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Research Article

Isolation and Identification of Probiotic Lactobacilli from Non-ruminant Animals

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ABSTRACT

Probiotic *lactobacillus* strains constitute a group of probiotic bacteria which confer health benefits on the host when consumed in appropriate amount. The aim of the current study was to isolate and identify naturally occurring probiotic *Lactobacillus* species in some non-ruminant animals including horses, donkeys and pigs to investigate interspecies differences in probiotic *Lactobacillus* contents. A total of 39 samples including rectal, buccal, and nasal swabs were collected under aseptic conditions from horses, donkeys and pigs. The samples were cultured on MRS medium and the isolated strains were identified using 16S rRNA multiplex polymerase chain reaction (PCR) analysis following DNA extraction from the isolated bacteria. A total of 37 isolates were identified as lactobacillus strains including 19, 8 and 10 isolates from horses, donkeys and pigs respectively. The pig samples showed the lowest variability in lactobacilli contents with the amplification of *L. rhamnosus*, *L. gasseri* and *L. delbrueckii.* The lactobacilli contents of both horses and donkeys were comparable; however, *L. casei* was isolated only from horses. The diversity of probiotic *lactobacillus* strains isolated from nun-ruminant animals including the close relatives, horses and donkeys, indicates the uniqueness of lactobacilli contents in different animals. Further studies are needed to investigate the beneficial effects of these strains, either separately or in combination, on the host. Understanding the interaction of these strains with other members of the bacterial community inside each host as well as their interaction with the host cells especially the cells of the immune system, will provide important information on how they function in both health and disease states.

Key words: *Lactobacillus*, Microbiota, Non-ruminant, Probiotics.

INTRODUCTION

Probiotics are recognized as living microorganisms that, when given in appropriate amounts, award benefits to the host (FAO/WHO 2011). They enhance the host's resistance against infection through the modulation of immune and inflammatory response in local mucosal surfaces, as well as inducing a positive effect on the systemic immune system in general (Kang and Im 2015). Probiotics have been prescribed for patients with gastrointestinal diseases due to their health promoting properties including the beneficial balance of the intestinal microbiota that can be also associated to other benefits to the host (Williams *et al.*, 2010).

Probiotics represent a heterogeneous group of microorganisms that are present in the gastrointestinal, respiratory and urogenital tract of humans and animals (Wallace and Milev 2017). Gut microbiota constitutes a large number of anaerobic microorganisms that assist the host to achieve numerous biochemical and physiological functions served by their metabolites. In turn, the intestinal microflora is involved in supplying metabolic nutrition to the host, sharing in growth promotion and immune regulation, clearing pathogenic microorganisms and keeping the integrity of intestinal barriers and normal homeostasis (Jones, 2016).

The microbiota of the respiratory tract likely acts as a gate guard that prohibits respiratory pathogens colonization. The respiratory microbiota might also be involved in the maturation and conservation of respiratory physiology and immunity stability (Man *et al.*, 2017).

The vaginal microbiota is commonly predominated by lactic-acid producing species. The output of lactic acid has been associated with the overall health of the vagina due to its direct and indirect influences on pathogens and host defense (Witkin and Linhares, 2017).

The target of the current study was the isolation and identification of naturally occurring probiotic lactobacillus species in some non-ruminant animals using multiplex PCR to investigate interspecies differences.

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MATERIALS AND METHODS

Ethical approval

Institutional Animal Ethics Committee and local laws and regulations were considered in applying our experiment.

Collection of samples

Three swabs from different body cavities of each animal including rectal, buccal and nasal swabs were collected under aseptic conditions from horse, donkey and pig. Vaginal swabs were also taken from pig. The samples were collected from private, individually owned healthy animals in Giza governorate, Egypt to ensure the diversity of bacterial strains. All samples were collected in the presence of the owners after oral acceptance and they were informed with the sampling procedure and very brief note about what the samples will be used for. The samples were collected in sterile carriers containing 5 ml MRS broth medium and stored on ice until delivery to the laboratory. Once delivered to the laboratory, they were incubated at 37°C for 48 h in anaerobic conditions.

Isolation of probiotic strains

After incubation, the sampling containers were shaken homogeneously and a total of 10µl of the liquid culture were transferred into test tubes containing 5 ml fresh MRS broth as selective media to grow Lactobacillus as well as other lactic acid bacteria (LAB). The tubes were shaken homogeneously and incubated at 37°C for 48 h in anaerobic conditions. Inoculum from each tube was sub-cultured at 37°C under anaerobic conditions in the presence of 10% CO_2 to remove unwanted bacteria. After seven subcultures, the bacterial culture was streaked onto MRS agar media. Finally, single colonies with different morphology were isolated and streaked again onto fresh MRS agar media. The resulting pure colonies were checked under an optical microscope after Gram staining and tested for catalase production. LAB was identified by being rod and coccoid shaped, Gram-positive and catalase negative bacteria (Silva *et al.,* 2015).

Gram staining test

Gram staining was performed by conventional procedure, and bacterial cells were observed microscopically (magnification × 1,000) (Brown, 2007).

Catalase test

A single isolated bacterial colony was streaked on a glass slide and mixed with one drop of 3% hydrogen peroxide (Merck, Germany). The effervescence of oxygen indicated the positive response of the bacteria to catalase test (Nanasombat *et al.,* 2017).

DNA extraction for molecular identification of probiotic lactobacillus strains

A total of 1.5 mL of overnight culture (of each of the mixed colony cultures representing the bacterial content of the original samples) in MRS broth was centrifuged at 5000 ×g for 10 min at room temperature. The cell pellet was used for extraction of total genomic DNA using the G-spin Total DNA extraction kit (Intron, Korea).

Molecular identification of probiotic strains

Common lactobacillus strains naturally occurring in different animal species were identified by 16S rRNA multiplex PCR analysis of genomic DNA extracted from mixed bacterial cultures. Multiplex PCR assays were performed with a mixture of seven species-specific or group-specific primers for *L. acidophilus*, *L. bulgaricus* (same as *L. delbrueckii* subsp. *bulgaricus*), *L. casei*-group *L. gasseri*, *L. plantarum*, *L. reuteri* and *L. rhamnosus* and two bacterial conserved primers (Table 1). The PCR products were detected by electrophoresis on 1.5% agarose gel, stained with RedSafe Nucleic Acid Staining Solution (Intron Biotechnology, Korea). Lactobacillus species was identified based on the size of the PCR product (Kwon *et al.,* 2004).

RESULTS

A total of 39 samples were collected from different non-ruminant species including horse (n=5), donkey (n=5), and pig (n=2). Three swabs from rectum, buccal cavity and nasal cavity were collected from each animal under aseptic conditions. The only exception in sampling was the pig as only 2 individuals were sampled; a male and female. Three swabs were collected like all other animal species in addition to vaginal swab from the female pig.

A representative picture of gram-stained lactobacilli isolated from different animal species is shown in figures 1-3. The multiplex PCR products of lactobacilli isolated from horse showed the presence of 19 isolates of lactobacillus strains. The lactobacilli content in the first horse were similar in both the buccal and the nasal swabs (*L. acidophillus*, *L. rhamnosus*, *L. gasseri*). The second horse showed the presence of *L. acidophillus*, and *L. plantarum* in the buccal swab. *L. plantarum* was also isolated from both the rectal swab of the third horse, and from both the nasal and rectal swabs of the fourth horse. The rectal swab of the fourth horse also showed the presence of *L. casei*, *L. rhamnosus*, *L. gasseri*, *L. delbrueckii.* (Fig. 4).

Table 1: Multiplex PCR primers

Target bacteria	Sequence (50 to 30)	Target site	Product (bp)
All Lactobacillus	CCACCTTCCTCCGGTTTGTCA	1178–1198	-
All Lactobacillus	AGGGTGAAGTCGTAACAAGTAGCC	1499–1522	-
L. casei-group	TGGTCGGCAGAGTAACTGTTGTCG	472–495	727
L. acidophilus	AACTATCGCTTACGCTACCACTTTGC	2079–2104	606
L. delbrueckii	CTGTGCTACACCTAGAGATAGGTGG	1015–1039	184
L. gasseri	ATTTCAAGTTGAGTCTCTCTCTC	1748–1770	272
L. reuteri	ACCTGATTGACGATGGATCACCAGT	94–118	1105
L. plantarum	CTAGTGGTAACAGTTGATTAAAACTGC	1900–1926	428
L. rhamnosus	GCCAACAAGCTATGTGTTCGCTTGC	1922–1946	448

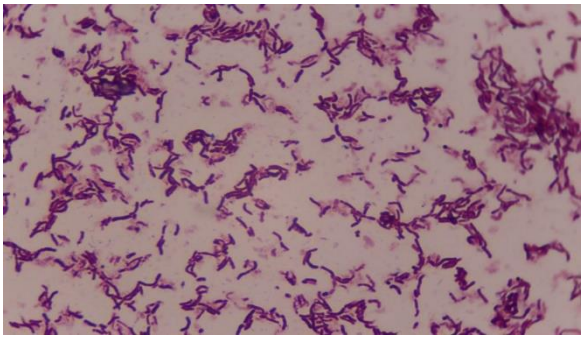

Fig. 1: Gram-positive bacilli isolated from nasal swab taken from pig.

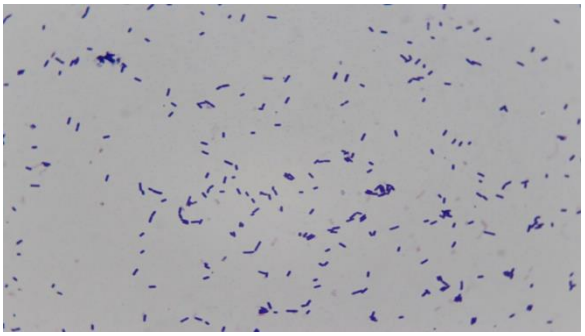

Fig. 2: Gram-positive bacilli isolated from buccal swab taken from donkey.

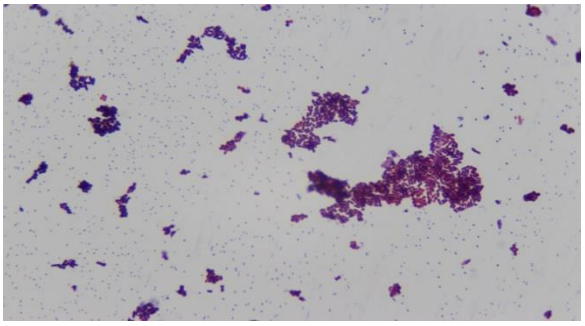

Fig. 3: Gram-positive bacilli isolated from rectal swab taken from horse.

The multiplex PCR products of lactobacilli isolated from donkey indicated the presence of 8 isolates of lactobacilli. The results showed the presence of *L. acidophilus and L. delbrueckii* in buccal swabs from the first and third donkey, while fecal samples showed amplification of *L. rhamnosus*, in the first and second donkey. *L. plantarum* was isolated from the rectal swab of the first donkey only while *L. gasseri* was isolated from the rectal swab of the second donkey only (Fig. 5).

Figure 6 shows the results of the multiplex PCR products of lactobacilli isolated from swabs taken from male and female pigs. A total of 10 isolates were identified as lactobacillus strains. The nasal and buccal swabs from the male pig as well as the nasal, rectal and vaginal swabs from the female pig showed the presence of *L. rhamnosus* and *L. gasseri.* The rectal swab of the male pig did not show the growth of any lactobacillus strains. The buccal swab of the female pig showed the amplification of *L. delbrueckii* which was isolated also from the nasal swab of the female pig.

DISCUSSION

The consumption of some beneficial microorganisms in traditional foods including yogurt, cheese and milk was associated with protection against diseases and extended lifespan. These microorganisms were identified as “Probiotics” and they have become the subject of study that drove attention of many scientists (Metchnikoff and Metchnikoff, 1908; Brown and Valiere, 2004).

There is growing evidence that supports the use of probiotics provides protection against infectious diseases (Hao *et al.,* 2011; Ozen *et al.,* 2015). The most commonly used strains as probiotics are members of *Lactobacilli*, *Enterococci* and *Bifidobacteria* groups which are families of Lactic acid bacteria (LAB). Lactic acid bacteria represent a heterogeneous group of microorganisms that are present in the normal diet of many people and also in the gastrointestinal and urogenital tract of animals.

In a previous study we reported the diversity of lactobacillus species isolated from milk and/or fecal samples of different animal species including cattle, buffalo, goat, sheep, camel and fish (Abdou *et al.,* 2018). The purpose of the current study was to isolate and identify probiotic lactobacillus strains which are naturally occurring in some non-ruminant animal species including horses, donkeys and pigs. To isolate naturally occurring probiotic strains, samples were collected from private, individually owned animals to ensure the diversity of bacterial strains. The diversity of probiotic strains, like most of the commensal bacteria which colonize animals’ body, is due to the variability of nutritional habits as well as the exposure to infections, antibiotics, stress and various disease conditions (Zhang *et al.,* 2015).

Although MRS media is selective and recommended for the isolation, enumeration and cultivation of Lactobacillus species which represent one of the most commonly consumed probiotics, it encourages the growth of other lactic acid bacteria including species of *Streptococcus, Lactococcus, Pediococcus* and *Leuconostoc* (Kacem *et al.* 2003; Wassie and Wassie 2016). For this reason, the isolated bacteria were subjected to repeated sub-culturing to remove unwanted bacteria.

Horses have a big and complicated gastrointestinal (GI) tract and obtain most of their energy demands through the degradation of constructional carbohydrates by fibrolytic bacteria. As a non-ruminant herbivore, this is accomplished by anaerobic fermentation in the cecum and large colon, generating volatile short fatty acids that are absorbed via the intestinal mucosa (Mshelia *et al.,* 2018). The variation in microbial content of fecal samples extracted from healthy horses, whether directly from the rectum or from fecal balls, was documented (Stewart *et al.,* 2018). Lactobacillus strains constituted the majority of bacteria tightly adhere to the mucosa and epithelium in horse stomach (Perkins *et al.,* 2012) and cecum (Moreau *et al.,* 2014). Furthermore, the predominance of Lactobacilli in fecal samples was also reported (Dougal *et al.,* 2012; Costa *et al.,* 2015). These lactic acid bacteria are responsible for the rapid fermentation of extra amount of grains rich in starch fed to horses (Costa *et al.,* 2015).

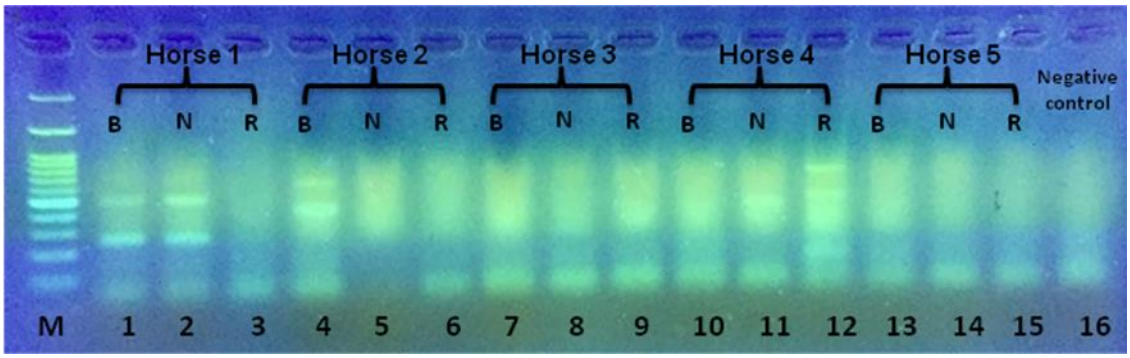

Fig. 4: Agarose gel electrophoreses of PCR products from multiplex PCR assays of genomic DNA extracted from mixed cell suspension of naturally occurring lactobacilli in swabs taken from horse. Lane 1 and 2, faint band of *L. acidophillus, L. rhamnosus, L. gasseri,* faint band of *L. delbrueckii;* lane 4, *L. acidophillus, L. plantarum*; Lane 7 and 9, faint band of *L. plantarum;* Lane 11, faint band of *L. rhamnosus, L. plantarum*; lane 12, *L. casei, L. rhamnosus, L. plantarum, L. gasseri, L. delbrueckii*; Lane 16, negative control; lane M, 100 bp-DNA ladder.

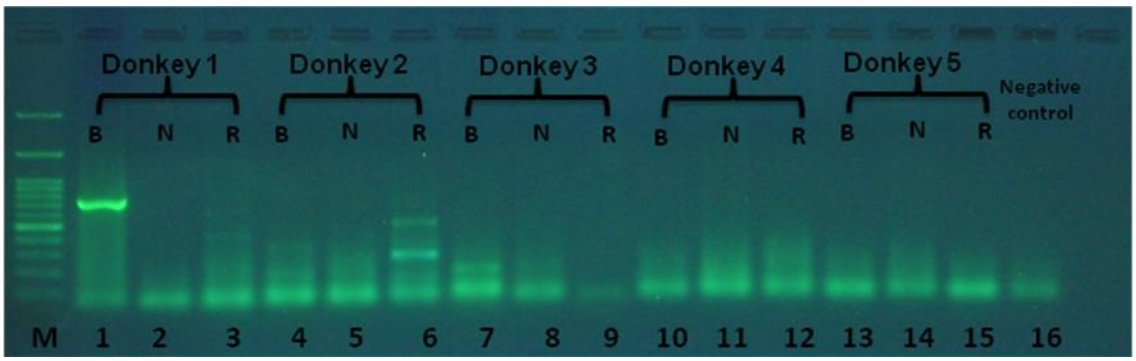

Fig. 5: Agarose gel electrophoreses of PCR products from multiplex PCR assays of genomic DNA extracted from mixed cell suspension of naturally occurring lactobacilli in swabs taken from donkey. Lane 1, *L. acidophillus*; lane 3, faint bands of *L. rhamnosus, L. plantarum;* Lane 4, faint bands of *L. gasseri, L. delbrueckii;* Lane 6, *L. rhamnosus, L. gasseri*; Lane 7, *L. delbrueckii*; lane 16, negative control; lane M, 100 bp-DNA ladder.

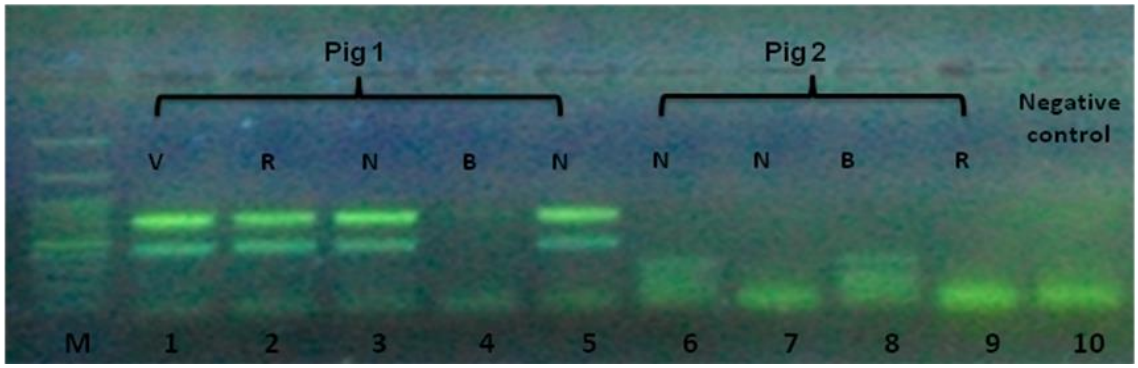

Fig. 6: Agarose gel electrophoreses of PCR products from multiplex PCR assays of genomic DNA extracted from mixed and single cell suspension of naturally occurring lactobacilli in swabs taken from pig. Lane 1, 2, 3, and 5, *L. rhamnosus, L. gasseri*; Lane 6, and 8, faint band of *L. delbrueckii*; Lane 10, negative control; lane M, 100 bp-DNA ladder.

Although it was believed that the lungs are sterile the lungs of horses found to be occupied by beneficial bacteria and there was a great similarity between the microbial communities in both upper and lower respiratory system. This similarity seemed to be due to the fact that horses breathe through their nose as they have complete separation of oral cavity and the nasopharynx. Meanwhile, bacterial content was different between healthy horses and horses with inflammatory airway disease (mild equine asthma) which suggests an important role of the normal bacterial flora in preventing the disease (Bond *et al.*, 2017). The bacterial community in the oral cavity was found to be associated with equine oral health (Kennedy *et al.*, 2016). In the current study we isolated *L. rhamnosus and L. plantarum* from oral samples collected from horse and these two strains were also isolated from the oral cavity of healthy people (Chervinets *et al.*, 2018).

As the donkey is a close relative to horse, similar lactobacillus strains were found in both its oral and fecal samples. Lactobacillus probiotic strains were also isolated from donkey milk which is gaining more attention due to its high nutritive contents and similarity to human milk to

the extent that it was used as a replacement for infants (Soto Del Rio Mde *et al.,* 2016; Soto Del Rio *et al.,* 2017).

Similar to human, pigs are omnivorous as they consume a variety of foods and their gut microbiota is not only responsible for carbohydrate metabolism but also fat metabolism. Any disturbance of microflora constitution is considered to be the major contributor in pigs' obesity (Yang *et al.,* 2018). The abundance of microbiota including lactobacilli was found to be health promoting and prevents post-weaning diarrhea in pigs (Dou *et al.,* 2017).

The dominance of lactobacilli in vaginal microbial ecosystem is thought to be critical to both host health and disease (Macklaim *et al.,* 2012). The dynamic equilibrium of the vaginal microbial ecosystem can be altered by external factors (Cruciani *et al.,* 2012) or by internal factors, and hormonal changes resulting in microbial imbalances in the vagina (Huang *et al.,* 2014).

Conclusions

The diversity of probiotic lactobacillus strains isolated from nun-ruminant animals including the close relatives, horses and donkeys, indicates the uniqueness of lactobacilli contents in different animals. Further studies are needed to investigate the beneficial effect of these strains either separately or in combination on the host. Understanding the interaction of these strains with other members of the bacterial community inside each host as well as their interaction with the host cells especially the cells of the immune system, will provide important information on how they function in both health and disease states.

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REFERENCES

Abdou AM, RH Hedia, ST Omara, MAE Mahmoud, MM Kandil and MA Bakry, 2018. Interspecies comparison of probiotics isolated from different animals. Vet World, 11: 227-230.

Bond SL, E Timsit, M Workentine, T Alexander and R Leguillette, 2017. Upper and lower respiratory tract microbiota in horses: bacterial communities associated with health and mild asthma (inflammatory airway disease) and effects of dexamethasone. BMC Microbiol, 17: 184.

Brown AC and A Valiere, 2004. Probiotics and medical nutrition therapy. Nutr Clin Care, 7: 56-68.

Brown AE, 2007. Staining and observation of microorganisms. In: Brown AE (ed) Benson`s microbiological applications. Laboratory manual in general microbiology, 10th edn. McGraw-Hill, New York, pp: 93-128.

Chervinets Y, V Chervinets, B Shenderov, E Belyaeva, A Troshin, S Lebedev and V Danilenko, 2018. Adaptation and Probiotic Potential of Lactobacilli, Isolated from the Oral Cavity and Intestines of Healthy People. Prob Antimicrob Prot 10: 22-33.

Costa MC, G Silva, RV Ramos, HR Staempfli, LG Arroyo, P Kim and JS Weese, 2015. Characterization and comparison of the bacterial microbiota in different gastrointestinal tract compartments in horses. Vet J, 205: 74-80.

Costa MC, HR Stampfli, E Allen-Vercoe and JS Weese, 2015. Development of the faecal microbiota in foals. Equine Vet J, 48: 681-688.

Cruciani F, P Brigidi, F Calanni, V Lauro, R Tacchi, G Donders, K Peters, S Guaschino and B Vitali, 2012. Efficacy of rifaximin vaginal tablets in treatment of bacterial vaginosis: a molecular characterization of the vaginal microbiota. Antimicrob Agents Chemother, 56: 4062-70.

Dou S, P Gadonna-Widehem, V Rome, D Hamoudi, L Rhazi, L Lakhal, T Larcher, N Bahi-Jaber, A Pinon-Quintana, A Guyonvarch, IL Huerou-Luron and L Abdennebi-Najar, 2017. Characterisation of Early-Life Fecal Microbiota in Susceptible and Healthy Pigs to Post-Weaning Diarrhoea. PLoSOne 12: e0169851.

Dougal K, PA Harris, A Edwards, JA Pachebat, TM Blackmore, HJ Worgan and CJ Newbold, 2012. A comparison of the microbiome and the metabolome of different regions of the equine hindgut. FEMS Microbiol Ecol, 82: 642-52.

FAO/WHO, 2011 ICMR-DBT guidelines for evaluation of probiotics in food. Indian J Med Res, 134: 22-25.

Hao Q, Z Lu, BR Dong, CQ Huang and T Wu, 2011. Probiotics for preventing acute upper respiratory tract infections. Cochrane Database Syst Rev, 9: CD006895.

Huang B, JM Fettweis, JP Brooks, KK Jefferson and GA Buck, 2014. The changing landscape of the vaginal microbiome. Clin Lab Med 34: 747-61.

Jones RM, 2016. The Influence of the Gut Microbiota on Host Physiology: In Pursuit of Mechanisms. Yale J Biol Med, 89: 285-297.

Kacem M, H Zadi-Karam and NE Karam, 2003. Identification of lactic acid bacteria isolated from milk and fermented olive oil in western Algeria. Actes Inst Agron Vet, 3: 141-135

Kang HJ and SH Im, 2015. Probiotics as an Immune Modulator. J Nutr Sci Vitaminol (Tokyo) 61 Suppl: S103-5.

Kennedy R, DF Lappin, PM Dixon, MJ Buijs, E Zaura, W Crielaard, L O'Donnell, D Bennett, BW Brandt and MP Riggio, 2016. The microbiome associated with equine periodontitis and oral health. Vet Res 47: 49.

Kwon HS, EH Yang, SW Yeon, BH Kang and TY Kim 2004. Rapid identification of probiotic Lactobacillus species by multiplex PCR using species-specific primers based on the region extending from 16S rRNA through 23S rRNA. FEMS Microbiol Lett, 239: 267-75.

Macklaim JM, CR Cohen, G Donders, GB Gloor, JE Hill, GP Parham, J Ravel, G Spear, J van de Wijgert and G Reid, 2012. Exploring a road map to counter misconceptions about the cervicovaginal microbiome and disease. Reprod Sci 19: 1154-62.

Man WH, WA de Steenhuijsen Piters and D Bogaert, 2017. The microbiota of the respiratory tract: gatekeeper to respiratory health. Nat Rev Microbiol, 15: 259-270.

Metchnikoff E and II Metchnikoff, 1908. The Prolongation of Life: Optimistic Studies. Our post human future. Consequences of the biotechnology revolution. New York, London: In: Chalmers Mitchell P (ed) GP Putnam's Sons.

Moreau MM, SC Eades, CR Reinemeyer, MN Fugaro and JC Onishi, 2014. Illumina sequencing of the V4 hypervariable region 16S rRNA gene reveals extensive changes in bacterial communities in the cecum following carbohydrate oral infusion and development of early-stage acute laminitis in the horse. Vet Microbiol, 168: 436-41.

Mshelia ES, L Adamu, Y Wakil, UA Turaki, IA Gulani and J Musa, 2018. The association between gut microbiome, sex, age and body condition scores of horses in Maiduguri and its environs. Microb Pathog 118: 81-86.

Nanasombat S, P Treebavonkusol, S Kittisrisopit, T Jaichalad, S Phunpruch, A Kootmas and I Nualsri, 2017. Lactic acid bacteria isolated from raw and fermented pork products: Identification and characterization of catalase-producing Pediococcus pentosaceus. Food Sci Biotechnol 26: 173-179.

Ozen M, G Kocabas Sandal and EC Dinleyici, 2015. Probiotics for the prevention of pediatric upper respiratory tract infections: a systematic review. Expert Opin Biol Ther, 15: 9-20.

Perkins GA, HC den Bakker, AJ Burton, HN Erb, SP McDonough, PL McDonough, J Parker, RL Rosenthal, M Wiedmann, SE Dowd and KW Simpson, 2012. Equine stomachs harbor an abundant and diverse mucosal microbiota. Appl Environ Microbiol, 78: 2522-32.

Silva LF, T Casella, ES Gomes, MC Nogueira, J De Dea Lindner and AL Penna, 2015. Diversity of lactic acid bacteria isolated from Brazilian water buffalo mozzarella cheese. J Food Sci, 80: M411-7.

Soto Del Rio Mde L, C Andrighetto, A Dalmasso, A Lombardi, T Civera and MT Bottero, 2016. Isolation and characterisation of lactic acid bacteria from donkey milk. J Dairy Res, 83: 383-6.

Soto Del Rio MLD, A Dalmasso, T Civera and MT Bottero, 2017. Characterization of bacterial communities of donkey milk by high-throughput sequencing. Int J Food Microbiol, 251: 67-72.

Stewart HL, D Pitta, N Indugu, B Vecchiarelli, JB Engiles and LL Southwood, 2018. Characterization of the fecal microbiota of healthy horses. Am J Vet Res, 79: 811-819.

Wallace CJK and R Milev, 2017. The effects of probiotics on depressive symptoms in humans: a systematic review. Ann Gen Psychiat, 16: 14.

Wassie M and T Wassie, 2016. Isolation and Identification of Lactic Acid Bacteria from Raw Cow Milk. Int J Adv Res Biol Sci, 3: 44-49.

Williams MD, CY Ha and MA Ciorba, 2010. Probiotics as therapy in gastroenterology: a study of physician opinions and recommendations. J Clin Gastroenterol 44: 631-6.

Witkin SS and IM Linhares, 2017. Why do lactobacilli dominate the human vaginal microbiota? Bjog 124: 606-611.

Yang H, Y Xiang, K Robinson, J Wang, G Zhang, J Zhao and Y Xiao, 2018 Gut Microbiota Is a Major Contributor to Adiposity in Pigs. Front Microbiol, 9: 3045.

Zhang YJ, S Li, RY Gan, T Zhou, DP Xu and HB Li, 2015. Impacts of gut bacteria on human health and diseases. Int J Mol Sci, 16: 7493-519.