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Isolation and characterization of lactic acid bacteria from the gut of the grasscutter (*Thryonomys swinderianus*)

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Abstract. *Agbove MM, Kayang BB, Futse JE. 2021. Isolation and characterization of lactic acid bacteria from the gut of the grasscutter (*Thryonomys swinderianus*). Asian J Trop Biotechnol 18: 79-85.* Intestinal microbiota can affect hosts either beneficially or harmfully. Many efforts have been made to identify and study the microbial community in the gastrointestinal tract of livestock. The grasscutter is a micro-livestock species whose intestinal microflora is yet to be explored. Lactic acid bacteria confer probiotic benefits among the intestinal microflora and are of special interest. This study was conducted to isolate and characterize lactic acid bacteria from the gut of grasscutters in Ghana. Fresh fecal samples were collected from a total of 26 grasscutters comprising nine domesticated grasscutters and 17 wild grasscutters from Ghana. The samples were cultured on MRS agar, and the DNA from 57 bacterial colonies was extracted and sequenced at the 16S rRNA gene to identify the bacteria at the species level using the Basic Local Alignment Search Tool in the National Centre for Biotechnology Information database. Some of the five genera comprising 15 species of lactic acid bacteria (LAB) were identified with ≥99% similarity. Those included *Lactobacillus fermentum* (n = 11), *L. formosensis* (n = 1), *L. salivarius* (n = 11), *L. ingluviei* (n = 9), *L. plantarum* (n= 7), *L. reuteri* (n = 2), *L. taiwanensis* (n = 1), *L. rhamnosus* (n= 1), *Pediococcus pentosaceus* (n = 5), *Enterococcus gallinarum* (n = 2), *E. faecium* (n = 2), *Staphylococcus homini* (n = 2), *Weissella cibaria* (n = 1), *E. hirae* (n = 2), and *W. paramesenteroides* (n = 1). Moreover, all five genera were isolated from the domesticated grasscutters, while only two genera (*Lactobacillus* and *Pediococcus*) were isolated from wild grasscutters. The isolation of *L. ingluviei* is very interesting since this species was originally isolated from birds and is associated with weight gain in mice. The bacteria identified in this study may be important in determining the intestinal health of the grasscutter and should be assessed for their potential as probiotics to improve grasscutter nutrition.

Keywords: Coastal savannah grasscutter, gut, Lactic Acid Bacteria, *Thryonomys swinderianus*

INTRODUCTION

The grasscutter (*Thryonomys swinderianus* Temminck, 1827) is a rodent that is a wild herbivore related to the African brush-tailed porcupine as well as the guinea-pig, chinchilla, and the capybara of South America (Baptist and Mensah 1986). The main habitat is grassland in West Africa, hence the name grasscutter. While in other parts of Africa, it is associated with a cane field called the cane rat (Eben 2004).

Grasscutters occur in Western, Central, and Southern Africa (Mohamed et al. 2011; Adu et al. 2017). In Ghana, the domestication of this rodent started in the 1970s, and it is the preferred game by people living in rural areas in West Africa (National Research Council 1991). In some West African countries, particularly in Ghana, grasscutter meat is a delicacy everyone enjoys, regardless of religion or tribe. Several studies have been conducted on the animal due to its delicacy, popularity, low-fat level, and a high source of protein (Rural Infrastructure and Agro-Industries Division 2012).

Promoting grasscutters as micro-livestock is expected to contribute significantly to reducing malnutrition, which is prevalent in Ghana. Grasscutter rearing depends largely on the peasant farmers who keep these animals in different forms of housing units. Although being the most preferred (Teye et al. 2020) and the most expensive game meat in West Africa (Baptist and Mensah 1986), it has the potential to contribute to both local and export earnings in West African countries. However, dependence on wild grasscutters does not allow for sustainability and planned production for consumption (Yaro et al. 2012), hence the importance of domesticating and rearing them as micro-livestock.

Grasscutters readily adapt to many diets, such as leguminous fodder, roots, food crops, fruits, and grasses (Eben 2004). In the wild, grasscutters derive their nutrients from a variety of feedstuff for the production and maintenance of their body (Wogar 2011). Therefore, the breeder must supply all the needed nutrients in the right proportions in captivity. However, the nutrient requirement of grasscutters is not well known yet. Among the major nutrients, protein is most likely to be deficient in the formulated feed of farm animals because of its diverse roles in the body of animals (McDonald et al. 1996). Furthermore, conventional protein sources, such as fish meals and oil seed cakes (soya bean meal), are very expensive and most farmers cannot afford them.

The unbalanced and low nutritional value of the natural forage and feed fed to the grasscutter usually results in poor performance of the animal. Feeding is essential in animal breeding because it results in good reproduction, health, and growth in the animal (Zougou-Tovignon 2005). Most of the food nutrients are made available to the grasscutter either by microbial or enzymatic activities in the

gastrointestinal tract (Yapi et al. 2012). Despite the grasscutter's good attributes, attempts at its domestication have been marred by malnutrition, under-nutrition, and a high death rate (Yaro et al. 2012).

As a herbivorous animal for potential domestication, sufficient knowledge and understanding of the intestinal micro-flora may provide the much-needed information on how to formulate feed with a suitable probiotic. Those can help increase the animal's feed conversion ratio leading to increased productivity, increased litter size as well as reduced under-nourishment (Adu and Wallace 2004) since the digestion and the absorption of nutrients are dependent on activities of normal microflora of the gastrointestinal tract (GIT) (Draser 1989). Lactic Acid Bacteria (LAB) are prominent among the microflora that promote the health and bioavailability of nutrients in the gut of the grasscutter.

LAB are a group of Gram-positive, anaerobic, non-sporulating, or facultative aerobic cocci or rods, which produce lactic acid as one of their main products of carbohydrate metabolism (Ghoddusi et al. 2011). LAB confers probiotic benefits among the intestinal microflora and is thus of special interest. Markowiak and Śliżewska (2017) defined a probiotic as a live microbial feed supplement that beneficially affects the host animal by improving its intestinal microbial balance. LAB forms part of an animal's GIT and boosts the immune system against invading pathogens, availing some nutrients, increasing growth rate, digestion rate, and absorption of nutrients in the intestine, and preventing the onset of some diseases (Florou-Paneri et al. 2013).

Currently, because of regulations restricting antibiotics as feed additives, there is a growing interest in employing probiotics as a suitable alternative in animal production because of issues of generating antibiotic-resistant bacteria. In this regard, LAB offers great promise for use as probiotics. However, it is important first to study the LAB profile in livestock to identify which LAB species to target for use as probiotics for a particular livestock species. Therefore, this study was conducted to isolate and characterize lactic acid bacteria from the gut of the grasscutter in Ghana.

MATERIALS AND METHODS

The sample collection

A sum of 26 fresh fecal samples was collected from both domesticated and wild grasscutters. The domesticated grasscutters (n = 9) were taken from the grasscutter facility at the Department of Animal Science, the University of Ghana, Ghana (n = 4), and Legon Staff village (n = 5). The wild grasscutters (n = 17) were taken from Ghana's Mankessim and Gomoa Abontin regions. Samples were collected using sterile tweezers and dropped into sterile tubes containing De Man, Rogosa, and Sharpe (MRS) broth (Becton, Dickinson and Company, Sparks, USA), then transported as soon as possible to the laboratory. Next, the collected samples were cultured overnight in an incubator at 37°C. The fecal samples were then removed from the MRS broth to get a microbial broth. The resultant MRS broth in the tubes was kept in a plastic bag with an AnaeroPack-Anaero gas generator (Mitsubishi Gas Chemical CO., INC., Japan) and stored at 4°C.

The isolation of LAB

The samples were homogenized and cultured on MRS agar using the streak method. Next, the MRS agar plates were incubated anaerobically using the AnaeroPack-Anaero gas generator at 37°C for 24 hrs. The dominant and discrete colonies were selected (based on morphological characteristics such as color, colony size, roughness or smoothness, and transparency), transferred onto a new MRS agar, and incubated at 37°C for 24 hrs. Next, sub-culturing was further carried out to obtain homogeneous colonies. The homogeneous colonies were then collected into sterilized skimmed milk and stored at -80°C until use.

DNA extraction

A sterilized loop was used for each homogenized colony to collect the colony into 200 µL of a 0.5% triton-X extraction buffer. The resultant solution therein was vortexed and kept at -20°C overnight. Next, the samples were removed, thawed, and placed in a bead beater for about 20 sec at a speed of about 5,000 rpm. Finally, the samples were centrifuged at 13,000 rpm for 5 mins at a temperature of 4°C. A sum of 30 µL of each resulting supernatant was pipetted into a 96 well plate and stored at -20°C until use.

PCR amplification

Amplification of the V3 region of the 16S rRNA gene from LAB was carried out by using primers 27F (5 '- AGA GTT TGA TCC TGG CTC AG 3 ') and 1492R (5 '- GGT TAC CTT GTT ACG ACT 3 ') (Lane 1991). Next, the PCR amplification was performed in a 50 µL reaction mixture containing 0.3 µL of each primer, 2.4 µL of dNTP, 7.5 µL 10×buffer, 3.35 µL distilled water, 0.15 µL Taq DNA polymerase, and 1 µL template DNA. First, PCR amplification was performed on Takara thermal cycler (Takara bio-medical Tokyo, Japan) with denaturing at 94°C for 10 sec, followed by 35 cycles with denaturing at 94°C for 10 sec, extension at 55°C for 30 sec, elongation at 72°C for 30 sec.

PCR product purification and sequence reaction

The PCR product to purify was topped up with distilled water into 100 µL, mixed thoroughly with 500 µL of binding buffer, and then transferred into spin columns. Next, the PCR product was centrifuged at 14,000 rpm for 1 min. Then, 500 µL of washing buffer was added and centrifuged at 14,000 rpm for 1 min. Finally, another 200 µL of washing buffer was added and centrifuged at 14,000 rpm for three mins. Next, spin columns containing the PCR products were transferred into new 1.5 ml tubes, and the flowthrough was discarded. Next, 50 µL of distilled water was added and centrifuged at 14,000 rpm for one minute to elute the purified PCR product. Again, the spin columns were discarded, and the purified PCR product was stored at 4°C.

The sequence reaction was performed in a 15 µL reaction mixture, which contains 3 µL Big Dye, 3 µL of

buffer, 1.5 µL of each primer, and 7.5 µL of purified PCR product. The cycling was carried out in a Takara thermal cycler (Takara bio-medical Tokyo, Japan) with denaturing at 96°C for 10 sec followed by 25 cycles of denaturing at 96°C for 10 sec, extension at 50°C for 5 sec, and elongation at 72°C for 1 min.

Ethanol precipitation and sequencing

The PCR product sequence reaction was purified by ethanol precipitation into a 100 µL reaction mixture, which contained 3 µL of NaOAc (3M), 24.5 µL of distilled water, 62.5 µL of EtOH (99.5%), and 10 µL of the sample. Next, the mixture was vortexed and kept at room temperature for 15 mins and later centrifuged at 3,100 x g for 20 mins. Next, 200 µL of 70% ethanol was added to the plate and mixed by inverting the plate slowly five times to wash the DNA. Next, the plate was centrifuged at 3,100 x g for 5 mins, after which the supernatant was carefully discarded. Next, the plate was wrapped with tissue and cellophane and centrifuged upside down at 800 x g to dry the DNA. After that, 4 µL HIDI formamide was added to each sample to re-suspend the DNA. Next, a sum of 10 µL HIDI formamide was pipetted into a sequencing plate, and 1 µL of each dissolved DNA sample was added. Next, the plate was incubated on a heat block at 95°C for 2 mins and quickly transferred onto a cold block for 5 mins. The samples were finally electrophoresed on an ABI prism 3100 DNA sequencer (Applied Biosystem Division, Foster City, CA, USA).

Sequence analysis

The 16S rRNA sequences method was edited to obtain the required length of about 500-600 bp using Finch TV (www.geospiza.com) and Mega 7 (Kumar et al. 2015) software. Next, the Basic Local Alignment Search Tool (BLAST) was used to align sequences in the GenBank database of the National Centre for Biotechnology Information (NCBI) for species assignment. The strains selected showed ≥99% similarity with 16s rRNA genes in the NCBI GenBank.

RESULTS AND DISCUSSION

Molecular identification of LAB isolates

The sums of fifty-seven isolates were cultured from 26 grasscutter samples and identified as belonging to the following five genera: *Lactobacillus, Weissella, Pediococcus, Enterococcus,* and *Staphylococcus* (Table 1). The domesticated grasscutters, which had 18 isolates of LAB, belonged to the above five genera. In comparison, the wild grasscutter had 39 isolates of LAB, which belonged to just two of the five genera namely *Lactobacillus* and *Pediococcus*. The aligned sequences were identified at the species level (Table 1). Moreover, all the five genera with their species isolated included, *Lactobacillus fermentum* (n = 11), *L. salivarius* (n = 11), *L. ingluviei* (n = 9), *L. plantarum* (n = 7), *Pediococcus pentosaceus* (n = 5), *Enterococcus gallinarum* (n = 2), *E. hirae* (n = 2), *E. faecium* (n = 2), *Staphylococcus homini* (n = 2), *L. reuteri* (n = 2), *L. taiwanensis* (n = 1), *L. formosensis* (n = 1), *L. rhamnosus* (n = 1), *Weissella cibaria* (n = 1) and *W. paramesenteroides* (n = 1).

The cultivable lactic acid bacteria from the gut of the grasscutter

The cultivable LAB from the gut of the grasscutters that were successfully isolated is presented in Table 2, which are 57 LAB isolates belonging to five genera and consisting of 15 species. The species that were found to be common and/or specific to domesticated and wild grasscutters are presented in Table 3. The four LAB species were isolated from both the wild and domesticated grasscutters. These were *L. salivarius, L. reuteri, L. plantarum* and *P. pentosaceus.* And then, four LAB species were also found to belong specifically to the wild grasscutter. These included *L. ingluviei, L. taiwanensis, L. formosensis, and L. fermentum.* However, the seven LAB species were found exclusively in the domesticated grasscutter. These were *L. rhamnosus, E. Hirae, W. cibaria, W. paramesenteroides, S. hominis, E. faecium,* and *E. gallinarum.* There was greater diversity in the domesticated grasscutter than in the wild grasscutter.

Table 4 represents the frequency of LAB isolated from all the grasscutters (domesticated and wild). *L. fermentum* and *L. salivarius* had the highest number of isolates (11 each), representing 19.2% of the total LAB isolates, respectively, with L. ingluviei isolated as the third most dominant isolate representing 15.7% of the total isolates. Next, *L. plantarum* was the fourth most dominant isolate and represented 12.3%. Finally, *L. formosensis, L. taiwanensis, L. rhamnosus, W. cibaria, W. paramesenteroides,* and *Enterococcus hirae* had the least number of isolates (1 each), which represented 1.8% each (Table 4).

The number of LAB isolated from wild and domesticated grasscutter

The domesticated grasscutter had all five genera of LAB that were isolated (Figure 1), while the wild grasscutter had only two genera (Figure 2). However, for the domesticated grasscutter, the five genera represented by 18 isolates included *Lactobacillus* (n = 8), *Enterococcus* (n = 5), *Weissella* (n= 2), *Pediococcus* (n = 1) and *Staphylococcus* (n= 2) (Figure 1) but the wild grasscutter which had two genera and were represented by 39 isolates included *Lactobacillus* (n = 39) and *Pediococcus* (n = 4) (Figure 2).

Discussion

In this study, all 57 isolates (Table 1) belonged to *Lactobacillus, Weissella, Pediococcus, Enterococcus,* and *Staphylococcus* genera. There were 15 individual species isolated as follows: *L. ingluviei, L. fermentum, L. salivarius, L. plantarum, L. formosensis, L. reuteri, L. taiwanensis, L. rhamnosus, W. cibaria, W. paramesenteroides, P. pentosaceus, E. gallinarum, E. hirae, E. faecium* and *S. hominis* (Table 2). Although within the isolated LAB genera, *Lactobacillus* had the highest proportion of bacteria in all the samples, followed by *Enterococcus* and *Pediococcus*. Conversely, *Staphylococcus* and *Wiessella* had the lowest proportion (Table 4).

Lactobacillus represented 75.4% (Table 4) of the overall LAB isolates. This finding follows the findings of Wang et al. (2014) regarding the isolation of LAB from the gastrointestinal tract of a chicken. Eight species represented the *Lactobacillus* genus (43 isolates): *L. ingluviei, L. fermentum, L. salivarius, L. plantarum, L. formosensis, L. reuteri, L. taiwanensis,* and *L. rhamnosus*.

Table 1. The basic local alignment search tool result of lactic acid bacteria isolated from the gut of domesticated and wild grasscutters

Source of animal	Sample ID	Colony ID	Sequence length (bp)	BLAST identity %	E-value	Species
Wild	28 TS	1	675	99	0	*Lactobacillus ingluviei*
Domesticated	13 TS	3	698	100	0	*Weissella cibaria*
		5	796	99	0	*Lactobacillus salivarius*
Wild	30 TS	7	680	99	0	*Lactobacillus ingluviei*
		8	641	100	0	*Lactobacillus fermentum*
Wild	31 TS	9	776	100	0	*Lactobacillus salivarius*
		10	588	99	0	*Lactobacillus ingluviei*
Wild	34 TS	12	565	100	0	*Lactobacillus fermentum*
		13	699	100	0	*Lactobacillus fermentum*
Wild	29 TS	15	686	100	0	*Lactobacillus plantarum*
		16	606	100	0	*Pediococcus pentosaceus*
		17	809	99	0	*Lactobacillus plantarum*
Wild	32 TS	18	579	100	0	*Lactobacillus ingluviei*
		19	576	100	0	*Lactobacillus ingluviei*
Wild	33 TS	20	580	100	0	*Lactobacillus fermentum*
		21	671	100	0	*Lactobacillus fermentum*
		22	576	100	0	*Lactobacillus fermentum*
Wild	43 TS	23	696	99	0	*Lactobacillus fermentum*
		24	704	99	0	*Pediococcus pentosaceus*
		25	680	99	0	*Lactobacillus fermentum*
Wild	41 TS	27	695	100	0	*Lactobacillus plantarum*
		28	690	99	0	*Lactobacillus plantarum*
Wild	42 TS	29	677	99	0	*Pediococcus pentosaceus*
Wild	36 TS	30	677	99	0	*Lactobacillus ingluviei*
		32	678	99	0	*Lactobacillus ingluviei*
		33	791	100	0	*Lactobacillus salivarius*
Wild	35 TS	34	671	100	0	*Lactobacillus fermentum*
		35	695	99	0	*Lactobacillus formosensis*
Wild	37 TS	37	786	99	0	*Lactobacillus salivarius*
		38	642	99	0	*Lactobacillus reuteri*
		39	778	99	0	*Lactobacillus salivarius*
Wild	38 TS	40	778	100	0	*Lactobacillus salivarius*
		41	666	100	0	*Lactobacillus salivarius*
		42	756	99	0	*Lactobacillus ingluviei*
Wild	39 TS	43	666	99	0	*Lactobacillus fermentum*
		44	753	100	0	*Pediococcus pentosaceus*
		45	752	99	0	*Lactobacillus fermentum*
Wild	44 TS	46	749	100	0	*Lactobacillus salivarius*
		47	657	99	0	*Lactobacillus salivarius*
		48	681	99	0	*Lactobacillus ingluviei*
Domesticated	02 TS	49	741	100	0	*Staphylococcus hominis*
		51	800	100	0	*Weissella paramesenteroides*
Domesticated	03 TS	52	691	100	0	*Staphylococcus hominis*
		53	733	99	0	*Enterococcus gallinarum*
Domesticated	01 TS	56	692	100	0	*Lactobacillus salivarius*
		57	777	100	0	*Lactobacillus reuteri*
Domesticated	07 TS	58	668	99	0	*Lactobacillus salivarius*
		59	605	99	0	*Lactobacillus plantarum*
Wild	40 TS	60	642	99	0	*Lactobacillus taiwanensis*
Domesticated	08 TS	61	670	99	0	*Lactobacillus plantarum*
		63	592	99	0	*Lactobacillus plantarum*
Domesticated	09 TS	64	753	100	0	*Pediococcus pentosaceus*
		65	742	100	0	*Enterococcus faecium*
Domesticated	10 TS	66	711	99	0	*Enterococcus hirae*
Domesticated	04 TS	67	728	99	0	*Enterococcus faecium*
		68	566	100	0	*Enterococcus gallinarum*
		69	669	99	0	*Lactobacillus rhamnosus*

Table 2. The cultivable lactic acid bacteria from the gut of the grasscutter

Genus	Species
Lactobacillus	*L. fermentum*
	L. salivarius
	L. ingluviei
	L. plantarum
	L. formosensis
	L. reuteri
	L. taiwanensis
	L. rhamnosus
Weissella	*W. cibaria*
	W. paramesenteroides
Pediococcus	*P. pentosaceus*
Enterococcus	*E. gallinarum*
	E. hirae
	E. faecium
Staphylococcus	*S. hominis*

Table 3. Species of lactic acid bacteria common and/or specific to domesticated and wild grasscutter

Species of LAB	Origin*	
	Domesticated	Wild
Lactobacillus salivarius	√	√
Lactobacillus reuteri	√	√
Lactobacillus plantarum	√	√
Pediococcus pentosaceus	√	√
Lactobacillus ingluviei	x	√
Lactobacillus taiwanensis	x	√
Lactobacillus formosensis	x	√
Lactobacillus fermentum	x	√
Lactobacillus rhamnosus	√	x
Weissella cibaria	√	x
Weissella paramesenteroides	√	x
Staphylococcus hominis	√	x
Enterococcus faecium	√	x
Enterococcus gallinarum	√	x
Enterococcus hirae	√	x

Note: *: √ = isolated, x = not isolated

Table 4. Frequency of lactic acid bacteria species isolated

Genus	Species of LAB	Frequency	Percentage (%)
Lactobacillus	*L. fermentum*	11	19.2
	L. salivarius	11	19.2
	L. ingluviei	9	15.7
	L. plantarum	7	12.3
	L. reuteri	2	3.5
	L. formosensis	1	1.8
	L. taiwanensis	1	1.8
	L. rhamnosus	1	1.8
Weissella	*W. cibaria*	1	1.8
	W. paramesenteroides	1	1.8
Pediococcus	*P. pentosaceus*	5	8.8
Enterococcus	*E. Faecium*	2	3.5
	E. gallinarum	2	3.5
	E. hirae	1	1.8
Staphylococcus	*S. hominis*	2	3.5

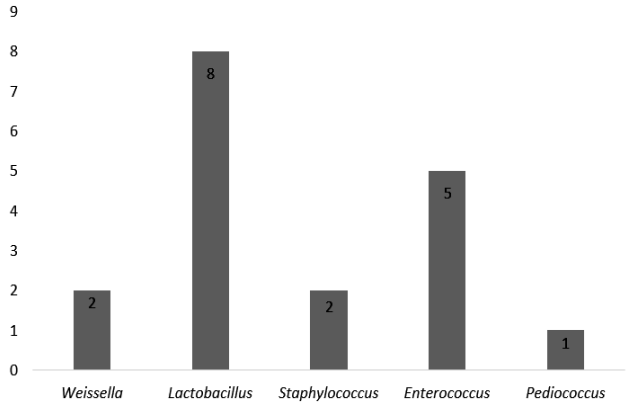

Figure 1. Number of LAB species isolated from domesticated grasscutter

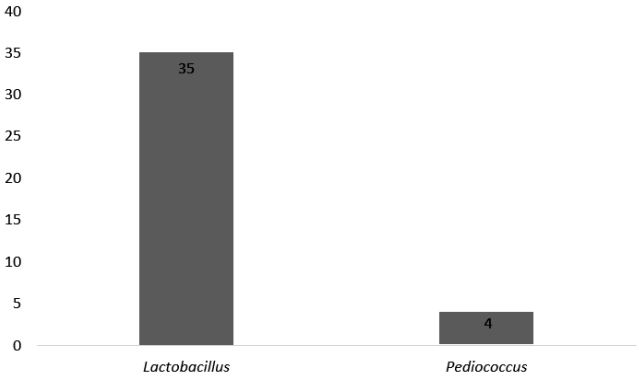

Figure 2. Number of LAB species Isolated from Wild Grasscutter

Lactobacillus fermentum and *L. salivarius* had the highest number of isolates, representing 19.2% of the overall LAB isolates (Table 4), respectively. This result is comparable to the findings of Kobierecka et al. (2017) pertaining to the study of LAB isolated from the chicken intestinal system. *L fermentum* maximized the digestibility of crude protein in piglets (López-Gálvez et al. 2020). *L fermentum* also increased average daily weight, improved intestinal immunity in neonatal pigs (Liu et al. 2014), improved feed conversion and weight gain, reduced diarrhea incidence, and improved meat quality in grower-finishing pigs (Suo et al. 2012). Therefore, it was not surprising to find *L. fermentum* as one of the most dominant lactic acid bacteria in this study since they dominate during the intermediate and final stages of the fermentation (Kogno et al. 2017).

Lactobacillus salivarius was isolated as one of the dominant species (19.2%) of the total LAB isolates (Table 4) as it is fast-growing and a bacteriocin producer. Therefore, from this study, *L. salivarius* was isolated as the dominant species, like the results from the study of LAB being isolated as the dominant species from chicken (Wang et al. 2014) and horse (Rafat et al. 2005). Furthermore, *L salivarius* has been known to develop at a temperature of 37°C. In addition, *L salivarius* has been observed to enhance the breakdown of fiber during fermentation (Yang

et al. 2006) and reduce glucosinolate and crude fiber (Aljoubori et al. 2014).

In the present study, *L. ingluviei* was isolated as the third most dominant LAB representing 15.7% (Table 4) of the total LAB isolates in the wild grasscutter, indicating it is one of the main lactic acid microbiotas of the wild grasscutter. *L ingluviei* may be one of the main lactic acid microbiota in the wild grasscutter. *L ingluviei* has only previously been isolated in birds, specifically in pigeons (Baele 2003) and ostriches (Khan et al. 2007). Quite recently, *L. ingluviei* has been shown to be responsible for weight gain in ducks and chicks (Angelakis and Raoult 2010) as well as in mice (Angelakis et al. 2012). Therefore, *L. ingluviei* would be a lactic acid bacterial species of interest since this is the first isolate from a mammalian species. *L plantarum* represented 12.2% of the total LAB isolates (Table 4).

This species has been widely studied for its numerous application. Wide adaptation capacity with most research focused on its response to the high concentration of lactic acid (Pieterse et al. 2005), low pH and ethanol (De Angelis et al. 2004), bile (Bron et al. 2006), and heat shock (De Angelis et al. 2004).

The least number of *Lactobacillus* species isolated were *L. reuteri*, *L. taiwanensis*, *L. rhamnosus*, and *L. formosensis. L. reuteri* represented 3.5% of the total LAB isolates, while *L. taiwanensis*, *L. rhamnosus*, and *L. formosensis* each represented 1.8% of the total LAB isolates (Table 4).

The genus *Enterococcus* had five isolates represented by three species: *E. Faecium, E. gallinarum*, and *E. hirea*, representing 8.8% of the total LAB isolates (Table 4). This result contrasts with 50% for *E. hirea* isolated from cow feces by Adeniyi et al. (2015) and the 22% reported by Devriese et al. (1987) concerning the isolation of LAB from the intestines of different farm animals. *Enterococcus* species, especially *E. faecalis* and *E. faecium*, have been studied and are known to produce a variety of bacteriocins against many pathogenic bacteria (Ogier and Serror 2008).

The genus *Pediococcus* also had five isolates represented by *P. pentosaceus*. That accounted for 8.8% of the total LAB isolates in this study (Table 4). The *P. pentosaceus* has been shown to reduce fatty liver and obesity (Zhao et al. 2012) in animals. It has also been employed as a food preservative due to its ability to produce antimicrobial agents (Martino et al. 2013).

The genus *Staphylococcus* was represented by *S. hominis*, which accounted for 3.5 % of the total LAB isolates (Table 4). The *S. hominis* is part of the normal microflora of the skin of humans and sometimes animals. Therefore, although it is one of the least isolated species, its presence in the grasscutter may indicate the level of contact between the grasscutters and humans. In addition, the *S. hominis* has been reported to produce bacteriocin, an antibacterial agent against pathogenic *S. aureus* (Sung et al. 2010; da Costa et al. 2016). Therefore, its presence in domesticated grasscutters could have beneficial health implications.

The genus *Weissella* was represented by *W. cibaria* and *W. paramesenteroides*. They constituted 3.5% (Table 4) of the total LAB isolates. Belda et al. (2011) showed that *Weissella* occurred at higher levels in the mid-gut of the European corn borer lab-reared populations than in the field population. *Weissella* species are also commonly found in habitats associated with the human or animal body (Nistal et al. 2012). So it was not surprising to isolate them from domestic but not wild grasscutters.

Although the number of samples collected was not even for both domesticated (n = 9) and the wild (n = 17) grasscutters, the number of LAB genera isolated from the domesticated grasscutter (n = 5) (Figure 1) dwarfed that of the wild grasscutter (n = 2) (Figure 2). The genera *Lactobacillus, Weissella, Enterococcus, Staphylococcus*, and *Pediococcus*, were isolated from the domesticated grasscutter (Figure 1), while only *Lactobacillus* and *Pediococcus* were isolated from the wild grasscutter (Figure 2). The difference in the profile can probably be attributed to the fact that domesticated grasscutters are fed with different feed with different microbial compositions and exposed to human contact, hence the higher number of LAB genera. In contrast, the wild grasscutters select their natural feed, mainly grass, hence the lower number of LAB genera. In addition, the animals' stress conditions (health and diet) were unknown at the time of the sample collection, and these factors usually negatively affect the lactic acid microbiota. Therefore, most species isolated from domesticated grasscutters and wild grasscutters were from the genera *Lactobacillus*.

Although the points of sample collection were also far apart, some LAB species were common to both the domesticated and wild grasscutter, notably *L. salivarius, L. reuteri, L. plantarum*, and *P. pentosaceus*. In addition, four species were specific to domesticated grasscutters, while seven other species were specific to the wild grasscutter (Table 3).

In conclusion, sequence analysis showed that a wide range of LAB species are available for isolation and characterization in the gut of the grasscutter. Furthermore, the domesticated grasscutter had more LAB diversity than the wild grasscutter. The difference in diversity could be attributed to the exposure of domesticated grasscutters to different types of feed and human contact. The isolation of *L. ingluviei* in this study is of great significance since it was only previously isolated from pigeons and ostriches. So this marks the first case of isolation in mammals.

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