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Study on the functional properties of potential indigenous probiotics isolated from human samples in West Africa

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ABSTRACT

A study was performed to isolate and evaluate potential indigenous probiotic strains in Ghana. A total of 99 strains were isolated from human breast milk (n = 29) and fecal samples (n = 70), which were identified and characterized using methods ranging from plating growth tests and presumptive analysis at species level using MALDI-TOF MS, prior to validation through 16S rRNA gene sequence analysis. Gastrointestinal tolerance and ability to form biofilms *in vitro* were determined. Results indicated that out of 99 isolates, 25 were Gram-positive, catalase-negative rods. More than one-third was identified as *Lactobacillus fermentum.* Others were identified to be *Lactobacillus (plantarum*, *rhamnosus*, *salivarius*, *reuteri), Enterococcus faecium*, *Weissella* spp. and *Pediococcus* spp. Among the 25 isolates, 9 had activity against both Gram-negative and Gram-positive pathogens including reference or local clinical isolates of *Pseudomonas aeruginosa, Salmonella* Typhi, *Staphylococcus aureus* and *Escherichia coli*. Almost all isolates were effective against *P. aeruginosa, E. coli* and *S.* Typhi. Thirteen isolates did not show activity towards *S. aureus*. The isolates were more effective against local pathogens tested than nonindigenous pathogens. The data showed survival of all studied isolate at pH 2, 3 and 6 followed by a successful growth of the co-cultured biofilm in a 3D Alvatex platform.

1. Introduction

During the 20th century, little was known about the beneficial attributes of some commensal bacteria before the noble laureate Elie Metchninkoff introduced the concept of probiotics and their boosting effects on human health (Metchnikoff, 1908). In view of this original contribution, various studies have then been implemented by microbiome researchers including the World Health Organization (WHO) to ascertain the health benefits of probiotics to the host. Among commensal microorganisms pertaining to fermented food and dairy industry (i.e., Yogurt, Kefir and cheese), lactic acid bacteria (LAB), primarily species from the *Lactobacillus* genera, have been extensively used for their fermentative ability and probiotic potential (FAO/WHO, 2001; Linares, Ross, & Stanton, 2016). Within LABs, some *Lactobacillus* strains constitute the most prevalent components of the gastrointestinal tract (gut) and vaginal microbiota (Metchnikoff, 1908; Westerik, Kort, Sybesma, & Reid, 2018). These strains are able to form biofilms in the host cells and protect them from infective bacteria such as mastitis pathogens, through producing bacteriocins, which act as antimicrobials, in addition to hydrogen peroxide and lactic acid (Ocana & Nader-Macias, 2004). The most common species of *Lactobacillus* used in food have been extensively documented (Salas-Jara, Ilabaca, Vega, & Garcia, 2016; Tamang, Watanabe, & Holzapfel, 2016). Furthermore, *Lactobacillus rhamnosus* GG ATCC 53103 (Valio), *Lactobacillus rhamnosus* GR-1, *Lactobacillus paracasei* Shirota (Yakult) along with *Bifidobacterium lactis* BB12 (Chr. Hansen) are the world's most documented probiotics, which also dominate the probiotic market (Linares et al., 2016). These species originating from America, Asia and Europe have shown high efficacy in the management of lactose intolerance, immune response modulation, protection against *Clostridium difficile*, *Helicobacter pylori* infections, diarrhea, urogenital, gut and respiratory tract infections (Linares et al., 2016; Westerik et al., 2018).

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Although these probiotics have also been extensively applied in Africa (Bisanz et al., 2014; Nduti et al., 2016), there is an unmet need to isolate indigenous strains, which have adapted to local conditions and have potential to be more specific to the local population (Mokoena, Mutanda, & Olaniran, 2016). For instance, *L. rhamnosus* GR-1 was demonstrated by Bisanz et al. (2014) to have a protective effect against further increases in heavy metal levels in pregnant women with high toxic metal exposure in Tanzania. *L. rhamnosus* GR-1 was also shown to reduce aflatoxin concentrations in urine among eastern Kenya school children when used together with locally isolated *Weisella cibaria* NN20 and *Streptococcus thermophiles* (Nduti et al., 2016). However, there is sparse data on indigenous probiotics in Africa, which has a less established market for probiotics relative to developed countries. In Ghana, there is limited information on indigenous probiotic microorganism isolated and documented (Owusu-Kwarteng, Tano-Debrah, Akabanda, & Jespersen, 2015). We believe that the indigenous flora has a high potential to be valuable in the management of enteric infections, boost immunity, and generally improve the wellbeing of local population that are often exposed to poor hygiene condition, malnutrition, and chronic enteric infections. Moreover, with our understanding of how an individual's genetics, environment, and diet influence their microbiota and the current trend towards personalized medicine (Vogenberg, Isaacson Barash, & Pursel, 2010), it has become very important to identify local strains, those which predominate in the specific population. This avoids the one-size-fits-all approach and generalization of the effectiveness of probiotic species, which often results in lack of efficacy (Allen et al., 2013; Ritchie & Romanuk, 2012). Locally sourced probiotics are of prime interest pertaining to their immediate availability in the local market and high antimicrobial specificity to residents' host cells.

The objective of this study is to isolate, identify and characterize the functional properties of local potential probiotics from human feces and breast milk samples in Ghana.

2. Materials and methods

2.1. Isolation and phenotypic identification of LAB species

The experimental protocol was approved by the College of Health Sciences Ethical and Protocol Review Committee, University of Ghana (Protocol Identification Number: CHS–Et/M.2-P5.15/2018–2019). Fecal samples were obtained from five adults, one adolescent, three children, and three infants. Milk was obtained from six breastfeeding mothers. 1 g of fecal sample was homogenized in 10 mL of phosphate buffered saline (PBS, pH 7.4) using a vortex. A loopful of the fecal suspension and breast milk sample was streaked onto separate de Man Rogosa Sharpe (MRS) agar (Oxoid) supplemented with 0.05% w/v L-cysteine hydrochloride and 0.002% w/v of bromophenol blue and incubated anaerobically at 37 °C for 48 h. Colonies with different morphological characteristics were picked from the agar plates and cultured on de Man Rogosa Sharpe (MRS) agar supplemented with 0.05% w/v L-cysteine hydrochloride (MRSc). The plates were incubated at 37 °C for 48 h anaerobically. Pure colonies obtained were Gram-stained and observed under the microscope for morphological characteristics. Biochemical characteristic of the isolates was performed using the catalase test. Colonies which were Gram-positive and catalase negative were selected for further studies.

2.2. Identification of isolates using MALDI-TOF MS

Isolates were identified by Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) using the MALDI Biotyper®. Briefly, a single colony of freshly grown isolated bacteria was smeared as a film onto individual spots on MALDI-target plate using 1 μL disposable loop and allowed to air dry. The film was overlaid with 1 μL of a 98% formic acid solution to allow on-plate extraction of cellular proteins. After drying, the film was further overlaid with 1 μL of a MALDI-TOF MS matrix comprising of α-cyano-4-hydroxycinnamic acid (CHCA) in 50% acetonitrile and 2.5% trifluoroacetic acid. The sample was further air-dried for 1–2 min and analyzed with the Bruker Microflex LT bench top MALDI-TOF mass spectrometer (Bruker Daltonics, Billerica, MA, USA), Bruker FlexControl 3.0 software, and MALDI Biotyper 3.1 software (Bruker Daltonics, Billerica, MA, USA). The reference strain was *Escherichia coli* ATCC 8739. Mass spectra for each spotted bacterial isolate was acquired with the instrument in a linear positive mode within a 2–20 kDa range, with ion source 1.0 at 20 kV, ion source 2.0 at 18.05 kV, the lens at 6.0 kV, and the linear detector at 2560 V. Mass spectra was analyzed and compared with the MALDI Biotyper 3.1 software database, comprised of 4970 distinct bacterial species, to determine the most likely microbial genus and species identification. A MALDI Biotyper score, generated as a level of probability by the software, of $\geq$1.7 was utilized as a threshold for reliable species identification, as recommended for assessment of anaerobic bacteria (Hsu & Burnham, 2014). The MALDI-TOF MS has previously been used for bacterial identification and established as an alternative to bacterial identification providing rapid determination (McElvania TeKippe and Burnham, 2014; Levesque et al., 2015; Mc).

2.3. Identification of isolates using 16S rRNA gene sequence analysis

The genomic DNA of the isolated bacteria was extracted using the GenElute™ Bacterial Genomic DNA Kit (Sigma) according to the manufacturer's protocol. The 16S rRNA gene was amplified using the following primers: 8F, 50-AGAGTTTGATCCTGGCTCAG-30, and 1492R, 50-GGTTACCTTGTTACGACTT-30. PCR was performed using the MJ Mini™ Thermal Cycler (Bio-Rad). The reaction contained in 50 μL total volume, 0.5 μM of each primer (0.5 μL of each), 1 μL of genomic DNA, 25 μL GoTaq® Master mix (Promega; reaction buffer, 400 μM dATP, 400 μM dGTP, 400 μM dCTP, 400 μM dTTP, 3 mM $MgCl_2$) and 23 μL RNase free water. The PCR was run under an initial activation at 94 °C for 2 min, followed by 30 cycles of denaturation step cycles at 94 °C for 30 s, an annealing step at 55 °C for 1 min, extension step at 72 °C for 1 min and final cycle at 72 °C for 10 min. 9 μL aliquots of the PCR samples were subjected to electrophoresis in 0.8% w/v agarose gel (stained with 0.5 μg/mL ethidium bromide), in TAE buffer. The gel was visualized under UV illumination and photographed. The PCR samples were further purified and sequenced. The sequences were analyzed and submitted to a search for similarity in the National Center for Biotechnology Information (NCBI) database using the nucleotide database tool (blastn) and the 16S rRNA sequence compared to known sequences in the NCBI Genbank database.

2.4. Antimicrobial activity of isolates

Antimicrobial activity of the isolated species was tested by the agar well diffusion assay against *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *E. coli* BAA-2471 (multi-drug resistant strain), *Salmonella* Typhi (local clinical isolate), *Pseudomonas aeruginosa* (local clinical isolate) and *P. aeruginosa* ATCC 10145. Briefly, 24 h culture of the isolated species in MRS broth supplemented with 0.05% w/v L-cysteine hydrochloride were centrifuged at 3500 *g* at 4 °C. The supernatant was collected and filter sterilized using a 0.2 μm membrane syringe filter. A lawn of each test microorganism was made by picking bacterial colonies and spreading on Mannitol Salt agar (Oxoid), MacConkey agar (Oxoid), Cetrimide agar (Oxoid), Bismuth sulphite agar (Oxoid) or Tryptic Soy agar (Sigma-Aldrich). Wells of 9 mm in diameter were made in the agar and filled with 150 μL of the filter-sterilized supernatant. The plates were kept on the bench for 2 h for diffusion of the supernatant and then incubated at 37 °C. Zones of inhibition were recorded after 48 h of incubation.

2.5. Gastrointestinal tolerance assay

Four different isolates belonging to different species from the *Lactobacillus* genus with antagonistic activity against both Gram-positive and Gram-negative tested pathogens were used for this study and subsequent study. Tolerance of the selected isolated species to simulated gastric fluid and bile salt were performed by inoculating 100 μL of a culture of each species in 1 mL solution pH adjusted (HCl/NaOH) to 1, 2, 3 and 6 and 0.3% w/v bile salt (Sigma-Aldrich). The cells were incubated at 37 °C and sample taken at 1 h, 2 h and 3 h, serially diluted in PBS (pH 7.4) and spread-plated on MRSc. Samples for bile salt test were further sampled at 4 h. Colonies were counted after incubation at 37 °C for 48 h.

2.6. Biofilm assay

The ability of selected potential species to form a successful biofilm growth *in vitro* was evaluated. The Alvatex strata 3D scaffold inserts (ReproCELL Europe Ltd., Glasgow, United Kingdom) were used for simulating the structural matrix found in the gut and mimicking *in vivo* biofilm growth of the bacteria. Each species was grown separately in MRS broth supplemented with 0.05% w/v L-cysteine hydrochloride. The 3D scaffold inserts were placed carefully into wells of a 24 well plate. The scaffolds were washed with 400 μL of 200-proof ethanol, then 550 μL of sterile PBS. 550 μL of sterile PBS was again poured carefully into the wells making sure the 3D fibrous scaffolds lay flat without any folding. The 3D fibrous scaffolds were UV sterilized for 45 min. The PBS was removed carefully from the wells and replaced with 550 μL of sterile MRS broth supplemented with 0.05% w/v L-cysteine hydrochloride, which was also carefully removed. 400 μL bacterial culture were inoculated into the wells containing the UV sterilized 3D fibrous scaffold and incubated at 37 °C for 5 days with addition of the broth as necessary. At the end of incubation the biofilms were stained with FilmTracer™ LIVE/DEAD™ (Invitrogen™) Biofilm Viability kit (a two-colour fluorescence assay of bacterial viability: SYTO® 9 green fluorescent nucleic acid stain and red-fluorescent nucleic acid stain, propidium iodide) and imaged using EVOS® FL Cell Imaging System at 40X resolution.

3. Results

3.1. Isolation and identification of isolates

Several isolates were obtained from fecal (adults, adolescent, children and infants) and breast milk samples on MRSc media. The isolates were Gram-stained, analyzed by microscopic observation and catalase test. To ensure that only species belonging to the lactic acid bacteria (LAB) or *Bifidobacterium* genera, which constitute the most important taxa of probiotics were selected, only Gram-positive, catalase negative rods were used for further analysis. Out of 99 colonies isolated including 29 colonies from breast milk samples and 70 from fecal samples, 25 were Gram-positive rods and catalase negative. The 25 Gram-positive catalase negative rods were submitted to MALDI-TOF MS and 16S rRNA gene sequencing analysis (Table 1). More than 80% of the isolated species were identified by MALDI-TOF MS or 16S rRNA gene sequencing as belonging to *Lactobacillus* genus. More than one-third of the species were identified as *L. fermentum* by both identification methods. Other species isolated included *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Lactobacillus salivarius*, *Lactobacillus reuteri*, *Enterococcus faecium*, *Weissella* spp. *and Pediococcus* spp. The congruency between the MALDI-TOF MS and 16S rRNA gene sequencing methods was almost 70%. Isolates FSC2-DC and FSC2-L were identified by both methods as *Weissella* and *Pediococcus* species respectively. However the *Weissella* species was identified as *Weissella confusa* with MALDI-TOF MS and *Weissella cibaria* with 16S rRNA gene sequencing whilst the *Pediococcus* species was identified as *Pediococcus pentosaceus* with MALDI-TOF MS and *Pediococcus acidilactici* with 16S rRNA gene sequencing. Another isolate identified as *L. reuteri* by MALDI-TOF MS was identified as *L. fermentum* by 16S rRNA gene sequencing whilst a *L. plantarum* was identified as *L. rhamnosus*.

3.2. Antimicrobial activity of isolates

The antimicrobial activities of isolated species were tested against Gram-negative *P. aeruginosa* (ATCC 10145 and local clinical isolate), *E. coli* (ATCC 25922 and BAA-2471 multi-drug resistant), *Salmonella* Typhi (local clinical isolate) and Gram-positive *S. aureus* (ATCC 29213).

Table 1
Identification of isolates.

Number	Source	Isolate	MALDI-TOF	Biotyper log score	16S rRNA gene	Sequence Identity
1	Feces of Adult	FSD1-D	*Lactobacillus salivarius*	1.78	[b]	
2	Feces of Adult	FSD2-TC	*Enterococcus faecium*	2.24	*Enterococcus faecium*	94.00%
3	Feces of Adult	FSD3-WC	*Lactobacillus plantarum*	1.97	*Lactobacillus plantarum*	98.75%
4	Feces of Adult	FSD3-LBC	*Lactobacillus plantarum*	2.07	*Lactobacillus rhamnosus*	98.54%
5	Feces of Adult	FSD3-DT	*Lactobacillus fermentum*	1.74	*Lactobacillus fermentum*	98.15%
6	Feces of Adult	FSD4-D	*Lactobacillus fermentum*	1.76	*Lactobacillus fermentum*	98.95%
7	Feces of Adult	FSD4-I	*Lactobacillus fermentum*	1.71	[a]	
8	Feces of Child	FSC2-DC	*Weissella confusa*	1.91	*Weisella cibaria*	86.26%
9	Feces of Child	FSC2-L	*Pediococcus pentosaceus*	2.14	*Pediococcus acidilactici*	94.85%
10	Feces of Child	FSC3-L	*Lactobacillus fermentum*	1.94	*Lactobacillus fermentum*	98.00%
11	Feces of Child	FSC3-D	*Lactobacillus fermentum*	1.95	*Lactobacillus fermentum*	99.43%
12	Feces of Child	FSC3-LBC	*Lactobacillus plantarum*	2.11	*Lactobacillus plantarum*	92.30%
13	Feces of Infant	FSI1-D	*Lactobacillus reuteri*	1.91	*Lactobacillus fermentum*	97.75%
14	Feces of Infant	FSI2-L	*Lactobacillus fermentum*	1.83	*Lactobacillus fermentum*	98.34%
15	Feces of Adult	FSI2-DT	*Lactobacillus fermentum*	1.70	*Lactobacillus fermentum*	98.86%
16	Feces of Infant	FSI2-D	*Lactobacillus fermentum*	1.71	*Lactobacillus fermentum*	98.86%
17	Feces of Infant	FSI3-D	*Lactobacillus fermentum*	1.76	*Lactobacillus fermentum*	97.52%
18	Feces of Infant	FSI3-LBC	[b]		*Lactobacillus fermentum*	98.32%
19	Feces of Infant	FSI3-L	*Lactobacillus fermentum*	1.78	*Lactobacillus fermentum*	98.70%
20	Feces of Adolescent	FSA1-TC	*Enterococcus fecium*	2.22	[a]	
21	Feces of Adolescent	FSA1-L	[b]		[a]	
22	Breast Milk	BMS5-D	*Lactobacillus fermentum*	1.86	*Lactobacillus fermentum*	98.49%
23	Breast Milk	BMS5-LBC	*Lactobacillus fermentum*	1.84	*Lactobacillus fermentum*	98.58%
24	Breast Milk	BMS6-D	[b]		*Lactobacillus fermentum*	98.85%
25	Breast Milk	BMS6-DBC	*Weissella confusa*	1.85	*Lactobacillus fermentum*	98.49%

[a] Undetermined.
[b] unidentified species.

Only 9 out of 25 isolates had activity against both Gram-negative and Gram-positive tested bacteria, Table 2. Isolates FSD1-D and FSI3-L showed highest activity against both the Gram-negative and Gram-positive bacteria, followed by FSD4-D, FSC3-L and FSD3-WC. All tested isolates had activity against *P. aeruginosa* and *E. coli*. Only two isolates, FSD4-I and BMS6-DBC did not show activity against *Salmonella* Typhi. Also 13 isolates (almost 60%) did not show zone of inhibition towards *S. aureus*. Isolate FSI3-L had highest activity against *S. aureus* whereas FSD1-D had highest activity against *Salmonella* Typhi. The relative activity of the isolates against *P. aeruginosa* was greater in the local isolate than the reference strain. The zone of inhibition against the multi-drug resistant *E. coli* strain was correspondingly smaller than in the reference strain.

3.3. Gastrointestinal tolerance assay

The viable counts of selected isolates to pH 1, 2, 3, 6 and 0.3% bile salt concentration are shown in Tables 3 and 4. The data shows maintained cell viability for all studied isolate at pH 2, 3 and 6. However, all isolates lost total viability during the acid test at pH 1. The exposure of the isolates to 0.3% bile salt concentration showed general maintenance of viability over the 4 h exposure period, Table 4.

3.4. Biofilm assay

The ability of selected isolates to form biofilm was evaluated using the Alvatex 3D scaffold insert (Supplementary data Fig. 1). The confocal imaging (Supplementary data Fig. 1b) of the 3D scaffold at 60x magnification shows randomly aligned fibers associating to form a fibrous mesh. The 3D scaffold was explored to simulate the structural matrix found in the gut and mimic the *in vivo* biofilm growth of bacteria. All tested species formed biofilm on the 3D scaffold (Supplementary data Fig. 2). The biofilm formed for all species comprised of both live and dead cells. All scaffolds maintained integrity during the period of assay except for scaffold used for culturing isolate FSD1-D.

4. Discussion

In the present investigation, we obtained ninety-nine morphologically distinct isolates from breast milk and fecal samples, which were subjected to Gram stain test and catalase test to obtain potential indigenous probiotic microorganisms, which are mainly species from the lactic acid (LAB) or *Bifidobacterium* genera. Out of the isolates determined to be Gram-positive, catalase negative, twenty-four were identified as *L. fermentum* (14), *L. plantarum* (2), *L. rhamnosus* (1), *L. salivarius* (1), *L. reuteri* (1), *Enterococcus faecium* (2), *Weissella* spp. (2) and *Pediococcus* spp. (1) using the MALDI-TOF MS and 16S rRNA gene sequencing analysis. All the identified isolates belonged to the lactic acid group of bacteria. The predominant species in both the breast milk and fecal samples was *L. fermentum*. Other studies have reported a similar profile for fecal samples (Mandal, Jariwala, & Bagchi, 2016), more diverse profile for breast milk samples (Damaceno et al., 2017; Khalkhali & Mojgani, 2017) or only one type of bacteria from breast milk (Martin et al., 2003; Rajoka et al., 2017).

16S rRNA gene sequencing analysis is often considered the gold standard for identification of bacteria and it is commonly combined with phenotypic methods/biochemical fermentation strips for identification of LAB, with the latter methods, streamlining the number of species to be identified. It has been previously suggested to provide more than 90% genus identification and 65%–83% in species identification (Janda & Abbott, 2007; Mignard & Flandrois, 2006). However, for routine use in laboratories, 16S rRNA gene sequencing analysis is limited by its time consuming nature and cost. MALDI-TOF MS offers the advantage of a rapid turn-around time and minimal cost and has been previously demonstrated to be highly congruent (86.1%) to 16S rRNA gene sequencing analysis (Garcia et al., 2016). The MALDI-TOF MS also requires no extraction of nucleic acids or sequencing steps but limited by the need for a fresh culture and conceivably a restricted MS-profile database relative to the 16S rRNA gene sequencing method. The present data suggests a good congruency (approximately 70%) between the two methods at the genus level and 84.62% for the identification of *L. fermentum*. However poor congruency was obtained for some species, which indicates limiting agreement between the two methods at the species level. For instance, *Weisella confusa* and *Weisella cibaria* very closely related species with very high 16S rDNA sequence similarity which have been previously misidentified (Bjorkroth et al., 2002) could not be differentiated by the two methods in the present study. *Pediococcus pentosaceus* and *Pediococcus acidilactici* which are also highly

Table 2
Antimicrobial activity of isolates.

No	ISOLATE	Antimicrobial activity (zone of inhibition in mm)					
		P. aeruginosa (clinical isolate)	*P. aeruginosa* ATCC 10145	*E. coli* ATCC 25922	*E. coli* (multi-drug resistant)	***S. typhi* (clinical isolate)**	***S. aureus* ATCC 29213**
1	FSD1-D	14.3 ± 3.4	7.7 ± 2.1	10.3 ± 1.0	4.67 ± 0.6	14 ± 1.4	9.75 ± 2.2
2	FSD2-TC	8.8 ± 3.4	7.4 ± 1.3	11 ± 0.8	5.8 ± 0.9	10.5 ± 0.7	0
3	FSD3-WC	12.5 ± 3.4	8.7 ± 1.2	10.5 ± 1.7	7.0 ± 0.0	13.5 ± 0.7	8.5 ± 0.7
4	FSD3-LBC	12.3 ± 4.4	8.7 ± 1.2	10.5 ± 0.6	3.7 ± 1.2	12 ± 1.4	7 ± 2.64
5	FSD3-DT	10.5 ± 3.7	5.3 ± 0.6	11.3 ± 0.5	4.3 ± 1.5	11 ± 1.4	0
6	FSD4-D	12.5 ± 4.5	7.3 ± 0.6	10.6 ± 0.5	5.3 ± 0.6	12 ± 1.4	10.5 ± 0.7
7	FSD4-I	9.0 ± 0.0	9.0 ± 1.0	11.3 ± 0.35	5.0 ± 1.0	0	0
8	FSC2-DC	7.7 ± 2.9	3.7 ± 2.1	11 ± 0.0	2.3 ± 1.5	10.5 ± 0.7	0
9	FSC2-L	9.0 ± 0.0	8.3 ± 1.5	10.3 ± 1	6.0 ± 1.0	11.5 ± 0.7	0
10	FSC3-L	12.8 ± 3.9	7.7 ± 0.6	12.5 ± 4.4	5.7 ± 0.6	10.5 ± 0.7	11 ± 1.4
11	FSC3-D	10.3 ± 2.1	7.7 ± 1.2	11.5 ± 0.6	2.3 ± 1.5	11.5 ± 0.7	0
12	FSC3-LBC	8.8 ± 4.3	8.7 ± 0.6	13.8 ± 3.3	5.3 ± 0.6	11 ± 1.4	10.5 ± 0.7
13	FSI1-D	7.8 ± 3.2	9.0 ± 1.0	10.6 ± 0.9	3.7 ± 0.6	10.5 ± 0.7	4.25 ± 1.06
14	FSI2-L	10.3 ± 5.1	9.0 ± 1.0	12.0 ± 2.2	6.7 ± 0.6	12 ± 0	0
15	FSI2-DT	10.4 ± 2.5	7.3 ± 1.2	10.0 ± 0.8	4.7 ± 0.6	10.0 ± 0.0	0
16	FSI2-D	10.1 ± 3.9	8.3 ± 1.2	11.4 ± 1.4	4.7 ± 1.2	13.5 ± 0.7	0
17	FSI3-D	7.5 ± 5.1	8.3 ± 0.6	9.8 ± 1.0	3.7 ± 0.6	10.5 ± 0.7	3.0 ± 4.24
19	FSI3-L	12.3 ± 3.8	5.3 ± 0.6	12.8 ± 2.9	4.0 ± 1.7	11 ± 1.4	13 ± 1.4
20	FSA1-TC	8 ± 4.6	*	11.8 ± 1.0	*	11 ± 1.4	0
22	BMS5-D	11.8 ± 4.1	7.3 ± 0.6	11.1 ± 0.9	6.3 ± 0.6	13.5 ± 2.1	0
23	BMS5-LBC	10.5 ± 1.3	6.7 ± 0.6	11.0 ± 0.8	6.7 ± 0.6	10.5 ± 0.7	0
25	BMS6-DBC	7.5 ± 0.7	8.0 ± 1.0	10.0 ± 0.0	5.7 ± 0.6	0	0

Table 3
Gastric tolerance of selected isolates.

Isolate	Log CFU/mL											
	Pre-acid tolerance	pH 1			pH 2			pH 3			pH 6		
	0 h	1 h	2 h	3 h	1 h	2 h	3 h	1 h	2 h	3 h	1 h	2 h	3 h
FSI1-D	8.21 ± 0.18	0	0	0	8.20 ± 0.11	8.15 ± 0.17	8.25 ± 0.11	8.24 ± 0.21	7.94 ± 0.14	8.05 ± 0.04	7.90 ± 0.12	8.18 ± 0.11	8.44 ± 0.22
FSI3-D	7.98 ± 0.03	0	0	0	7.81 ± 0.07	7.86 ± 0.06	7.80 ± 0.10	8.01 ± 0.27	8.01 ± 0.07	8.09 ± 0.03	8.02 ± 0.20	8.05 ± 0.14	7.98 ± 0.03
FSD1-D	8.11 ± 0.16	0	0	0	8.01 ± 0.05	8.01 ± 0.03	7.95 ± 0.01	8.32 ± 0.24	7.95 ± 0.34	8.18 ± 0.21	7.94 ± 0.19	8.06 ± 0.13	8.20 ± 0.14
FSC3-LBC	8.02 ± 0.24	0	0	0	8.14 ± 0.08	7.98 ± 0.06	7.90 ± 0.10	7.90 ± 0.12	8.02 ± 0.20	7.94 ± 0.19	7.82 ± 0.20	7.80 ± 0.18	7.93 ± 0.24

Table 4
Bile salt test.

Isolate	Log CFU/mL				
	0 h	1 h	2 h	3 h	4 h
FSI1-D	8.22 ± 0.09	8.27 ± 0.22	8.18 ± 0.25	7.98 ± 0.03	8.25 ± 0.38
FSI3-D	7.75 ± 0.05	7.69 ± 0.36	7.92 ± 0.37	7.90 ± 0.11	7.74 ± 0.17
FSD1-D	8.39 ± 0.12	8.33 ± 0.04	8.23 ± 0.24	8.23 ± 0.20	8.20 ± 0.11
FSC3-LBC	8.13 ± 0.24	7.96 ± 0.06	8.13 ± 0.17	8.15 ± 0.15	8.25 ± 0.22

phylogenetically related; 16S rRNA sequence homology of 98.3% (Collins, Williams, & Wallbanks, 1990; Mora, Fortina, Parini, & Manachini, 1997) could not be differentiated by the methods. Still, isolate FSI1-D was identified as *L. reuteri* by MALDI-TOF MS and as *L. fermentum* by 16S rRNA gene sequencing analysis (97.75% identity score). It must be noted that *L. fermentum* and *L. reuteri* were previously classified as a single species, because *L. fermentum* is closely related phenotypically but have subsequently been separated (Klein, Pack, Bonaparte, & Reuter, 1998). It is interesting that in the present study, one of the two methods could not differentiate the species. The use of MALDI-TOF MS in bacteria identification is recently soaring and showing promise to be used as a fast identification in the characterization of cultured bacteria, supplementing phenotypic methods. Duskova, Sedo, Ksicova, Zdrahal, and Karpiskova (2012) reported a superior success rate of species level identification of Lactobacilli with MALDI-TOF MS (93%) than polymerase chain reaction (PCR). In the study, the identified isolates yielded Biotyper log scores of between 1.71 and 2.24 indicating very reliable identification at the genus level although for some isolates, no significant similarity of their spectrum with the MALDI-TOF MS database were obtained. Overall, the data suggest the fact that either single identification method may not be sufficient to identify accurately at the species level. The use of the MALDI-TOF MS for bacterial identification may require further validation against other standard molecular identification techniques.

It is important that potential probiotic candidates produce extracellular antimicrobial compounds such as organic acids, hydrogen peroxide, bacteriocins, low-molecular mass peptides and enzymes to kill pathogenic bacteria and mitigate infectious diseases. When evaluated for the antimicrobial activity against both Gram-negative and Gram-positive bacteria, with the exception of FSD4-I and BMS6-DBC, which did not show activity against *Salmonella* Typhi, all the isolates demonstrated inhibitory activity against the tested Gram-negative bacteria. The several isolated species of *L. fermentum* exhibited different inhibitory profile against the tested bacteria suggesting strain specificity of activity also observed by Arena et al. (2016) and McCoy and Gilliland (2007). In the present study, it was noted that the isolates were generally more effective against the local clinical isolate of *P. aeruginosa* than *P. aeruginosa* ATCC 10145. This observation supports that indigenous isolates may be more effective in the treatment of native infections and also more specific to the microbiota of the local population. To the best of our knowledge, research on the effectiveness of indigenous probiotic stains against local pathogens is scarce. Halder, Mandal, Chatterjee, Pal, and Mandal (2017) demonstrated very strong inhibitory activity of indigenous lactic acid bacteria against clinical pathogens, which included *S.* Typhi, *E. coli*, *Proteus vulgaris* and *Acinetobacter baumannii* from different clinical samples although they did not test comparatively with reference pathogenic strains. In another study, Hasslof, Hedberg, Twetman, and Stecksen-Blicks (2010) while using commercial lactobacilli probiotics, did not observe any significant difference in the inhibitory activity against reference strains and clinical isolates of mutas streptococci (MS). Further studies therefore need to be done to explore the comparative effectiveness of indigenous probiotics against local pathogens. The species were also more effective against the Gram-negative species than the Gram-positive *S. aureus*. However, this greater effectiveness towards Gram-negative bacteria was not reported by Mandal et al. (2016) when they assessed isolated LAB against *E. coli*, *P. aeruginosa*, *Salmonella* Typhi, *Shigella dysenteriae, Proteus vulagaris* and *S. aureus* in a similar study. Tharmaraj and Shah (2009) reported a higher inhibition of Gram-positive bacteria including *S. aureus* than Gram-negative bacteria while Tirloni et al. (2014) reported a higher susceptibility of Gram-negative bacteria to antimicrobial activities of *L. animalis* and *L. paracasei* although *P. aeruginosa* was one of the least susceptible species (Tirloni et al., 2014). It was also demonstrated that the isolates were effective against the multi-drug resistant *E. coli.* This concurs with a previous study which demonstrated the antagonistic effect of locally isolated LAB towards six out of seven antibiotic resistant uropathogens tested including *E. coli* (Manzoor, Ul-Haq, Baig, Qazi, & Seratlic, 2016). It was however noted in the present study that the general effectiveness of inhibition against the resistant *E. coli* was lesser relative to the reference *E. coli* tested.

For probiotic species to exert their activity, they must first colonize the gastrointestinal tract. The ability of selected isolates to survive gastrointestinal tolerance was tested in pH 1 to 6 and 0.3% w/v bile salt concentration for 3 h and more. None of the tested isolates survived at pH 1 for the period tested and for 30 min after inoculation (results not shown). All tested isolates were resistant to pH 2 and above and the bile salt. The resistance of the isolates was better than previously reported (Garcia et al., 2016; Jacobsen et al., 1999). Although most of the harsh conditions of the gastric environment is contributed by pH, other enzymatic and digestive substance contained in the gastric fluid as well as the presence of food and the delivery matrix for the probiotic bacteria could affect the survival of the cells during gastrointestinal transit (Fredua-Agyeman & Gaisford, 2015). The results indicate that the tested isolates may survive passage through the gastrointestinal tract at those pH and bile salt concentration; however, a more biorelevant test may be required.

The establishment of the biofilm forming potential of the isolates is very important, as the bacterial association within the gut predominantly exist as biofilms. Biofilm provide colonization resistance; they prolong the residence of ingested bacteria, suppress pathogenic microorganisms and interact with the host cells to regulate immunity (de Vos, 2015; Sassone-Corsi & Raffatellu, 2015). The examined species in the present study all showed biofilm forming potential on 3D nanofibrous scaffold. It was however observed that some of the species were self-inhibitory, having a greater proportion of dead than live cells at the end of incubation period. Although quantification of metabolic products such as organic acids, hydrogen peroxide was not made in the present study, the pH measurements of supernatants produced by the species suggest organic acids may have played a role. The lowest pH (pH of 3.8) was recorded for FSD1-D, which digested the 3D scaffold (Supplementary data Fig. 3) followed by FSC3-LBC (pH 4.2). The digestion of the scaffold however could not be replicated in HCl at similar pH.

Ultimately, the indigenous microbiota proved to be the most effective antimicrobial for residents in addition to their potential to regulate host serotonin biosynthesis in the gut as was previously documented by Yano et al. (2015). Our research study herein, demonstrated the capability of some indigenous strains of *Lactobacillus* to effectively inhibit pathogens and withstand severe conditions, suggesting their combination with commercialized probiotics in order to optimize the indigenous efficiency. They have exceptional ability to withstand extreme pH conditions. Although our preliminary data on the antibiotic resistance profiling of some strains (results not shown) revealed the presence of some strains carrying resistance, future research studies and directions will be focused towards further investigation on the antibiotic resistance profiling to ensure the selection of strains that are free from resistance, which will warrant the benefits and safe-use of this natural therapy. Perhaps, the biofilm will also be enhanced towards the fourth-generation encapsulation to ensure greater *in vivo* bioavailability and potency towards pathogens in the gut.

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CRediT authorship contribution statement

Mansa Fredua-Agyeman: Conceptualization, Funding acquisition, Resources, Investigation, Methodology, Supervision, Formal analysis, Writing - original draft, Writing - review & editing. **Malvin Ofosu-Boateng:** Investigation, Methodology, Formal analysis. **Adelaide Ohenasi Offei:** Investigation, Methodology, Formal analysis. **Mausam Mehta:** Investigation, Methodology, Writing - review & editing. **Simon Gaisford:** Funding acquisition, Conceptualization, Writing - review & editing. **Alya Limayem:** Resources, Methodology, Supervision, Validation, Writing - review & editing.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Appendix A. Supplementary data

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