Isolation of Potential Novel Probiotic Bacteria from Canada Goose Feces

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ABSTRACT

Removal of antibiotics during food-animal production, e.g. poultry, will require developing innovative strategies to maintain animal health and optimize nutrition. Utilizing naturally occurring bacteria as probiotics has several advantages including viability, ability to compete against ecologically similar taxa and a natural rationale for regulatory approval. Indigenous species of spore-forming, fiber-fermenting bacteria promote anti-inflammatory responses in the mammalian gut by activating immune cells and these bacteria make up a large proportion of the monogastric animal gastrointestinal microflora. Enriching avian feces for chloroform-resistant bacteria will select for bacterial spores that represent potentially non-toxin producing bacteria that could be utilized as probiotics for poultry or another avian species. Therefore, our hypothesis is that selecting spore-forming bacterial taxa closely related to known pathogens offers potential for competitive exclusion of pathogenic bacteria. Also, isolation of potential probiotic bacteria from a variety of avian species could be of value for commercial poultry production and the minimal result will be discovery of previously undiscovered bacteria. Three percent chloroform treatment of Canada goose (*Branta canadensis*) feces was completed for one hour to remove vegetative bacterial cells, followed by anaerobic and aerobic culture of surviving spores. Twenty-six axenic, endospore containing isolates were obtained that were distinct from one another morphologically and phenotypically based on Gram staining. Twelve anaerobic Gram positive (3) and negative (9) isolates were obtained by culturing on Brucella blood with vitamin K and hemin or reinforced clostridial hiveg hydrolysate with L-cysteine, Na acetate and starch agars. Fourteen aerobic Gram positive (4) and negative (10) isolates were cultured using the two aforementioned agars and nine (9) aerobes could subsequently be propagated on lysogeny broth (LB). Results from these investigations further demonstrate that newly identified, potential probiotic bacterial cultures can be isolated from free-ranging species and identified for future use to improve animal health.

SUMMARY & METHODS

Indigenous species of non-toxin producing anaerobic bacteria (Gram-positive spore-forming *Clostridium* spp.) promote anti-inflammatory immune responses in the mammalian gut by activating T-regulatory cells and these bacteria make up a large proportion of the monogastric animal intestinal microflora (Atarashi et al., 2011). Rational selection of an anaerobic spore-forming mixture of bacteria utilizing chloroform extraction of mouse feces was completed to develop seventeen (17) strains of bacteria that can be orally administered to mice for attenuating colitis and allergic diarrhea (Atarashi et al., 2013; Narushima et al. 2014).

Fresh feces was suspended in phosphate-buffered saline (PBS; Wise and Siragusa, 2007) from geese, then treated with 3% chloroform for 30 mins to eliminate vegetative bacterial cells (Itoh and Mitsuoka, 1980; Atarashi et al., 2011, 2013; Honda K, personal communication; van Asselt and Zwietering, Int J Food Microbiol, 2006), then cultured under anaerobic conditions with sachets (Oxoid, AnaeroGenTM) for four days or aerobically for two days utilizing brucella agar with blood and vitamin K-hemin (BBHK) and reinforced clostridial agar with L-cysteine, Na acetate, starch without polymyxin B (Chapin and Murray, 1999).

RESULTS

Aerobic Bacterial Isolates OSU Cascades Biology:

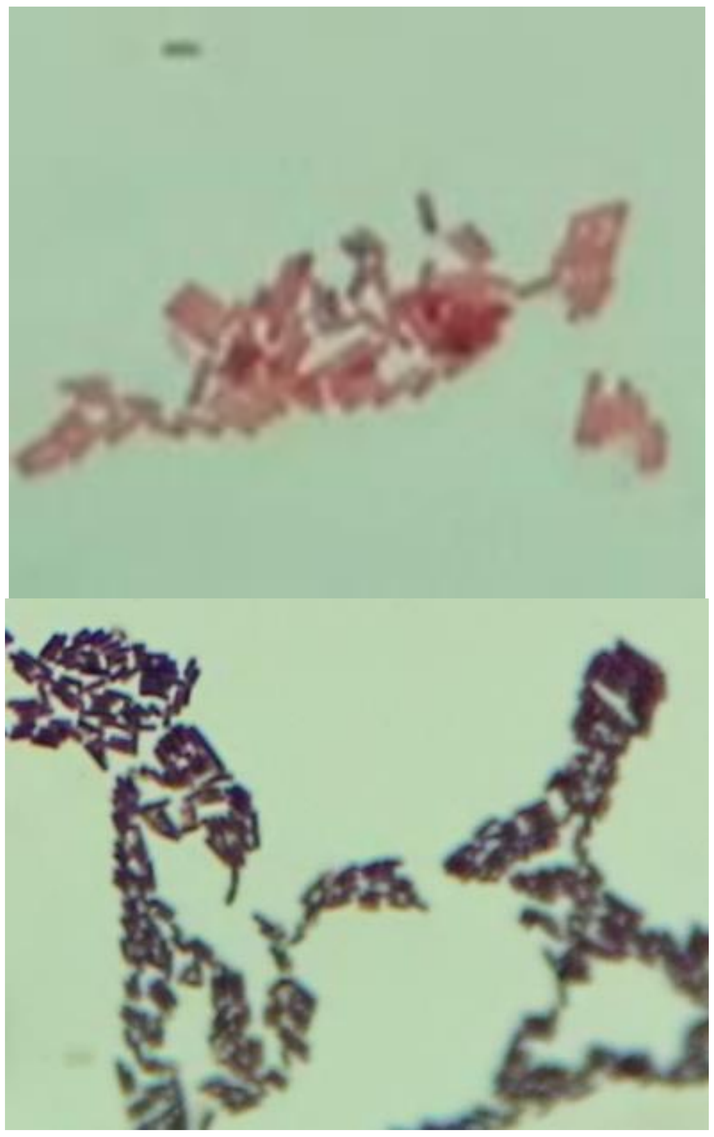
Isolate No.	Gram Stain	TSA/BBHK[1]	Clostridial[2]	LB[3]	Morphology
01	Pos	Yes	Yes	Yes	1 & 2 similar
02	Neg	Yes	Yes	Yes	
04	Neg	Yes	Yes; slow	No	Small Colonies
06	Pos	Yes	Yes, slow	No	Small, H α
07	Neg	Yes	Yes	Yes	
08	Neg	Yes	Yes	Yes	Chromogenic
09	Neg	Yes	Yes	Yes	Small Colonies
10*	Neg	Yes	Yes	Yes	Small
11*	Neg	Yes	No	No	Motile?
12a	Pos	Yes	?	Yes	Motile?
12b	Pos	Yes	?	Yes	Bkg Gram Pos
13	Neg	Yes	Yes	No	Small
14	Neg	Yes	Yes	Yes	Small
15	Neg	Yes	Yes	No	Small, H α

[1]BBHK – Brucella blood w/ Vitamin K and Hemin, VWR source from Hardy Diagnostics, TSA - tryptone soy agar

[2]Clostridial – Reinforced clostridial agar hiveg hydrolysate w/ L-cysteine, Na acetate, starch
NOTE: VWR source is the HIMEDIA version

[3]LB – Standard 'Lysogeny Broth' (LB) commonly utilized in the lab for propagating bacteria; See: https://en.wikipedia.org/wiki/Lysogeny_broth

Representative Aerobe Gram Stains



Gram staining was completed by standard procedures following aerobic culture

Anaerobic Bacterial Isolates OSU Cascades Biology:

Isolate No.	Gram Stain	BBHK[1]	Clostridial[2]	LB[3]	Morphology
01	Positive	Yes	No	No	Hemolysis (H) β
02	Positive	Yes	Low	No	H β
03	Positive	Yes	Low	No	H β
04	Negative	Yes	No	No	Small, H α
05	Negative	Yes	Yes	No	H α
06	Negative	Yes	Yes	No	H α
07	Negative	Yes	Yes	No	H α
08	Pos/Neg	Yes	No	No	H α
09	Negative	Yes	Yes	No	H α
10	Negative	Yes	Yes	No	H α
11*	Negative	Yes	No	No	H β
12*	Negative	Yes	No	No	H β

*Passage resulted in very little growth; most recent passaged plates back in the incubator and new passage on to BBHK from original plates, incubated from 28 to 30 June.

Clostridium acetobutylicum, Gram-positive bacillus, non-H (control)

Clostridium butyricum, Gram-positive bacillus, non-H; used as a probiotic (control)

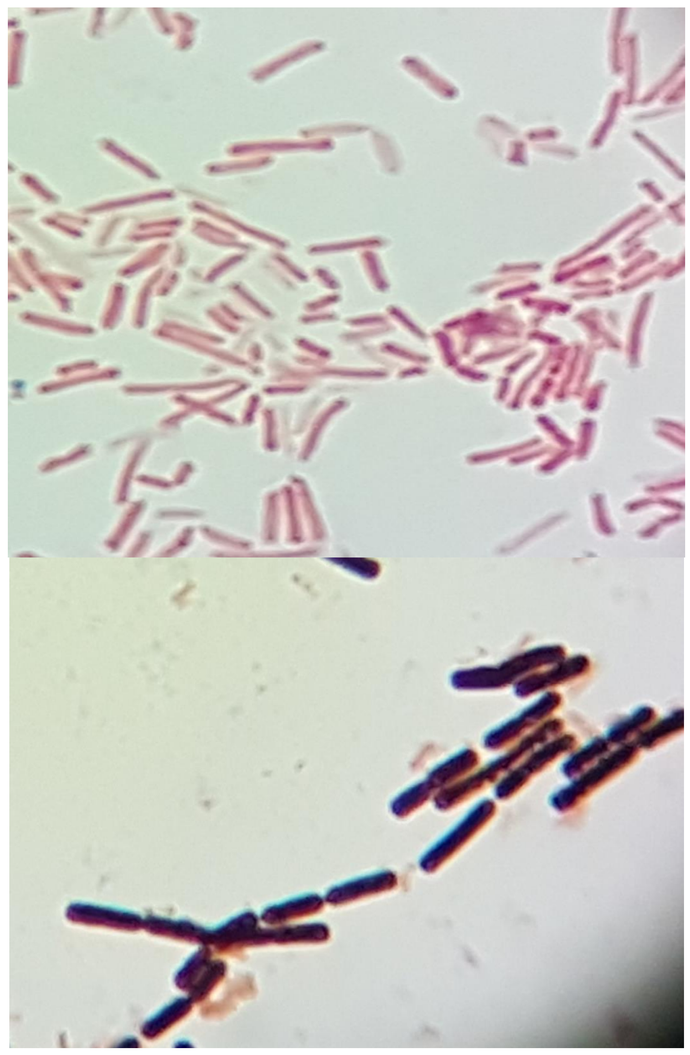
[1]BBHK – Brucella blood w/ Vitamin K and Hemin, VWR source from Hardy Diagnostics

[2]Clostridial – Reinforced clostridial agar hiveg hydrolysate w/ L-cysteine, Na acetate, starch
NOTE: VWR source is the HIMEDIA version

[3]LB – Standard 'Lysogeny Broth' (LB) commonly utilized in the lab for propagating bacteria; See: https://en.wikipedia.org/wiki/Lysogeny_broth

RESULTS CONTINUED

Representative Anaerobe Gram Stains



Gram staining was completed by standard procedures following anaerobic culture

CONCLUSIONS

Although bacteria have been identified in geese (Wang et al., 2016), there are a variety of both Gram-negative (Yutin and Galperin, 2013) and Gram-positive (Galperin, 2012) bacteria that remain to be further characterized in culture.

This led us to *'bio-prospect'* for potential new probiotic spore-forming bacteria from a free-ranging goose species. The next step is to identify these isolates by 16S rRNA gene sequencing and assay for inhibitory properties to known pathogenic microbes.

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