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Review

Antimicrobial activity of *Lactobacillus acidophilus* against pathogenic and food spoilage microorganisms: A review


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Abstract. The purpose of this review is to summarize the information regarding the antimicrobial activity of *Lactobacillus acidophilus*, an important species of lactic acid bacteria. Lactic acid bacteria are constituents of many beneficent for the consumer's health food products. They are considered potentially promising in the strategy to combat infections and prevent the growth of spoilage microorganisms, and also have antimutagenic, anticarcinogenic, hypolipidemic and hypcholesterolemic properties, improve the lactose metabolism, stimulate the immune system, etc. In the recent years *Lactobacillus acidophilus* is considered the main probiotic species in the intestinal tract of healthy humans and is widely used in functional dairy foods. It produces a variety of metabolic products with antimicrobial properties, including organic acids and bacteriocins, such as lactocin B and F, acidophilin, acidocin, acidophilucin, acidophilicin, which are active against many pathogenic and spoilage microorganisms - *Escherichia coli* (including *Escherichia coli* 0157:H7), *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Vibrio parahaemolyticus*, *Vibrio cholerae*, *Helicobacter pylori*, *Clostridium*, *Mucor*, *Aspergillus*, *Fusarium*, *Trichoderma* and *Candida* spp., etc. Because of the above mentioned reasons *Lactobacillus acidophilus* could be used as an alternative therapeutic agent against infections caused by susceptible microorganisms. On the other hand *Lactobacillus acidophilus* based antimicrobial products (mainly bacteriocins and pure cultures) could also be applied to food products to prevent the growth of spoilage microorganisms and food-borne pathogens. To better understand the mode of action and the spectrum of antifungal activity more clinical and laboratory studies of different *Lactobacillus acidophilus* strains are required.

Keywords: *Lactobacillus acidophilus*, antimicrobial activity, spoilage microorganisms, pathogens

Introduction

In modern society there is increasing demand for safe foods and high quality preservative-free products. On the other hand, the food-borne diseases are major cause for morbidity and mortality in the population of the world. Most of the deaths occur in developing and tropical countries, although they are not limited to them (Arias et al., 2013). To meet the demands of food industry and to restrict the occurrence of the food-borne diseases lactic acid bacteria (LAB) and LAB-produced bacteriocins are often used (Vignolo et al., 2012). LAB consist of a few genera, which include *Streptococcus*, *Enterococcus*, *Lactococcus*, *Leuconostoc*, *Lactobacillus* and *Pedococcus*. Based on similarities in physiology, metabolism and nutritional needs, these genera are grouped together. The primary similarity is that all members produce lactic acid as a main end product of the carbohydrates fermentation. LAB are considered as a good choice to prevent the growth of spoilage and pathogenic microorganisms (Al-Chezzy et al., 2011). They are the most widely used bacteria as starter cultures for the industrial processing of fermented dairy, meat, vegetable and cereal products. Reduction of pH and conversion of sugars to organic acids are the main mechanisms for biopreservation of fermented foods (Vignolo et al., 2012).

Because of the irresponsible use of antibiotics to treat human and animal infections some bacteria have developed resistances (Besler and Essack, 2010). In order to avoid the frequent use of antibiotics and to control the rate of proliferation of potentially pathogenic gastrointestinal bacteria, probiotics could be successfully employed (Arias et al., 2013). LAB produced antimicrobials have been successfully used to prevent the formation of biogenic amines (Joosten and Nunez, 1996) and they also have the ability to inhibit mastitis pathogens (Ryan et al., 1998), food-borne pathogens (Abo-Amer, 2013), fungal pathogens (Demkova et al., 2013; De Seta et al., 2014) and the growth of *Helicobacter pylori* (Michetti et al., 1999). Recently some LAB strains have been successfully applied in human medicine for the treatment of various chronic and cardiovascular diseases such as Parkinson’s, Alzheimer’s, diabetes, obesity, urogenital complications, cancer, hypertension, liver disorders, etc. (Woo et al., 2014).

*Lactobacillus acidophilus* (*L. acidophilus*) is considered the predominant *Lactobacillus* species in the intestinal tract of healthy humans (Ray, 1996). Among the different *Lactobacillus* species *L. acidophilus* strains are most frequently used as probiotics (Klaenhammer and Kullen, 1999). *L. acidophilus* is often resistant to routinely used antibiotics. This resistance is often intrinsic and non-transmissible. Sometimes however, inherently antibiotic-resistant probiotic strains may benefit patients whose normal intestinal microbiota is unbalanced or greatly reduced in numbers due to the application of various antimicrobials (Emami and Bazargani, 2014). Because of its many beneficial properties *L. acidophilus* is widely...
used as a starter culture in fermented dairy products (Ahmed et al., 2010).

Considering the increasing significance of the LAB as antibiotics alternative, the knowledge of the antimicrobial activity of the main LAB species and *L. acidophilus* in particular has especially high importance. The antimicrobial activity of *L. acidophilus* can give us a lead if the corresponding *L. acidophilus* products can be helpful in the treatment of the particular infection. Also, because the main food-borne pathogens and food spoilage microorganisms are already well-known, the supplementation of *L. acidophilus* to the food could be made on the basis of the antimicrobial activity of the particular *L. acidophilus* strain. Thus, the purpose of this review is to summarize the information regarding the antimicrobial activity of *Lactobacillus acidophilus*, which could be useful in medical practice and food industry.

**Antimicrobial substances produced by *L. acidophilus***

*Bacteriocins*

Bacteriocins are ribosomally synthesized antimicrobial peptides produced by a variety of bacteria, including LAB. Some of them have considerable potential in food preservation and can greatly reduce or eliminate the necessity for addition of chemical preservatives or the intensity of processing the food and in that way can satisfy the consumer demand of safe and high-quality food products (Perez et al., 2014). Most of the bacteriocins kill the susceptible bacteria by membrane permeabilization or by interactions with essential enzymes (Wen et al., 2016). Moreover, bacteriocins are degraded by the proteolytic enzymes of the gastrointestinal tract and seems to be non-toxic and non-antigenic to animals and humans (Amenu, 2013). According to Zacharof and Lovitt (2012) the LAB bacteriocins are classified into 3 major classes: (1) Class I (the lantibiotics); (2) class II (the non-lantabiotics); and (3) Class III (bacteriocins).

*L. acidophilus*-produced bacteriocins are lactacin B, F; acidophilin 801; acidocin A, B, 1B, CH5, J1229, J132, 8912, LF221 A, LF221 B, D20079; acidophilinic A; acidophilin LA-1 (Ahmed et al., 2010). Barefoot and Klænhammer (1983) conducted the first study on the production of bacteriocins by *L. acidophilus*. They named the first bacteriocin lactacin B. It has narrow spectrum of activity against selected members of *Lactobacillaceae* family. The following studies found many bacteriocins with considerably broader spectrum of activity against food spoilage and pathogenic bacteria, such as acidocin 1B, CH5, D20079, LF221 A, LF221 B, etc. (Ahmed et al., 2010). Some bacteriocins, such as acidocin CH5 and acidocin D20079 could be used in fermented milk or meat products, respectively to inhibit the growth of the food spoilage bacteria (Collins and Aramaki, 2001). Gerez et al. (2013) reported that *L. acidophilus* strains can produce hydrogen peroxide in sufficient concentrations to inhibit the growth of some food spoilage bacteria (Collins and Aramaki, 2001). Gerez et al. (2013) reported that *L. acidophilus* strains can produce hydrogen peroxide in sufficient concentrations to inhibit the growth of some food spoilage bacteria (Collins and Aramaki, 2001).

**Organic acids**

Organic acids lower the local pH and therefore inhibit the growth of bacteria (especially Gram-negative bacteria) sensitive to acidic conditions (Makras and De Vuyst, 2006). Many experiments showed that organic acids are one of the main LAB compounds exerting antimicrobial effects (Piper et al., 2001; De Muynck et al., 2004; Denkova et al., 2013; Gerez et al., 2013). Because *L. acidophilus* belongs to the homofermentative *Lactobacillus* species it is fermenting carbohydrates mainly into lactic acid. During LAB fermentation lactic acid is in equilibrium between its undissociated and dissociated forms, and the extent of the dissociation depends on pH. At low pH, a large amount of lactic acid is in undissociated form and it is toxic to many bacteria, fungi and yeasts. However, different microorganisms vary significantly in their sensitivity to lactic acid. At pH 5.0 lactic acid is inhibitory towards spore-forming bacteria but is ineffective against yeasts and moulds (Amenu, 2013).

Benzonic acid is the most commonly applied preservative in the food industry. It is used primarily as antifungal agent. Dairy products can contain natural benzoic acid as some LAB could transform some acids naturally present in milk into benzoic acid. Some authors found strains of *L. acidophilus* that can produce benzoic acid in fermented milk (Reis et al., 2012).

**Hydroxy fatty acids**

The antifungal effects of the 3-hydroxy fatty acids are due to detergent-like properties of the compounds that alter cellular membrane structure in the target organisms (Sjögren et al., 2003). There are some studies on LAB-produced fatty acids that have a broad spectrum of antifungal activity (Sjögren et al., 2003; Dalé et al., 2010). Recently Hirata et al. (2015) reported that *L. acidophilus* NTV001 has the ability to convert linoleic acid to hydroxy fatty acids through production of fatty acids hydratase.

**Hydrogen peroxide**

Hydrogen peroxide is produced by LAB in the presence of oxygen as a result of the action of flavoprotein oxidases or NADH peroxidase. The antimicrobial effect of hydrogen peroxide could result from the oxidation of sulfhydryl groups which cause denaturation of a variety of enzymes and from the peroxidation of membrane lipids that leads to increased membrane permeability (Amenu, 2013). Hydrogen peroxide can be a precursor for the synthesis of bactericidal free radicals such as superoxide (O2-) and hydroxyl radicals (OH) which can damage DNA (Byczkowski and Gessner, 1988). It is well known that in certain conditions some *L. acidophilus* strains can produce hydrogen peroxide in sufficient concentrations to inhibit the growth of some food spoilage bacteria (Collins and Aramaki, 2001). Gerez et al. (2013) reported that hydrogen peroxide does not exert any antifungal activity.

**Antimicrobial activity of *L. acidophilus***

**Antibacterial activity**

Experimental data show that *L. acidophilus* is active against many Gram-negative pathogenic and food spoilage microorganisms - *Escherichia coli* (including multidrug-resistant enterogastric *Escherichia coli* and *E. coli* 0157:H7), *Pseudomonas aeruginosa*, *Klebsiella*, *Salmonella*, *Shigella* spp., etc. (Table 1). *L. acidophilus* exerts antibacterial activity also against a variety of Gram-positive bacteria, including important pathogens - *Bacillus cereus*, *Bacillus subtilis*, *Clostridium perfringens*, *Staphylococcus aureus* and *Listeria monocytogenes* (Table 2). It is important to emphasize that the antimicrobial activity of *L. acidophilus* (and the other LAB) is strain specific and not species- or genus specific (Deney et al., 2015). That means there are only some *L. acidophilus* strains that inhibit specific strains of the aforementioned bacteria. There are three mechanisms that could explain the antimicrobial efficacy of LAB and *L. acidophilus* in particular: 1) the production of bacteriocins; 2) the yield of organic acids and some other inhibitory substances such as hydroxy fatty acids and hydrogen peroxide; 3) and the competition for nutrients (Magnusson et al., 2003).
It is well established that Gram-negative bacteria are intrinsically resistant to bacteriocins produced by LAB and 
*L. acidophilus* in particular. This occurs due to the presence of the external membrane, which constitutes a physical barrier to the passage and binding of bacteriocins (Pehrson et al., 2015). However, it has been reported that the destabilization of the outer membrane could make Gram-negative bacteria susceptible to bacteriocins. It is found that lactic acid acts as a permeabilizer of the outer membrane of Gram-negative bacteria, thus increasing their susceptibility to antimicrobials (including bacteriocins), allowing their molecules to penetrate the bacteria (Alakomi et al., 2000). Initially most of the reports revealed that the bacteriocins were active against narrow spectrum of Gram-positive bacteria (mainly *Lactobacillus* spp.), but in the following years some authors also reported activity against Gram-negative bacteria from *Enterobacteriaceae* (Ahmed et al., 2010).

Regarding the antibacterial activity of *L. acidophilus*-produced substances it is considered that organic acids exert a strong inhibition effect on Gram-negative bacteria (Makras and De Vuyst, 2006). The probiotic-mediated inhibitory activity on *Escherichia coli* and *Salmonella enteritidis* increased proportionally to the concentration of organic acids in the medium. The authors observed that the low pH may not be the only cause for the observed inhibition effects. This however could be an important condition for the passage of organic acids through the membrane to the intracellular environment, where they will accumulate and exert inhibitory activity.

### Table 1. Antimicrobial activity of *L. acidophilus* strains against Gram-negative bacteria

<table>
<thead>
<tr>
<th>Spectrum of <em>L. acidophilus</em> activity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aeromonas spp.</td>
<td>Al-Chezzy et al. (2011)</td>
</tr>
<tr>
<td><em>A. hydrophila</em></td>
<td>Aly et al. (2008)</td>
</tr>
<tr>
<td>Bacteroides thetaiotaomicron</td>
<td>Dubourg et al. (2015)</td>
</tr>
<tr>
<td>Enterobacter spp.</td>
<td>Coconnier et al. (1997)</td>
</tr>
<tr>
<td><em>E. aerogenes</em></td>
<td>Aween et al. (2012)</td>
</tr>
<tr>
<td><em>E. cloacae</em></td>
<td>Karaoğlu et al. (2003)</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>Coconnier et al. (1997); Demblé et al. (1998); Ogawa et al. (2001); Klewicka and Libudzisz (2004); Abo-Amer (2006); Ogunshe et al. (2007); Ogunbanwo and Okanlawon (2008); Medellin-Peña and Griffiths (2009); Wang et al. (2010); Omem and Faniran (2011); Nigam et al. (2012); Al-Chezzy et al. (2011); Aween et al. (2012); Elamathy and Kanchana (2013); Pyar et al. (2013); Bharal and Sohpal (2013); Dixit et al. (2013); Pehrson et al. (2013); Arias et al. (2013); Abo-Amer (2013); El-Kholy et al. (2014); Emami and Bazargani (2014); Venkadesan and Sumathi (2015); Saad et al. (2015); Kumar et al. (2016)</td>
</tr>
<tr>
<td>Helicobacter pylori</td>
<td>Michetti et al. (1999)</td>
</tr>
<tr>
<td>Klebsiella spp.</td>
<td>Al-Chezzy et al. (2011); Elamathy and Kanchana (2013)</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>Coconnier et al. (1997); Ogunshe et al. (2007); Omem and Faniran (2011); Dixit et al. (2013); Emami and Bazargani (2014)</td>
</tr>
<tr>
<td>Proteus spp.</td>
<td>Al-Chezzy et al. (2011)</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>Coconnier et al. (1997); Karaoğlu et al. (2003); Klewicka and Libudzisz (2004); Ogunshe et al. (2007); Nigam et al. (2012); Bharal and Sohpal (2013); Dixit et al. (2013); Abo-Amer (2013); Emami and Bazargani (2014)</td>
</tr>
<tr>
<td><em>P. fluorescens</em></td>
<td>Klewicka and Libudzisz (2004); Aly et al. (2008)</td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>Al-Chezzy et al. (2011); Salleh et al. (2014)</td>
</tr>
<tr>
<td><em>S. choleraesuis</em></td>
<td>Lin et al. (2008)</td>
</tr>
<tr>
<td><em>S. enteritidis</em></td>
<td>Pehrson et al. (2013)</td>
</tr>
<tr>
<td><em>S. paratyphi</em></td>
<td>Abo-Amer (2006); Abo-Amer (2013)</td>
</tr>
<tr>
<td><em>S. typhi</em></td>
<td>Bharal and Sohpal (2013); Venkadesan and Sumathi (2015); Dixit et al. (2013)</td>
</tr>
<tr>
<td><em>S. typhimurium</em></td>
<td>Coconnier et al. (1997); Aween et al. (2012); Arias et al. (2013); Abo-Amer (2013)</td>
</tr>
<tr>
<td><em>Shigella flexneri</em></td>
<td>Coconnier et al. (1997)</td>
</tr>
<tr>
<td><em>Shigella sonnei</em></td>
<td>Pehrson et al. (2013); Abo-Amer (2006); Abo-Amer (2013)</td>
</tr>
<tr>
<td><em>Vibrio parahaemolyticus</em></td>
<td>Wang et al. (2010)</td>
</tr>
<tr>
<td><em>V. cholerae serotype Inaba</em></td>
<td>Wang et al. (2010)</td>
</tr>
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</table>
(Fooks and Gibson, 2002).

Because some *L. acidophilus* strains were effective against meticillin-resistant *Staphylococcus aureus* and multidrug-resistant enterococcal *Escherichia coli*, they can serve as alternative therapeutic agents against the specific infections (Karska-Wysocki et al., 1998; Kumar et al., 2016). It has been reported that *L. acidophilus* reduce the levels of harmful bacteria and yeasts in the small intestine and the toxic amines in the blood of dialysis patients with small bowel bacterial overgrowth (Sanders and Klaenhammer, 2001).

### Antifungal activity

Researches on that aspect show that *L. acidophilus* is active against a variety of pathogenic and food spoilage moulds and yeasts - *Aspergillus*, *Fusarium*, *Mucor*, *Trichoderma*, *Candida* spp., etc. (Table 3). Some authors attributed the antifungal activity of LAB on the metabolic activity of acidification of the cytoplasm, which affects the proton motive force of the membrane, thus directly inhibiting fungal growth (Piper et al., 2001). De Muynck et al. (2004) pointed out that the excellent antifungal effect of *L. acidophilus* LMG 9433 is probably due to either organic acids or other pH-dependent antifungal compounds. Gerez et al. (2013) also found that the main metabolites are the organic acids – lactic, acetic and phenyllactic. Other authors confirmed that *L. acidophilus* exerts a biostatic effect on the fungal growth due to the accumulation of organic acids (Denkova et al., 2013). De Muynck et al. (2004) pointed out that the excellent antifungal effect of *L. acidophilus* LMG 9433 is probably due to either organic acids or other pH-dependent antifungal compounds. Gerez et al. (2013) also found that the main metabolites are the organic acids – lactic, acetic and phenyllactic. Other authors confirmed that *L. acidophilus* exerts a biostatic effect on the fungal growth due to the accumulation of organic acids (Denkova et al., 2013).

### Table 3. Antifungal activity of *Lactobacillus acidophilus* strains against *Aspergillus*

|-------------------------------------|---------------|-----------|-----------------|------------|----------------------|---------------------|---------------------|---------------------|-------------|------------|-----------------|-----------------|----------------|-----------|-------------------|----------|----------------|-----------------|-----------|-----------|-----------|

References

*References*
spoilage factors the use of antifungal LAB as biopreservatives is very promising (Schnürer and Magnusson, 2005). However, to avoid ambiguities considering the antifungal activity of L. acidophilus more studies of this probiotic bacteria are needed.

### Conclusion

The literature review on the problem showed that L. acidophilus could be used as a biocontrol agent in the gastrointestinal ecosystem of humans and animals, because of the broad spectrum of antimicrobial activity against many pathogens. The supplementation of L. acidophilus strains or its bacteriocins (lactacin B and F; acidophilin 801; acidocin A, B, 1B, CH5, Jl32, 8912, LF221 A, LF221 B and D20079; acidophilicin A; acidophilicin LA-1) to many food products could be done to prevent the growth of certain undesirable bacteria and fungi. To better understand the mode of action and the possible applications of L. acidophilus against pathogenic and food spoilage fungi more clinical and laboratory studies of different strains are required.

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Review

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**Thesis:**

Hristova D, 2013. Investigation on genetic diversity in local sheep breeds using DNA markers. Thesis for PhD, Trakia University, Stara Zagora, Bulgaria, (Bg).

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