability of binding to the intestinal mucosa cells (B)

Antibiotic Susceptibility

The antibiotic sensitivity of *Lactobacillus* isolates was tested with seven antibiotics (Table 2). All the isolates showed susceptibility to cephalothin, chloramphenicol, erythromycin, and ampicillin, while resistant to vancomycin and norfloxacin. The susceptibility to streptomycin is varied between each isolate, from sensitive, intermediate, and resistant. The isolates BC 4/5.3, BS 3/3.2, and LS 6/8.2 shown sensitivity, isolate LS 6/8.14 shown intermediate and isolates BC 1/3.4, BC 1/5.5, BC 4/4.2, BC 4/5.2, BS 1/3.4 shown resistance.

Table 2 Antibiotic susceptibility of *Lactobacillus* isolates strain

Isolates No.	Antibiotic Susceptibility (mm)						
	CEP	CHL	ERY	STR	AMP	VAN	NOR
BC1/3.4	S	S	S	R	S	R	R
BC1/5.5	S	S	S	R	S	R	R
BC4/4.2	S	S	S	R	S	R	R
BC4/5.2	S	S	S	R	S	R	R
BC4/5.3	S	S	S	S	S	R	R
BS1/3.4	S	S	S	R	S	R	R
BS3/3.2	S	S	S	S	S	R	R
BS3/3.3	S	S	S	S	S	R	R
LS 6/8.2	S	S	S	S	S	R	R
LS6/8.14	S	S	S	I	S	R	R

*cephalothin (CEP), chloramphenicol (CHL), erythromycin (ERY), streptomycin (STR), ampicillin (AMP), vancomycin (VAN), norfloxacin (NOR).

**I: intermediate (zone diameter, 12.5–17.4 mm) R: resistant (zone diameter, ≤12.4 mm) S: susceptible (zone diameter, ≥17.5). Erythromycin results based on R≤ 13 mm; I:13–23 mm; S ≥ 23 mm. Gentamycin results based on R ≤ 6 mm; I: 7–9 mm; S ≥ 10 mm. Vancomycin results based on R ≤ 12 mm; I: 12–13 mm; S ≥13 mm (CLSI, 2020).

Molecular Sequencing and Phylogenetic Tree

Four from 139 isolates, showing powerful probiotic properties with broad spectrum antimicrobial activity, was identified with 16S rDNA sequencing and phylogenetic analysis, as shown in figure 3. The isolate BC 1/3.4, BC 4/4.2, BS 3/3.2, and BS 3/3.3 identified as *L. plantarum*, *L. salivarius*, *L. paracasei*, and *L. paraplantarum* with 99.53 %, 99.08 %, 99.30%, and 99.29 % identity, respectively (Fig. 3), demonstrate to have probiotic properties.

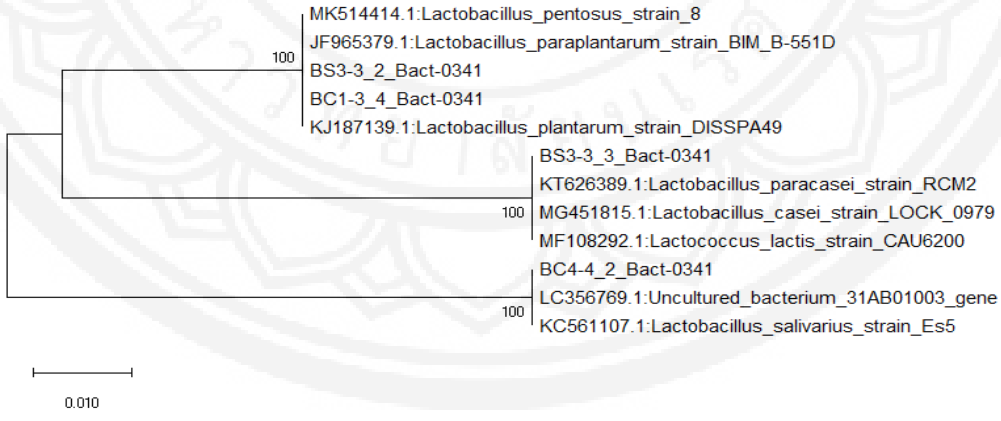

Figure 3 Phylogenetic tree of lactic acid bacteria isolated from native pig feces by MEGA program by the maximum likelihood method (1000 bootstrap)

Discussion

Probiotics are live organisms that provide health benefits to the host when consumed in adequate amounts by balancing the intestinal tract's microorganism. (Fung et al., 2009; Musa, Wu, Zhu, Seri, & Zhu, 2009; Salminen et al., 2010). However, before incorporating strains into products, their efficacy should be carefully assessed. To prove the probiotic properties of these strains scientifically, they have to fulfill series of selective criteria (Feng, Qiao, Liu, Yao, & Gao, 2017). Almost all displayed those prominent lactobacilli properties that presented to play a role in preventing the host from a bacterial infection (Reid & Bruce, 2001).

The primary criteria for selecting the *Lactobacillus* were antibacterial activity against six pathogenic bacteria (EHEC SC2451-1, EPEC SC2451-2, *S. aureus* ATCC 25923, *K. pneumoniae* ATCC700603, *P. aeruginosa* ATCC27853, and *S.* Typhimurium SC2451-3), since these pathogens are the common pathogen causing gastrointestinal tract infection in pigs (Bidewell et al., 2018; Lertworapreecha, Noomee, Sutthimusik, Utarapichat, & Tontikapong, 2016; Malik, Tóth, & Nagy, 2012). Moreover, these pathogens are the primary source of food-borne disease in humans. Besides, the *Salmonella* spp. is indicator bacteria that not allowed to contaminating export meat products (Rodríguez & Suárez, 2014). Although from all 139 isolates just only ten isolates qualified this assay, all the ten isolates exhibited highly effectively to inhibit at least five pathogenic bacteria. It is relatively high compared to the previous *Lactobacillus* study isolated from swine (Balasingham, Chinnamani, Radhakrishnan, & Balasuramanyam, 2017; Yun et al., 2009). Typically, the *Lactobacillus* strain's antibacterial activity resulted from two mechanisms; organic acid production and antimicrobial peptides production (Sablon, Contreras, & Vandamme, 2000; Tachedjian, Aldunate, Bradshaw, & Cone, 2017). Since the cell-free supernatant did not adjust the pH before testing to inhibit bacteria, we assume that the ability to inhibit pathogenic bacteria is primarily a result of acid production. The organic acids of selected isolates were produced and reduced the media's pH, which is known to inhibit the pathogens through a disruption in vital cell functions (Marteau, Minekus, Havenaar, & Veld, 1997).

The ability to survive in extreme conditions of *Lactobacillus* was assayed by acid and bile tolerance. The results found two isolates showed high tolerance in pH 3.0 with a 3-hour survival rate of up to 100%. In contrast, most isolates were highly resistant to bile salt. Both properties indicate that the isolated *Lactobacillus* can withstand severe conditions in the pig digestive tract. Tolerance of acid in gram-positive bacteria is based on the F0F1-ATPase mechanism, which act as channels for proton transport (Marteau et al., 1997; Gotcheva et al., 2002; Cotter & Hill, 2003). While bile salt tolerance results of bile efflux and bile hydrolysis mechanisms (Ruiz, Margolles, & Sánchez, 2013). The isolates that can withstand such conditions may result from highly effective enzymes.

The LAB isolates are resistant to some antibiotics tested in this study. The results of antibiotic susceptibility are similar to previous studies that have also reported the absence of acquired resistance in the LAB isolated from naturally fermented food samples (Tynkkynen, Singh, & Varmanen, 1998; Vidhyasagar & Jeevaratnam, 2013). Before using these isolates in feed, the virulence and antimicrobial resistance genes will be confirmed to protect the antibiotic resistance gene transfer to gut microbiota.

Due to their competitive exclusion to bind to the gastrointestinal tract, it is crucial to select *Lactobacillus* strain as probiotics; therefore, the adhesion ability of Lactobacillus is the one desired characteristics of probiotics (Cueva et al., 2010; Velez et al., 2007). The cell surface hydrophobicity

demonstration constrained the colonization and adhesion of probiotic bacteria to epithelial cells, which leads to the prevention of colonization of pathogens interaction (de Wouters, Jans, Niederberger, Fischer, & Ruhs, 2015). The results show high hydrophobicity of isolates BC 4/4.2 and LS 6/8.14 with 88.38 % ± 3.4 and 82.70 % ± 3.05 was present through cell surface interaction with hydrocarbon.

Among all the isolates tested, the isolates BC 1/3.4, BC 4/4.2, BS 3/3.2, and BS 3/3.3 identified as *L. plantarum*, *L salivarius*, *L. paracasei*, and *L. paraplantarum* with 99.53 %, 99.08 %, 99.30 %, and 99.29 % identity, respectively by 16S rDNA sequencing and phylogenetic analysis. Many studies have been conducted demonstrating the probiotic potential of *L. plantarum*, *L salivarius*, *L. paracasei*, and *L. paraplantarum* isolated from a wide variety of other samples. The LAB strains (BC 1/3.4, BC 4/4.2, BS 3/3.2, and BS 3/3.3) isolated from the native pig feces exhibited high probiotics properties in vitro, substantiating their potential to further develope as a probiotic in animal feed.

Conclusion

This study indicated that the antagonistic effects of the bacteria on pathogenic microorganisms play an important role in animal health. The *Lactobacillus* isolates isolated from native pig feces demonstrated a desirable tolerance to low pH and high bile salts, propitious anti-pathogen activity, and acceptable antibiotic susceptibility. Consequently, it is expected that these bacteria have the potential to be further developed as probiotic products for pigs.

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References

Balasingham, K., Chinnamani, V., Radhakrishnan, L., & Balasuramanyam, D. (2017). Probiotic characterization of lactic acid bacteria isolated from swine intestine. *Veterinary World*, *10,* 825-829. https://doi.org/10.14202/vetworld.2017.825-829

Bidewell, C. A., Williamson, S. M., Rogers, J., Tang, Y., Ellis, R. J., Petrovska, L., & AbuOun, M. (2018). Emergence of *Klebsiella pneumoniae* subspecies pneumoniae as a cause of septicaemia in pigs in England. *PLoS One*, *13*(2), e0191958. https://doi.org/10.1371/journal.pone.0191958

Campana, R., van Hemert, S., & Baffone, W. (2017). Strain-specific probiotic properties of lactic acid bacteria and their interference with human intestinal pathogens invasion. *Gut Pathogens*, *9,* 1-12. http://doi.org/10.1186/s13099-017-0162-4

Carlson, J., & Slavin, J. (2016). Health benefits of fibre, prebiotics and probiotics: a review of intestinal health and related health claims. *Quality Assurance and Safety of Crops & Foods*, *8,* 539-553. https://doi.org/10.3920/Qas2015.0791

CLSI. (2020). *Performance Standards for Antimicrobial Susceptibility Testing M100* (30th ed.). Wayne, PA: Clinical and Laboratory Standards Institute.

Collado, M. C., Meriluoto, J., & Salminen, S. (2008). Adhesion and aggregation properties of probiotic and pathogen strains. *European Food Research and Technology*, *226,* 1065-1073. https://doi.org/10.1007/s00217-007-0632-x

Cotter, P. D., & Hill, C. (2003). Surviving the acid test: responses of gram-positive bacteria to low pH. *Microbiology and Molecular Biology Reviews*, *67*(3), 429-453. https://doi.org/10.1128/mmbr.67.3.429-453.2003

Cueva, C., Moreno-Arribas, M. V., Martin-Alvarez, P. J., Bills, G., Vicente, M. F., Basilio, A., & Bartolome, B. (2010). Antimicrobial activity of phenolic acids against commensal, probiotic and pathogenic bacteria. *Research in Microbiology*, *161,* 372-382. https//:doi.org/10.1016/j.resmic.2010.04.006

de Wouters, T., Jans, C., Niederberger, T., Fischer, P., & Ruhs, P. A. (2015). Adhesion Potential of Intestinal Microbes Predicted by Physico-Chemical Characterization Methods. *Plos One*, *10*(8), https://doi.org/ARTNe013643710.1371/journal.pone.0136437

Ehrmann, M. A., Kurzak, P., Bauer, J., & Vogel, R. F. (2002). Characterization of lactobacilli towards their use as probiotic adjuncts in poultry. *Journal of Apply Microbiology*, *92*(5), 966-975. https//:doi.org/10.1046/j.1365-2672.2002.01608.x

Feng, J. C., Wang, L. H., Zhou, L. X., Yang, X., & Zhao, X. (2016). Using In Vitro Immunomodulatory Properties of Lactic Acid Bacteria for Selection of Probiotics against *Salmonell*a Infection in Broiler Chicks. *Plos One*, *11*(1), https://doi.org/ARTN e014763010.1371/journal.pone.0147630

Feng, Y. Y., Qiao, L., Liu, R., Yao, H. M., & Gao, C. B. (2017). Potential probiotic properties of lactic acid bacteria isolated from the intestinal mucosa of healthy piglets. *Annals of Microbiology*, *67,* 239-253. https://doi.org/10.1007/s13213-017-1254-6

Fung, W. Y., Woo, Y. P., Wan-Abdullah, W. N., Ahmad, R., Easa, A. M., & Liong, M. T. (2009). Benefits of probiotics: Beyond gastrointestinal health. *Milchwissenschaft - Milk Science International*, *64,* 17-21.

Galdeano, C. M., de LeBlanc, A. D., Vinderola, G., Bonet, A. E. B., & Perdigon, G. (2007). Proposed model: Mechanisms of immunomodulation induced by probiotic bacteria. *Clinical and Vaccine Immunology*, *14,* 485-492. https://doi.org/10.1128/Cvi.00406-06

Gotcheva, V., Hristozova, E., Hristozova, T., Guo, M. R., Roshkova, Z., & Angelov, A. (2002). Assessment of potential probiotic properties of lactic acid bacteria and yeast strains. *Food Biotechnology*, *16,* 211-225. https://doi.org/10.1081/Fbt-120016668

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., & Glockner, F. O. (2013). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Research*, *41*(1), https://doi.org/ARTN e110.1093/nar/gks808

Lertworapreecha, M., Noomee, S., Sutthimusik, S., Utarapichat, B., & Tontikapong, K. (2016). Multidrug resistant and extended spectrum beta-lactamase producing *Salmonella enterica* isolated from food animals in Phatthalung, Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health*, *47*(6), 1257-1269.

Lunden, J. M., Autio, T. J., & Korkeala, H. J. (2002). Transfer of persistent *Listeria monocytogenes* contamination between food-processing plants associated with a dicing machine. *Journal of Food Protection*, *65,* 1129-1133. https://doi.org/10.4315/0362-028x-65.7.1129

Malik, A., Tóth, I., & Nagy, B. (2012). Colonization of conventional weaned pigs by enteropathogenic Escherichia coli (EPEC) and its hazard potential for human health. *Acta Veterinaria Hungarica*, *60*(3), 297-307. https://doi.org/10.1556/AVet.2012.025

Marteau, P., Minekus, M., Havenaar, R., & Veld, J. H. J. H. I. (1997). Survival of lactic acid bacteria in a dynamic model of the stomach and small intestine: Validation and the effects of bile. *Journal of Dairy Science*, *80,* 1031-1037. https://doi.org/10.3168/jds.S0022-0302(97)76027-2

Musa, H. H., Wu, S. L., Zhu, C. H., Seri, H. I., & Zhu, G. Q. (2009). The Potential Benefits of Probiotics in Animal Production and Health. *Journal of Animal and Veterinary Advances*, *8,* 313-321.

Patterson, J. A., & Burkholder, K. M. (2003). Application of prebiotics and probiotics in poultry production. *Poultry Science*, *82,* 627-631. https://doi.org/10.1093/ps/82.4.627

Prabhurajeshwar, C., & Chandrakanth, R. K. (2017). Probiotic potential of lactobacilli with antagonistic activity against pathogenic strains: An in vitro validation for the production of inhibitory substances. *Biomedical Journal*, *40,* 270-283. https://doi.org/10.1016/j.bj.2017.06.008

Reid, G., & Bruce, A. W. (2001). Selection of *Lactobacillus* strains for urogenital probiotic applications. *Journal of Infectious Diseases*, *183,* S77-S80. https://doi:Doi 10.1086/318841

Rodríguez, D. M., & Suárez, M. C. (2014). *Salmonella* spp. in the pork supply chain: a risk approach. *Revista Colombiana de Ciencias Pecuarias, 27*, 65-75.

Ruiz, L., Margolles, A., & Sánchez, B. (2013). Bile resistance mechanisms in *Lactobacillus* and *Bifidobacterium*. *Frontiers in Microbiology*, *4,* 396-396. https://doi.org/10.3389/fmicb.2013.00396

Sablon, E., Contreras, B., & Vandamme, E. (2000). Antimicrobial Peptides of Lactic Acid Bacteria: Mode of Action, Genetics and Biosynthesis. *Advances in Biochemical Engineering/Biotechnology,* 68, 21-60. htts://doi.org/10.1007/3-540-45564-7_2

Salminen, S., Nybom, S., Meriluoto, J., Collado, M. C., Vesterlund, S., & El-Nezami, H. (2010). Interaction of probiotics and pathogens-benefits to human health? *Current Opinion in Biotechnology*, *21,* 157-167. https://doi.org/10.1016/j.copbio.2010.03.016

Tachedjian, G., Aldunate, M., Bradshaw, C. S., & Cone, R. A. (2017). The role of lactic acid production by probiotic *Lactobacillus* species in vaginal health. *Research in Microbiology*, *168*(9), 782-792. https://doi.org/10.1016/j.resmic.2017.04.001

Tynkkynen, S., Singh, K. V., & Varmanen, P. (1998). Vancomycin resistance factor of *Lactobacillus rhamnosus* GG in relation to enterococcal vancomycin resistance (van) genes. *International Journal of Food Microbiology*, *41,* 195-204. https://doi.org/10.1016/S0168-1605(98)00051-8

van der Aar, P. J., Molist, F., & van der Klis, J. D. (2017). The central role of intestinal health on the effect of feed additives on feed intake in pig and poultry. *Animal Feed Science and Technology*, *233,* 64-75. https://doi.org/10.1016/j.anifeedsci.2016.07.019

Velez, M. P., Hermans, K., Verhoeven, T. L. A., Lebeer, S. E., Vanderleyden, J., & De Keersmaecker, S. C. J. (2007). Identification and characterization of starter lactic acid bacteria and probiotics from Columbian dairy products. *Journal of Applied Microbiology*, *103,* 666-674. https://doi.org/10.1111/j.1365-2672.2007.03294.x

Vidhyasagar, V., & Jeevaratnam, K. (2013). Evaluation of *Pediococcus pentosaceus* strains isolated from Idly batter for probiotic properties in vitro. *Journal of Functional Foods*, *5,* 235-243. https://doi.org/10.1016/j.jff.2012.10.012

Yamaguchi, T., Okihashi, M., Harada, K., Konishi, Y., Uchida, K., Do, M. H. N., . . . Yamamoto, Y. (2015). Antibiotic Residue Monitoring Results for Pork, Chicken, and Beef Samples in Vietnam in 2012-2013. *Journal of Agricultural and Food Chemistry*, *63,* 5141-5145. https://doi.org/10.1021/jf505254y

Yun, J. H., Lee, K. B., Sung, Y. K., Kim, E. B., Lee, H. G., & Choi, Y. J. (2009). Isolation and characterization of potential probiotic lactobacilli from pig feces. *Journal of Basic Microbiology*, *49*(2), 220-226. http://doi.org/10.1002/jobm.200800119

Zhao, Y., Su, J. Q., An, X. L., Huang, F. Y., Rensing, C., Brandt, K. K., & Zhu, Y. G. (2018). Feed additives shift gut microbiota and enrich antibiotic resistance in pig gut. *Science of the Total Environment*, *621,* 1224-1232. https://doi.org/10.1016/j.scitotenv.2017.10.106