

《Research Note》

Isolation of *Lactobacillaceae* bacteria from feces of ostrich (*Struthio camelus*)

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The ostrich (*Struthio camelus*) is an herbivorous bird with a long and developed hindgut. In the hindgut, there is a dense and highly diverse population of anaerobic bacteria, and active fermentation produces high concentrations of short-chain fatty acids. Bacteria in the hindgut of the ostrich are considered vital for both their nutritional contribution and health benefits, such as benefits to the immune and defense system of the host. We attempted to isolate *Lactobacillaceae*, which might be involved in improving immune function and in inhibiting pathogens. The number of colonies from ostrich feces observed on LBS agar medium was 3.64×10^3 per gram of feces. Three strains of *Lactobacillaceae* were isolated from the feces. Nearly the entire length of the 16S ribosomal RNA gene of these isolates was sequenced, and a homology search showed high identity with *L. brevis* (identity=99.93%), *L. coryniformis* (98.39%), and *L. paracasei* (100.0%). These isolates may be deemed potential probiotics for the ostrich.

Key words: feces, health, *Lactobacillaceae*, ostrich, probiotics

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Introduction

Unlike other birds, the ostrich (*Struthio camelus*) is an herbivorous bird with a long and well-developed hindgut (Matsui *et al.*, 2010b). In its hindgut, there is a dense and highly diverse population of anaerobic bacteria (Matsui *et al.*, 2010a, b), and active fermentation produces high concentrations of short-chain fatty acids (Skadhauge *et al.*, 1984; Swart *et al.*, 1993), components which provide 76% of the metabolizable energy to the host (Swart *et al.*, 1993). Bacteria in the hindgut of the ostrich are vital for both their nutritional contribution and health benefits, such as benefits to the immune and defense systems of the host.

Among the gastrointestinal microorganisms, lactic acid bacteria, identified as *Lactobacillaceae*, contribute to the host's defense system by improving immune function and by suppressing pathogens. The genus *Lactobacillus* is now classified into 25 genera (Zheng *et al.*, 2020). If these genera can be isolated from the gastrointestinal tract of ostrich and characterized, the contribution of *Lactobacillaceae* can be clarified. Furthermore, isolates of *Lactobacillaceae* from ostrich may serve as probiotics for the animal.

The objective of this study was to isolate and identify members of the *Lactobacillaceae* from ostrich feces.

Materials and Methods

Animals and Feed

Three adult ostriches (two males and one female) reared at Hisai Norin High School (Mie Prefecture, Japan) were used in this study. The birds were fed with a self-mixed feed. The ingredients and composition of the feed are listed in Table 1. Water was provided *ad libitum*.

Sample Collection

Sample collection was performed in a non-invasive manner. Fecal samples that were released/excreted were collected at approximately 11:00 am. The fecal samples were immediately placed in a clean plastic bag and kept at 39°C. The collected samples were transferred to the laboratory within 30 minutes.

Isolation of Lactobacillaceae

Lactobacillaceae members were isolated as per protocols described by Tsuchida *et al.* (2014) with a few modifications. One gram of feces from each ostrich was diluted in 9 mL of sterilized saline and mixed thoroughly. The slurry was further diluted to 10^{-2} with sterilized saline. A portion (100 μ L) of the diluted samples was spread onto an LBS agar plate (Becton, Dickinson and Company, Sparks, MD, USA). The plates were then transferred into the BBL GasPak Anaerobic System (Becton, Dickinson and Company) and incubated anaerobically for 6 days at 39°C. Colonies on the agar plate were selected and inoculated into 2 mL of MRS broth (Becton, Dickinson and Company). The culture was then incubated for 4 d at 39°C. A full loop (10 μ L) of the culture fluid was again

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Table 1. **Ingredients and composition of the feed.**

Ingredients	Composition (%)
Maize	27.23
Wheat bran	11.98
Soybean meal	2.18
Lucerne pellet	43.57
Lucerne meal	10.89
Calcium phosphate	1.09
Calcium carbonate	2.18
Sodium chloride	0.44
Vitamin premix	0.44

streaked onto LBS agar and incubated anaerobically in a GasPak anaerobic system at 39°C. Finally, a single colony was selected, inoculated into 10 mL of MRS broth, and incubated for 4 d at 39°C.

Extraction of DNA and Sequencing

Ten milliliters of the culture fluid was centrifuged (8,000 ×g for 10 min, 4°C), and the pellet was used for genomic DNA extraction using the NucleoSpin Microbial DNA Kit (Macherey-Nagel GmbH & Co. Düren, Germany). The 16S ribosomal RNA gene fragment was amplified as per methods described in our previous study (Hattori and Matsui 2008). The reaction mixture was purified using ExoProStar (GE Healthcare, Buckinghamshire, UK). The purified DNA was sequenced with B35rev (5'-GGTATGGGATGAGCTTGC-3'; Guettler *et al.*, 1999), C01rev (5'-GGTTGCGCTCGTTGC-GGG-3'; Guettler *et al.*, 1999), and 926f (5'-AAACTCAA-GGAATTGACGG-3'; Leser *et al.*, 2002). The sequencing chemistry and sequencer used have been described previously (Abrar *et al.*, 2016). Sequenced fragments were combined into one sequence using the DNADynamo software (Blue Tractor Software Ltd, UK). The combined DNA sequence obtained was explored for homology in BlastN (Altschul *et al.*, 1997).

Results and Discussion

To the best of our knowledge, this is the first report on the isolation of *Lactobacillaceae* members from the hindgut of ostrich.

The number of colonies enumerated based on observation on the LBS agar plate was $3.64 \times 10^3 \pm 1.65 \times 10^3$ per gram of feces (mean ± SE, $n=3$). Nikravesh-Masouleh *et al.* (2018) reported that the number of *Lactobacillus* bacteria in the feces of ostrich chicks ranged between 10^6 and 10^8 colony forming units/g. This difference may be attributable to the age of the birds. Videvall *et al.* (2019) reported that the composition of *Lactobacillus* decreased with the growth of ostrich chicks. The present results support this finding. In our previous study conducted on microbial diversity in the hindgut of adult ostrich, there was no *Lactobacillaceae* clone sequence observed in the 16S ribosomal RNA gene library (Matsui *et al.*, 2010b). Therefore, *Lactobacillaceae* is a minor constituent of the adult ostrich hindgut.

Table 2. **Sequence identity of the 16S ribosomal RNA gene of isolates from ostrich feces against nucleotide database**

Isolate	Nearest known species	Identity (%)
HNost011	<i>Levilactobacillus</i> (formerly <i>Lactobacillus</i>) <i>brevis</i>	99.93
HNost031	<i>Loigolactobacillus</i> (formerly <i>Lactobacillus</i>) <i>coryniformis</i>	98.39
HNost055	<i>Enterococcus faecium</i>	100
HNost056	<i>Enterococcus faecium</i>	100
HNost057	<i>Enterococcus faecium</i>	100
HNost058	<i>Enterococcus faecium</i>	100
HNost059	<i>Enterococcus faecium</i>	100
HNost062	<i>Enterococcus faecium</i>	100
HNost065	<i>Bacillus coagulans</i>	100
HNost066	<i>Enterococcus faecium</i>	100
HNost067	<i>Bacillus coagulans</i>	99.79
HNost069	<i>Enterococcus faecium</i>	100
HNost076	<i>Bacillus coagulans</i>	100
HNost078	<i>Bacillus coagulans</i>	100
HNost082	<i>Lacticaseibacillus</i> (formerly <i>Lactobacillus</i>) <i>paracasei</i>	100
HNost085	<i>Enterococcus faecium</i>	100
HNost086	<i>Enterococcus faecium</i>	100

A total of 87 colonies were selected for analysis. Within these colonies, the partial 16S ribosomal RNA gene was sequenced using the B35rev primer of 17 isolates. Three *Lactobacillaceae* isolates (HNost011, HNost031, and HNost082) were obtained from ostrich feces (Table 2). The almost full-length 16S ribosomal RNA genes of these isolates were sequenced and searched for homology against the public database. HNost011, HNost031, and HNost082 showed high identity with *Levilactobacillus* (formerly *Lactobacillus*) *brevis* (identity=99.93%), *Loigolactobacillus* (formerly *Lactobacillus*) *coryniformis* (98.39%), and *Lacticaseibacillus* (formerly *Lactobacillus*) *paracasei* (100.0%). *Lactobacillaceae* isolates from chicken cloaca and feces were identified as *Ligilactobacillus* (formerly *Lactobacillus*) *agilis*, *Lactobacillus crispatus*, *Latilactobacillus* (formerly *Lactobacillus*) *curvatus*, *Limosilactobacillus* (formerly *Lactobacillus*) *fermentum*, *Lactobacillus gallinarum*, *Limosilactobacillus* (formerly *Lactobacillus*) *ingluviei*, *Lactobacillus johnsonii*, *Lactobacillus kitasatonis*, *Lactiplantibacillus* (formerly *Lactobacillus*) *plantarum*, *Ligilactobacillus* (formerly *Lactobacillus*) *salivarius*, and *Limosilactobacillus* (formerly *Lactobacillus*) *vaginalis* (Kobierecka *et al.*, 2017). Experimentally, *L. plantarum* showed the ability to decrease colonization of the *Campylobacter jejuni* strain (Kobierecka *et al.*, 2017). None of the ostrich isolates overlapped with the isolates from chickens. One reason for this is the small number of isolated *Lactobacillaceae* members. Isolation of more *Lactobacillaceae* members should be performed in the future.

The body weight (BW) and BW gain of ostrich chicks that received commercial probiotics (not for ostrich) were higher than those of chicks fed with a control diet (Karimi-Kivi *et*

al., 2015). Moreover, the abundance of *Lactobacillaceae* was significantly higher in chickens in the better feed efficiency group (Yan *et al.*, 2017). If the development of probiotics designated for ostrich can be achieved, ostrich productivity may be improved. The *Lactobacillaceae* isolates in the present study may be deemed potential candidates for this purpose.

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Conflicts of Interest

The authors declare no conflict of interest.

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