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Lactobacillus pentosus for Animal Nutrition. Review article

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ABSTRACT

The aim of this review was to assess the role of Lactobacillus pentosus in feeds. Several journals of poultry production, and others associated with animal production in general, were consulted. The main indicator was Lactobacillus. It was found that *L. pentosus* is a versatile species found in a variety of environmental niches; it plays a significant role during preparation of many fermented foods, as well as in secreting various antimicrobial substances, and it contributes to reduce environmental pollution. Little research has beed done on *L. pentosus* role in agricultural production of meat and poultry.

Key words: *meat production, bacteriocins, biosurfactants, probiotic*

INTRODUCTION

Lactic acid bacteria (LAB) are known for their use and activity in food preservation (milk, meat, greens, and bread) due to its fermentative and safety capacities, both alone and with other conventional treatments (Powthong and Suntornthiticharoen, 2015).

The Lactobacillus genus comprises a heterogeneous group of microorganisms with the shape of cobacillus or sticks with low G+C base content, Gram positive, usually negative catalase, non-spore-forming and anaerobic (Bernardeau *et al*., 2008). The most representative probiotic microorganisms belong to that genus (Para-Huertas, 2010).

Hasan *et al.* (2015) pointed that the most widely used probiotics in animal production are L. *acidophilus* and *S. cerevisiae*. *Lactobacillus pentosus* is only mentioned in relation to equines; however, recent reports of possible use as probiotics have been published.

The species Lactobacillus pentosus was removed from the Approved Lists of Bacterial Names, and was included within the *Lactobacillus plantarum*. However, Zanoni *et al*. (1987) made molecular and biochemical studies that demonstrated that the strains isolated from maize forage silage, named L. *pentosus* NCDO 363 (= ATCC 8041) were different from *Lactobacillus plantarum*.

The first studies made in Cuba to achieve probiotics referred to the genus *Lactobacillus*, were made at the Cuban Institute of Sugar Cane By-product Research (ICIDCA). *Lactobacillus salivarius* strains and *Bacillus spp*. were used in broiler chickens (Brizuela *et al*., 2001; Brizuela, 2003). Recently, *Lactobacillus pentosus* proved positive modulation to the immune response of chicken (Garcia-Hernández *et al*., 2016). This review intends to assess the comprehensive role of *Lactobacillus pentosus* in food production.

DEVELOPMENT

Isolations from plant and animal based foods

Plant foods

Today, fermented greens are considered a source and vehicle of probiotic microorganisms. Plant probiotics have the potential to attract more consumers that demand functional products, because greens provide access to new formulations of probiotics to organisms that are lactose intolerant, or require low cholesterol diets. (Pérez *et al*., 2012).

Olives

Strain *L. pentosus* DSM 16366 was isolated during fermentation of green olives. It was relevant, technologically, because it could be used as a primer strain in cultures (Papadelli *et al*., 2015).

The lactic acid bacteria that prevail during fermentation of green olives is *Lactobacillus pentosus* (Maldonado-Barragán *et al*., 2011), a bacteriocin-producing bacterium with biotechnological and probiotic properties. *Lactobacillus pentosus* MP-10 was isolated from green olives in brine, naturally fermented, and it showed a probiotic potential, as pathogen inhibition in humans (*Salmonella enterica*, *Listeria monocytogenes*, *Bacillus*

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cereus, *Staphylococcus aureus*, and *Enterococcus faecalis*) (Abrionel *et al*., 2011).

Two strains of *Lactobacillus* were isolated from industrially fermented olives: *L. pentosus* B281 and *L. plantarum* B282, (Doulgeraki *et al.*, 2013). *L. pentosus* B281 was more effective because it survived at the end of fermentation, at 8-10% in brine, besides colonizing the surface of Spanish olive, from 6.0 to 7.0 log 10 colony forming units (CFU) g^{-1} (Blana *et al*., 2014).

Rodríguez-Gómez *et al.* (2013) observed the potential of *L. pentosu*s TOMC-LAB2 as a probiotic, for its resistance against digestion of gastric and pancreatic acids, selfaggregation, hydrophobicity, production of bacteriocins, and deconjugation of biliary salts. A new matrix to supply probiotics was developed from this strain, especially for lactose intolerant consumers who require low cholesterol diets (Rodríguez-Gómez *et al*., 2014).

Forage

L. pentosus ITA23 and *L. acidipiscis* ITA44 were isolated from mulberry forage (*Morus alba*) preserved in silos. The strains were very tolerant to acid and bile, had antioxidant activity and ability to produce fatty acids (Altaher *et al*., 2015).

Fermented saw dust

Lactobacillus pentosus LMG10755T was found in fermented saw dust. The strain was used as reference to characterize *Lactobacillus xiangfangensis sp*., isolated from pickles in China, which accounted for 98.9% identity with the 16S rRNA sequence (Gu *et al*., 2012).

Fruits and fermented foods

Three *lactobacillus* strains were isolated from fermented fruits and foods in Malaysia: *Lactobacillus fermentum* Te007, *Pediococcus pentosaceus* Te010, and *Lactobacillus pentosus* G004. All of them showed antifungal activity against *Aspergillus oryzae* and *Aspergillus niger* (Muhialdin *et al*., 2011). The best combination was *Lactobacillus pentosus* G004 and *Lactobacillus fermentum* Te007, with total growth inhibition of both microorganisms.

Sánchez Ortiz *et al.* (2013) isolated *Lactobacillus sp*. IC1 from lactic acid fermentations of *Brassica oleracea*, were confirmed as the predominant genus in these kinds of isolations. The strain was able to stimulate the immune system and growth in *Litopenaeus vannamei* shrimps (Sánchez Ortiz *et al*., 2013).

Fermented beverages and batter

L. pentosus ST712BZ is present in boza, a Bulgarian traditional beverage made from different cereals. The strain produced bacteriocins (Todorov and Dicks, 2006).

Strain L. *pentosus* SJ65A was isolated from fermented Uttapam batter, used to make pancakes in southern India. The strain can produce effective antibacterial compounds against *Staphylococcus aureus* MTCC 737, *Listeria monocytogenes* MTCC 657, (Saraniya and Jeevaratnam, 2014).

Foods from animal origin

Sausages

L. pentosus strain MF1300 and other two strains of *L. plantarum* (MF1291 and MF1298) were identified in Scandinavian fermented sausages. Their presence inhibited pathogenic bacterial growth, contributing to product preservation (Klingberg and Budde, 2006). In foods, it can work as a probiotic delivery systems for humans (Cavalheiro *et al*., 2015).

The *L. pentosus* strain MR 483 was isolated from 99 strains of *Lactobacillus* found in certain Ethiopian fermented meat sausage (Wakalim). It was less useful as a probiotic due to its low tolerance to biliary salts (0.3%) *in vitro* (Bacha *et al*., 2009).

Milk, butter, cheese and yogurt

L. pentosus KCA1 was used for milk to process yogurt. As a fermentation finishing agent, it increased lactic acid production, as pH decreased, surviving to storage conditions of 4ºC for 49 days, at 5.5 x 106 log 10 cfu ml^{-1} (Anukam and Olise, 2012).

Lactobacillus pentosus B231 was isolated from crude cow milk used for traditional PDO Portuguese cheese. Bacteriocin B231 produced by the microorganism was characterized, and it turned out to be highly dependent from the culture medium used to grow the lactobacillus (Guerreiro *et al*., 2014).

In Xinjiang, China, a strain classified as *L. pentosus* (ATCC 8041) was isolated from fermented milk products by 16S rRNA gene sequencing. Then it was used to ferment milk and produce yogurt; however, xylose fermentation affected the taste of yogurt (Pan *et al*., 2014).

In a region of Iran, two strains of *Lactobacillus*: *L. brevis* LB32 and *L. pentosus* LP05 were isolated from sour sheep milk butter. The two species were confirmed by 16S rRNA sequencing. They were identified as negative, non-motile heterofermentative catalase and oxidase (producing acid and gas from glucose), growing at 15ºC. Both strains inhibited growth of *Listeria monocytogenes*, *Salmonella enteritidis*, *Shigella dysenteriae*, *Staphylococcus aureus*, and *Streptococcus pneumonia*, likely to be used later in dairy products or the medical industry.

Manufacture of enzymes, surfactants, antimicrobial substances and bacteriocins

The lactic acid bacteria (LAB) are GRAS microorganisms (generally regarded as safe), that produce substances with bactericidal effects, with a strong antagonic activity against several pathogens and microbes that deteriorate foods (Bhattachararya and Das, 2010). Their antimicrobial properties owe to the formation of organic acids, ethanol, diacetyl, H_2O_2, and protein compounds known as bacteriocins.

Enzymes

L. pentosus strain MP-10 has probiotic and nutritional properties thanks to the release of enzymes like phytase, tannase, hydrolase of biliary salts, and hydrolysis of stachyose and raffinose, which is important during digestion (Abriouel *et al*. , 2011). Recently, a proteomic analysis of strain MP-10, mentioned the proteins involved in resistance mechanisms for stressful environments (Muñoz *et al*., 2016).

It was disclosed that tannase (tannin acyl hydrolase, CEE 3.1.1.20) is a specific catalyzer of hydrolysis of galloyl ester bonds in tannins, widely recurrent in plants, which is considered a protection strategy against microbial attacks (Aguilar *et al*., 2007).

The inclusion of probiotics in the intestinal tract of birds may lead to the secretion of amyolytic, cellulolytic, proteolytic and lipolytic enzymes that might reinforce the activity of enzymes that catalyze endogenous enzymes, to release more energy contained in food ingredients (Owosibo *et al*., 2013).

Surfactants

Surfactants have been used by the food processing industry for many centuries. The combination of particular features, like emulsifiers, antiadhesives, and their antimicrobial activity suggest their potential application as multipurpose ingredients or emulsifying additives in foods (Kralova and Sjöblom, 2009).

Recently, they were characterized using a biosurfactant produced by *L. pentosus* CECT-4023 T (ATCC-8041) to accelerate biorehabilitation of soils polluted with octane. It improved its solubility in aqueous phase of soil, it produce even better results than the ones achieved with dodecyl sodium sulphate, a synthetic ammonium surfactant, after 15 days (Moldes *et al*., 2013); (Vecino *et al*., 2015).

Substance production

Lactic acid bacteria play a key role in most food fermentations, besides inhibiting pathogenic bacterial growth in foods. It might be due to pH reduction caused by organic acids produced, or due to its ability to produce a variety of antimicrobial substances (Iranmanesh *et al*., 2015).

Bacteriocins

L. pentosus —whose bacteriocin titer is 1 600 SU ml^{-1} — has an important role within lactic acid bacteria isolated from yogurt, milk whey, and milk from sheep, on two locations of Azarbayan-e-sharqui, northern Iran (Iranmanesh *et al*., 2015). That strain has high inhibitory effects against several Gram positive and Gram negative bacteria (*Staphylococcus aureus*, *Listeria monocytogenes* and *Salmonella enteriditis*).

Lactobacillus (*L. pentosus* 31-1) used as probiotics and bioprotectors produce bacteriocins that improve safety and functionality of sturgeon sausage, with better storage safety and way to deliver the probiotic (Wang *et al*., 2015). Moreover, *L. pentosus* B231 produces bacteriocin B231, a small protein with a relative mass of approximately 5 kDa. It also acts against several wild strains of *Listeria monocytogenes*, *Listeria ivanovii*, and *Listeria innocua* (Guerreiro *et al*., 2014).

L. pentosus ST712BZ growth is influenced by the medium used. Triptone was used as an important source of nitrogen, also necessary for optimum production of bacteriocin ST712BZ. It inhibited the growth of *Escherichia coli*, *Pseudonomas aeruginosa*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, and *Lactobacillus casei* and *curvatus* (Todorov and Dicks, 2007). The growth of *L. pentosus* TV35b in MRS medium (Biolab) produced pentosine TV35b which was active against *Clostridium sporogenes*, *Clostridium tyrobutyricum*, *L. curvatus*, *L. fermentum*, *Lactobacillus sake*, *L. innocua*, *Propioni bacterium acidipropionici*, *Propioni bacterium* sp. and *Candida albicans* (Okkers *et al*., 1999).

Environmental contamination

A large amount of by-products and agroindustrial residues are generated every day, causing diverse environmental issues when they are not properly recycled or processed. Biotechnological treatment of residues is a method to deal with pollution. They are mostly lignocelluloses that could be used for biotechnological production of several additives in foods, like lactic acid. Additionally, they must be submitted to various fractioning stages; for instance, from hydrolyzed hemicellulose derived from vine pruning made to the trunks of grapes with *L. pentosus* CECT-4023T (ATCC-8041) (Bustos *et al*., 2004).

Industrial processing of potatoes generates large amounts of residues that pollute the water, air and soils, with no apparent solution. In that sense, *L. casei*, *L. plantarum* and *L. pentosus* were used to hydrolyze starch from potato residues and produce lactic acid, with subsequent polymeration in polylactic acid and xanthan, precursors of renewable and biodegradable polymers, to replace petroleum-based plastics (Bilanovic *et al*., 2011). Intensive production and use of hydrocarbons cause environmental pollution, with greenhouse gases and cancerous polycyclic compounds. The use of previously hydrolyzed lignocellulose residues, in fermentation with *L. pentosus* CECT-4023T (ATCC-8041) produced biosurfactants, mediated by biotechnologies. It means that agricultural residue burning in the fields can be prevented, including the giving off certain gases, like CO_2, CH_4, and N_2O, with greenhouse effects (Moldes *et al*., 2007).

Production and health in monogastrics

The probiotic capacity of bacteria and yeasts largely owes to stimulation of the immune system and growth. For strain selection, several *in vitro* assays can predict the possibility of using them as probiotics (tolerance to acidity, antagonistic activity against pathogens, antibiotic resistance, and others). Mahasneh *et al.* (2015) analyzed 17 *Lactobacillus* strains from plant fragments. Three of them were *L. pentosus*.

Aquatic organisms

The inclusion of *L. pentosus* LB-31 in the diet of rainbow trouts (*Oncorhynchus mykiss*) at a concentration of 107 cfu/g-1β ($P \leq 0.05$), IL-8 ($P \leq 0.001$), and TNF-α (NS), led to a significant production of IL-1 cytokines. Besides, the behavior of cytokines when the *L. pentosus* LB-31 mixture with *Wickerhamomyces anomalus* LV-6, in a second treatment had similar values for IL-1β and TNF-α, with immunomodulating action of both organisms (Sánchez, 2016). The authors detected greater presence of *L. pentosus* in the intestine of all trouts. So it had the capacity to colonize the intestines of fish, contrary to *W. anomalus*, which remained undetected.

In intensive shrimp culture, chemical agents (antibiotics) were used without regulation in the presence of any symptoms of a disease. Indiscriminate use of antibiotics may lead to multiresistant antibacterial strains, with harmful effects on consumer health (Soto-Rodríguez *et al*., 2012). Several species of *Lactobacillus* have been tested in shrimp, usually from vertebrates (Kongnum and Hongpattarakere, 2012). *Lactobacillus sp*. Strain IC1 supplied every 24h to ponds with *Litopenaeus vanamei* shrimp, at a concentration of 1-2 x 104 cfu ml^{-1}, produced weight gains and increased enzymatic activity, which improved postlarvae immunological behavior (phenol oxidase and superoxide dismutase) (Sánchez Ortiz *et al*., 2013).

Strain PL11 was isolated from Japanese eel, and identified as L. *pentosus*. PL11 had a probiotic potential, with tolerance to acid and bile, along with digestive enzyme production, antibacterial activity, pathogen inhibition and intestinal mucus adhesion (Lee *et al*., 2015). Previously, this strain had demonstrated to stimulate the immune system of Japanese eel. When it was challenged with Edwardsiella, the antioxidant capacity delayed reduction of superoxide dismutase, catalase, and heat stress (HSP70) (Lee *et al*., 2013).

The strain of L. *pentosus* H16 was isolated from intestinal treatment to hake, and its partial rRNA sequence 16S of H16, showed 100% match to the strain of *L. pentosus* JCM 1558T. The strain reduced the adhesion of *Vibrio alginolyticus* and *Aeromona salmonicida* (ictiopathogens). Additionally, it also produced organic acids. This strain can be bioencapsulated and be supplied as a probiotic in aquaculture (Garces *et al*., 2015).

Equines, swine and birds

Few studies have reported the efficacy of probiotics to prevent or treat enteric diseases in equines (Boyle *et al*., 2013). To prevent neonatal diarrhea, a group of hoals was treated with an oral dose of 2x 10011 cfu of dehydrated *L. pentosus* WE7, for 7 days. The results did not show probiotic capacity of the animal clearly, perhaps for not having a developed intestinal microflora to tolerate a high dose of *L. pentosus* (Weese and Rousseau, 2005). These authors claimed that probiotics may fail to colonize the gastrointestinal tract of horses, so it is unlikely that they can act beyond their administration period.

Swine

A mixture of five strains of *Lactobacillus murinus* DPC6002 and DPC6003, *Lactobacillus salivarius* DPC6005, *L. pentosus* DPC6004 and *P. pentosaceous* DPC600 was applied to weaned pigs. It resulted in lower incidence, severity and duration of diarrhea when a strain of *Salmonella typhimurium* (Casey *et al*., 2007) was challenged.

In Yorkshire-Landrace x L35 pigs, with an average initial weight of 7 and 8 kg, recently weaned, *L. pentosus* LB-31, at 108 cfu g^{-1} of feed was included in the daily feed ration. On day 42 the pigs reached significant increases ($P < 0.05$) of final live weight, as a result of mean daily gain, with lower feed conversion. In addition to it, diarrhea was reduced ($P < 0.001$) (Sánchez, 2016).

Birds

L. pentosus LB-31, *W. anomalus* LV-6 were isolated from fermented chicken stools. They were included in the diet of broiler chicken as a single feed, or mixed, at 109 cfu and 108 cfu ufc·g^{-1}, respectively. At sacrifice, on day 42, the relative weight, live weight of cecum and liver did not differ among themselves. The lowest weight of empty small intestine corresponded to the treatment with *L. pentosus*, and the highest values of abdominal fat. The concentrations of *Lactobacillus*, yeasts and coliforms of cecal content was not different, which indicated a state of intestinal eubiosis. The concentration of volatile fatty acids and pH of cecal content behaved similarly. No significant values were detected in hemoglobin and hematocrite contents in blood and total serum protein. Regarding productive indicators, consumption and conversin of feeds did not differ. Live weight and live weight gain between the control and the *L. pentosus*-added group (LB-31) were similar. Carcass and breast in the experimental groups were higher ($P \leq 0.05$), regarding the control. The results indicated that the inclusion of *L. pentosus* LB-31, *W. anomalus* LV-6, and their non-antagonic mixture in the diet of broiler chicken, caused a probiotic response in animal health and growth indicators (García-Hernández *et al*., 2016).

In Malaysia, productive indicators were measured in commercial broiler chicken (Cobb 500), which received a solid diet of 0.1% a mixture of *L. pentosus* ITA23, and *Lactobacillus acidophyllus* ITA44, each at 109 cfu cells kg^{-1} of the feed. Feed ingestion and feed conversion were significant ($P \leq 0.0$, whereas body weight and weight gain did not differ in chicken sacrificed at 35 days of age. The authors noted that *Lactobacillus spp*. probably acts as a probiotic, by removing pathogenic bacteria, thus keeping a better intestinal environment for digestion and absorption of nutrients (Altaher et al., 2015).

CONCLUSIONS

Lactobacillus pentosus is present in the preparation of several fermented foods of animal and plant origin.

The feature of stimulator of digestion, antimicrobial and de-pollutant of the environment owes to the secretion of intracellular substances.

There are few studies on its use as additive for poultry and livestock meat productions.

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