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Lactococcus piscium : a psychotrophic lactic acid bacterium with bioprotective or spoilage activity in food - a review

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Abstract :

The genus *Lactococcus* comprises twelve species, some known for decades and others more recently described. *Lactococcus piscium*, isolated in 1990 from rainbow trout, is a psychrotrophic lactic acid bacterium (LAB), probably disregarded because most of the strains are unable to grow at 30°C. During the last 10 years, this species has been isolated from a large variety of food: meat, seafood and vegetables, mostly packed under vacuum (VP) or modified atmosphere (MAP) and stored at chilled temperature. Recently, culture-independent techniques used for characterization of microbial ecosystems have highlighted the importance of *L. piscium* in food. Its role in food spoilage varies according to the strain and the food matrix. However, most studies have indicated that *L. piscium* spoils meat, whereas it does not degrade the sensory properties of seafood. *L. piscium* strains have a large antimicrobial spectrum, including Gram-positive and negative bacteria. In various seafood, some strains have a protective effect against spoilage and can extend the sensory shelf-life of the products. They can also inhibit the growth of *Listeria monocytogenes*, by a cell-to-cell contact-dependent. This article reviews the physiological and genomic characteristics of *L. piscium* and discusses its spoilage or protective activities in food.

Keywords : lactic acid bacteria, biopreservation, spoilage, meat, seafood, cold adaptation.

Introduction

Lactic acid bacteria (LAB) constitute a heterogeneous group of Gram-positive bacteria, primarily non-sporulating, anaero-aerotolerant, and producing lactic acid as the principal end metabolite from carbohydrate fermentation. LAB can dominate the natural microbiota of many fermented foods where they play a key role in the development of the sensory properties (flavor and texture) and safety. In an appropriate environment, LAB can also colonize non-fermented products from plant or animal origin (Stiles and Holzapfel, 1997). Among LAB, the genus *Lactococcus*, and

particularly *Lactococcus lactis,* has been extensively studied, as some species are of major economic importance for the food bio-transformation industry (Stiles, 1996). The species *L. piscium* was isolated and characterized for the first time from diseased rainbow trout in 1990 (Williams et al., 1990). However, during the last 10 years its presence has been reported in various food and this species is gaining the interest of scientists. *L. piscium* is described either as a bioprotective or a spoilage microorganism depending on the strains and food matrix in concern.

This review deals with the characteristics of this species and its importance in food.

Taxonomy

Although many bacterial species produce lactic acid, the LAB group is restricted to fourteen genera, five of them constituting the core group (*Lactobacillus, Lactococcus, Leuconostoc, Pediococcus* and *Streptococcus*). The genus *Lactococcus* belongs to the phylum *Firmicutes*, Class *Bacilli*, Order *Lactobacillales* and Family *Streptococcaceae* (Samarzija et al. 2001). This genus is known for the ability to produce L-lactic acid from glucose. *Lactococcus* was proposed by Schleifer et al. (1985) to reclassify some species of the genus *Streptococcus* formerly included in the N-Lancefield group (lactic streptococci), according to DNA-DNA hybridization, 16S rRNA gene sequencing and basic physiological studies. Five species were initially described : *L. lactis, L. piscium, Lactococcus garvieae, Lactococcus raffinolactis* and *Lactococcus plantarum.* Recently, six new species have been described: *Lactococcus chungangensis* (Cho et al., 2008), *Lactococcus fujiensis* (Cai et al., 2011), *Lactococcus taiwanensis* (Chen et al., 2013), *Lactococcus formosensis* (Chen et al., 2014), *Lactococcus hircilactis* and *Lactococcus laudensis* (Meucci et al., 2015).

Phylogenetic analysis performed by the authors has revealed that the genus *Lactococcus* formed two significantly distinct phylogenetic groups (bootstrap ≥ 98 %) (Figure 1). The four subspecies of *L. lactis* (subsp. *lactis, cremoris, tractae* and *hordniae*), as well as *L. taiwanensis, L. hircilactis, L. fujiensis, L. garvieae* and *L. formosensis* formed the first group whereas *L. laudensis, L. raffinolactis,*

L. chungangensis, *L. plantarum* and *L. piscium* grouped close related together. Interestingly, this phylogenetic analysis indicated that *L. piscium* clustered with *L. plantarum* supported by a bootstrap value of 92% and 99% sequence similarity. This result confirmed those obtained by Rahkila et al. (2012) who obtained two distinct phylogenetic groups of *Lactococcus* using two different analysis (i) 16S rRNA gene sequences of twenty-two LAB strains and (ii) partial sequences of the housekeeping genes *rpo*A and *phe*S of seventy-one LAB. In the same study, they showed that numerical analyses of *Eco*RI and *Cla*I ribopatterns and phylogenetic sequence analyses of *rpo*A and *phe*S genes were reliable tools in species level identification of meat lactococci. In addition, the pangenome tree made on thirty lactococci genome revealed 3 major clades: (i) species of environmental or animal origin (*L. piscium*, *L. raffinolactis*, *L. chungangensis* and *L. garvieae*); (ii) *L. lactis* subsp. *lactis* strains and (iii) *L. lactis* subsp. *cremoris* strains (Andreevskaya et al., 2015).

Some strains, mainly *L. lactis*, are widely applied in industrial processes as starter cultures (Kelly et al., 2010), probiotics (Daniel et al., 2009) and protective cultures (Sarika et al., 2012). *L. lactis* is generally recognized as safe (GRAS) by the US FDA and considered by the EFSA to be suitable for the Qualified Presumption of Safety (QPS) approach to safety (EFSA, 2011). It has been used for decades by the dairy industry, and has thus been extensively studied as a model microorganism. Biochemical, physiological, and genetic aspects of *L. lactis* are widely described in the literature (for a review see Von Wright, 2012; Cavanagh et al., 2015). Over the years, interest has grown in the other four species initially described in the genus: *L. garvieae*, *L. raffinolactis*, *L. plantarum* and *L. piscium* (Boucher et al., 2003; Alomar et al., 2008; Matamoros et al., 2009a; Rahkila et al., 2012).

Habitat

L. piscium was described for the first time by Williams et al. (1990) in diseased rainbow trout. Its direct involvement in the disease has never been evidenced and, to the best of our knowledge, *L. piscium* has never again been isolated or identified by culture-independent techniques in the fish intestine microbiota. However, in marine farmed fish, other *Lactococcus* species have been shown to

be involved in epizoothics, such as *L. garvieae* responsible for septicemias, opthalmias and hemorrhages (Eldar et al., 1996; Vendrell et al., 2006) and *L. raffinolactis*, which has been identified as a fish commensal and also an opportunistic pathogen (Michel et al., 2007).

During the last 10 years, *L. piscium* has been isolated in a variety of chilled, modified atmosphere (MAP) and vacuum packed (VP) food (Table 1), including beef meat (Sakala et al., 2002a; Ferrocino et al., 2015; Jääskelänen et al., 2016), MAP marinated broiler meat leg (Björkroth et al., 2005), MAP skinned and boned broiler products (Vihavainen et al., 2007), raw salmon under MAP (Matamoros et al., 2009a; Macé et al., 2012), VP and MAP pork (Jiang et al., 2010; Rahkila et al., 2012), fermented turkey sausage (Kesmen et al., 2012), raw and cooked Belgium meat, tartar steak and ready-to-eat minimally processed vegetable salads (Delhalle et al., 2016; Pothakos et al., 2014a,b).

More recently, the use of culture independent techniques has revealed the presence of *L. piscium* in other products, although strains have not always been isolated. Chaillou et al. (2015a) conducted 16S rRNA gene pyrosequencing on 160 samples of fresh and spoiled foods to compare the bacterial communities associated with four meat products (ground veal and beef, diced bacon and poultry sausage) and four seafood products (salmon and cod fillet, cold-smoked-salmon (CSS) and cooked shrimp). *L. piscium* was the dominant species in ground veal and ground beef stored at 4 and 8°C under MAP (70% O_2, 30% CO_2). *L. piscium* was also in the top five species of the microbiota of MAP salmon fillets (50% O_2, 50% CO_2) and in the top twelve in CSS (Chaillou et al., 2015b).

The presence of *L. piscium* has also been reported in dairy products such as raw milk, using a novel multiplex PCR (Odamaki et al., 2011), and cheese, using 16S rRNA library sequencing (Carraro et al., 2011), as well as in human feces by rRNA-Targeted Reverse-PCR (Kubota et al., 2010).

Genomic characteristics of *Lactococcus piscium*

The complete genomes of numerous *Lactococcus* species, have been sequenced e.g. *L. lactis* (Bolotin et al., 2001; Makarova et al., 2006), *L. raffinolactis* (Meslier et al., 2012), *L. garvieae* (Morita et al., 2011), *L. piscium* (Marché et al., 2014; Andreevskaya et al., 2015), *L. fujiensis*

(http://www.ncbi.nlm.nih.gov) and *L. chungangensis* (https://www.patricbrc.org) (Table 2). Those species have genomes ranging between 1950 to 2641 kbp and contained 1947 to 2476 coding DNA Sequences (CDS). This indicates variability in genome size between species of *Lactococcus* up to 750 kbp. Genome-based analysis performed by Passerini et al. (2010) revealed that there are a genome size variability up to 600 kbp even within *L. lactis* subsp. *lactis* strains.

Concerning *L. piscium*, two different strains have been sequenced: *L. piscium* MKFS47, a spoiling strain of meat isolated from MAP broiler fillet strips (Andreevskaya et al., 2015) and *L. piscium* CNCM I-4031 (also named *L. piscium* EU2241), a bioprotective strain in seafood isolated from MAP raw salmon (Marché et al., 2014). The *L. piscium* MKFS47 genome size is ~ 2.5 Mb and GC% content is 38.79%. It contains one chromosome with 2394138 bp (2289 CDS) and two plasmids with 55671 bp (66 CDS) and 53257 bp (64 CDS). Annotated genomic nucleotide sequences are accessible through the European Nucleotide Archive (http://www.ebi.ac.uk/ena) under accession numbers LN774769-LN77477. The functions of deduced CDS-encoded proteins have been attributed to (i) proteins involved in primary and secondary metabolism and transport; (ii) transcription, translation, ribosomal structure and DNA replication and repair; (iii) cell division, envelope biogenesis and cell motility; (iv) metabolism, energy production and conversion; (v) signal transduction mechanism and (vi) proteins of unknown function (Andreevskaya et al., 2015). The *L. piscium* CNCM I-4031 genome size is ~ 2.26 Mb with 2239 CDS and GC% content is 39%. It contains one chromosome and one plasmid of 20 Kb. Annotated genomic nucleotide sequences are not accessible yet. A major part of its CDS-encoded proteins are classified as proteins of unknown function, 24% as enzymes, 20% represent transporters, regulators and factors, and 16% fall under components of cell processes and miscellaneous categories (Marché et al., 2014).

Physiological characteristics of *Lactococcus piscium*

L. piscium is a facultative anaerobic, Gram-positive, catalase- and oxidase-negative and non-motile cocci from 0.5 to 1 µm in diameter. Cells are spherical or ovoid and appear individually, in pairs or in short chains. A scanning electron microscopy picture is presented in Figure 2. Cai et al.

(2011) and Sakala et al. (2002b) showed that *L. piscium* was not able to grow at 30 g l^{-1} NaCl or higher. Leroi et al. (2012) later demonstrated that the $NaCl_{max}$ growth of *L. piscium* CNCM I-4031 was 23 $g.l^{-1}$. The optimal pH for growth was neutral and *L. piscium* could not grow at pH below to 4.8 (Cai et al., 2011; Leroi et al., 2012; Meucci et al., 2015).

Biochemical analysis, metabolic profiling and the genome analysis (Williams et al., 1990; Sakala et al., 2002b; Andreevskaya et al., 2015; Saraoui et al., 2016) revealed that *L. piscium* is a homo-fermentative bacterium that can ferment many carbon sources. Although only few *L. piscium* strains have been tested according to the literature, authors have shown that the following carbohydrates are fermented: glucose, fructose, lactose, galactose, gluconate, gentiobiose, mannose, maltose, melobiose, trehalose, arbutin, L-arabinose, *N*-acetylglucosamine, salicin, and D-raffinose. In addition, some strains can use saccharose, D-turanose, D-xylose, melezitose, α-methyl-D-glucoside, α-methyl-D-mannoside, mannitol and amygdalin (Williams et al., 1990; Sakala et al., 2002b; Fall, 2011; Chen et al., 2013). The fermentation of ribose is variable and strain-dependent (Sakala et al., 2002b; Andreevskaya et al., 2015). Sequencing and annotation of the genome of *L. piscium* MKFS47 by Andreevskaya et al. (2015) has shown that many catabolic pathways are predicted, including the degradation of monosaccharides (glucose, fructose, mannose, mannitol and xylose) and disaccharides (saccharose, maltose, lactose, trehalose and cellobiose). These authors also demonstrated that *L piscium* MKFS47 can grow on glycerol as a unique carbon source with a growth rate comparable to that obtained on glucose.

A metabolic profile of *L. piscium* strain CNCM I-4031 in a synthetic medium called “modified shrimp medium” (MSMA) containing glucose and other components showed that this strain catabolizes cysteine, histidine and glycine and, in lesser quantities, isoleucine, lysine and leucine, as well as the nucleic bases (adenine, guanine and uracil) and a vitamin (riboflavin) (Saraoui et al., 2016). In addition to these catabolic capacities, *L. piscium* harbors genes for the biosynthesis of all amino acids, except phenylanine, of purine/pyrimidine and also several cofactors/vitamins, such as riboflavin, folate, CoA, NAD, lipoate and polyprenyls (Andreevskaya et al., 2015). The *L. piscium* strains are able to hydrolyze aesculin but not arginine and they have no urease activities. Starch hydrolysis is

slow and weak while H_2S is not produced (Williams et al., 1990). Acetic acid production is strain-dependent. In fact, the strain CNCM I-4031 does not produce acetic acid (Fall, 2011) whereas this metabolite is produced by the strains R-46592 and MKFS47 (Pothakos et al., 2014c; Andreevskaya et al., 2015).

Cold-adaptation of *Lactococcus piscium*

The adaptation of LAB to different environmental conditions makes them of great importance in the food industry. For example, these bacteria can survive different environmental stresses caused by various steps in industrial processes, such as low temperature, high salt concentration, presence of preservative agents such as organic acids, and high CO_2 concentrations (Tsakalidou and Papadimitriou, 2011). *L. piscium* has long been disregarded in food, probably because the enumeration temperature commonly used for LAB enumeration has been 30°C. Since lower temperatures have been recently tested more frequently, *L. piscium* has been isolated in various chilled VP or MAP meat and seafood. Several strains of *L. piscium* were isolated on Elliker agar plates incubated at 8°C (Matamoros et al., 2009a). *L. piscium* is a psychrotrophic species, able to grow at 0°C with an optimum growth temperature at 24-26°C and a maximum growth temperature below 27-29°C, which is not common among LAB (Matamoros et al., 2009a; Leroi et al., 2012). Growth at 30°C is weak and variable among the strains. Another *L. piscium* isolated from raw salmon failed to grow at 30°C (Leroi et al., 2012) whereas *L. piscium* type strain grew at 30°C but not at 35°C (Williams et al., 1990). Growth at 5°C was observed for all of the 20 strains tested (isolated from fresh VP beef) and development was weak and variable at 30°C (Sakala et al., 2002b). Despite the absence of growth at 37°C (Williams et al., 1990; Sakala et al., 2002b; Leroi et al., 2012; Andreevskaya et al., 2015), it is noteworthy that *L. piscium* has been isolated from human intestine (Kubota et al., 2010). All the other *Lactococcus* species are mesophilic microorganisms with an optimum growth temperature around 30°C (Cavanagh et al., 2015; Meucci et al., 2015). *L. lactis, L.*

garvieae, *L. plantarum* and *L. raffinolactis* continued to grow at 35°C (Leroi et al., 2012). This characteristic may help to differentiate *L. piscium* from other *Lactococcus* species.

The unusual temperature-growth profile among Lactococci suggests that *L. piscium* is adapted to cold temperatures. Garnier et al. (2010) showed that the growth kinetics of *L. piscium* CNCM I-4031 at its optimum growth temperature (26°C) and after a cold-shock (0 or 5°C for 1 to 2 h) were similar (same growth rate and no lag phase). Additionally, no lag phase was observed when cultures were carried out at 5°C, after a pre-culture at 26°C, contrary to most *Lactococcus* species and other psychrotrophic LAB (Hamasaki et al., 2003). The specific result suggested that the proteins involved in the cold-shock response are constitutively produced. This is supported by the fact that the gene coding for the major cold-shock protein (CspE protein) was present in the *L. piscium* genome but its expression has been shown not to be regulated by cold-shock (Matamoros, 2008; Garnier et al., 2010). In other psychrotrophic bacteria, such as *Pseudomonas fragi* and *Bacillus subtilis*, the Csp protein was up-regulated in response to cold-shock and did not persist after the stress (Michel et al., 1997; Graumann and Marahiel, 1998). The comparison of proteome profiles of *L. piscium* at 26°C and after cold-adaptation (5°C) showed that the production of proteins involved in general and oxidative stress responses and in fatty acid and energetic metabolism was enhanced in cold conditions (Garnier et al., 2010). This can be explained by the fact that the Csp proteins play a significant role in many cellular processes such as general stress, cellular growth, nutrient stress and the stationary phase (Graumann and Marahiel, 1998).

Cold-adaptation constitutes an important advantage for bacterial competition in chilled food, especially against spoilage and pathogenic psychrotrophs, providing a promising perspective for food preservation.

Role of *Lactococcus piscium* in food spoilage

The quality of food can be determined by different sensory parameters such as appearance, odor, flavor and texture. The deterioration of freshness occurs progressively during storage due to internal reactions between food components, reactions of the components with water and air and,

mainly, the growth and metabolic activity of uncontrolled microorganisms (Lupien, 1997). The products become spoiled and unfit for human consumption and therefore have to be discarded. This process leads to significant economic losses and is a major problem for the food industry. Food waste at the consumer level in industrialized countries (222 million tons) is almost as high as the total net food production in sub-Saharan Africa (230 million tons) (Gustavsson et al., 2011). As a result, some strategies have already been adopted to prevent or delay this degradation, such as storage at chilled temperature, VP, MAP or addition of preservative agents (Borch et al., 1996). The microbial selection caused by these technologies gradually reduces the number of species present at the time of spoilage. As an example, in 160 samples of various meat and seafood products, the initial number of operational taxonomic units drastically decreased during MAP and VP storage. LAB and *Brochothrix* became dominant at the time of spoilage in meat, and LAB and *Photobacterium* in seafood (Chaillou et al., 2015a).

L. piscium has recently been shown to be one of the predominating species in chilled packed food, but its spoilage capability has to be demonstrated by challenge tests. In fact, it has clearly been established that in a food microbial ecosystem, only some microorganisms are involved in spoilage. This led to the concept of the specific spoilage organisms (Dalgaard, 1995; Leroi et al., 2015). The spoilage effect of *L. piscium* has been studied in different food matrixes by inoculating different strains into sterile or low contaminated food matrixes (Table 1). In sterile raw salmon fillets stored at 8°C under MAP (50% CO_2 - 50% N_2), the concentration of *L. piscium* increased from 3 to 9 Log (CFU g^{-1}) in 12 days and the samples were described as not spoiled by 56% of trained judges and lightly spoiled by 44 %. The weak odors associated were buttery and/or fatty fish–like (Macé et al., 2013). This low spoilage effect and characteristic off-odors are in accordance with other studies performed with different strains of *L. piscium* on CSS or cooked shrimp (Matamoros et al., 2009b; Fall et al., 2012; Leroi et al., 2015). In pork, *L. piscium* has a lightly spoiling effect (Rahkila et al., 2012). Two strains of *L. piscium* were inoculated on pork meat packed under MA conditions (71% O_2 - 22% CO_2 - 7% N_2) and stored at 6°C. The concentration of both strains reached approximately 8 Log (CFU g^{-1}). The products inoculated were characterized by buttery and sour odors after 2 weeks of

storage. The buttery off-odor was related to diacetyl/acetoin formation, which is frequently associated with the spoilage of food (Vihavainen et al., 2007; Jääskeläinen et al., 2015). After 48 h in modified Man-Rogosa-Sharpe (MRS) medium without acetate and with 2% glucose, a final concentration of diacetyl and acetoin produced by *L. piscium* reached 8.5 mM (Andreevskaya et al., 2015). In MAP (70% O_2 - 30% CO_2) ground veal at 8°C, *L. piscium* was shown to modify the color greatly, from red to gray (Denis et al., 2014). In ground beef under the same conditions, *L. piscium* acidified the meat (lowering 0.45 units the pH) and deteriorated the color, which became gray/green and released a strong rancid odor (personal communication from Souad Christeans, 2014).

The spoilage effect of three strains of *L. piscium* isolated from beef, pork and sweet peppers was studied in bell pepper simulation medium under three different conditions of gas composition: (i) 100% N_2; (ii) air: 21% O_2 and 79% N_2; (iii) MAP_1: 30% CO_2 and 70% N_2 and (iv) MAP_2: 50% O_2 and 50% CO_2 (Pothakos et al., 2014a). In the first three conditions, all strains reached about 7-9 Log (CFU g^{-1}) with some differences in growth speed between the strains. For the MAP_2 condition, only the strain isolated from beef was able to grow and reached about 8 Log (CFU g^{-1}) at the end of storage, suggesting that the combination of high O_2 and CO_2 concentration had a significant inhibitory effect on *L. piscium.* Only one strain, showing the best growth in all packing conditions, had a significant spoiling effect. This effect was correlated to the production of some metabolites that are involved in spoilage, such as ethanol after 7 days in 100% N_2 and MAP_1 conditions, acetic acid after 7 days in air and 2,3 butanedione (diacetyl) after 13 days in the MAP_2 condition (Pothakos et al., 2014c). These results are supported by the presence in the *L. piscium* genome of four predicted pathways for pyruvate utilization: acetoin/diacetyl, pyruvate dehydrogenase, L-lactate dehydrogenase and pyruvate-formate lyase pathways (Andreevskaya et al., 2015). Many significant spoilage substances, such as acetoin/diacetyl and acetate, are produced by these pathways.

Protective effect of *Lactococcus piscium*

During the recent years, consumers have shown a great interest in ready-to-eat, minimally processed and fresh-tasting food. In this context, chilled storage and modification of the gaseous environment of food have been developed and have become important and acceptable methods for food preservation (Cortesi et al., 2009). However, the drawback of these technologies is that the safety and quality of the product has to be maintained throughout a significantly-increased storage time (Ross et al., 2002). The physico-chemical characteristics of these products allow the development of a wide range of undesirable microorganisms, like pathogenic and spoilage bacteria. Biopreservation, which consists of inoculating food with selected protective bacterial strains that can inhibit undesirable components of the microbiota, is an increasing practice in the food industry. Many studies have demonstrated the interest of LAB such as *Carnobacterium*, *Lactobacillus*, *Lactococcus* and *Leuconostoc* for this purpose (for a review, see Rouse and van Sinderen, 2008; Lacroix, 2010; Ghanbari et al., 2013). In this context, the role of *L. piscium* has been extensively studied in recent years, mainly in seafood.

The positive effect of *L. piscium* on the sensory quality of VP cooked and peeled tropical shrimp was demonstrated for the first time by Matamoros et al. (2009a). After 28 days of storage at 8°C, the shrimps inoculated with *L. piscium* CNCM I-4031 and EU2229 were not spoiled whereas the control released very strong off-odors described as “cheese and feet” by sensory panel. These authors also showed that the strain CNCM I-4031 improved the sensory quality of VP CSS. However, the protective effect seems to be strain-dependent as *L. piscium* EU2229 had no effect on the sensory quality of the same batch of VP CSS. In another set of experiments, Leroi et al. (2015) confirmed the beneficial effect of *L. piscium* CNCM I-4031 in one batch of naturally contaminated CSS out of two batches tested, from different smokehouses, suggesting that the protective effect of this strain may vary according to its interaction with the spoiling microorganisms.

Antimicrobial activity of *Lactococcus piscium*

The antimicrobial activity of *L. piscium* has not been commonly tested. The inhibitory capability of *L. piscium* CNCM I-4031 and EU2229 against Gram-positive and -negative spoilage bacteria relevant in meat and seafood was tested using a diffusion test on Petri dishes (Matamoros et al., 2009a; Fall et al., 2010a). These strains had a large activity spectrum towards strains of *Brochothrix, Lactobacillus, Carnobacterium, Vagococcus, Enterococcus, Psychrobacter, Shewanella, Pseudomonas* and *Serratia* (Table 3). The inhibitory activity was confirmed on a seafood matrix for *B. thermosphacta,* which is considered a major spoilage bacteria in VP and MAP meat and seafood. *L. piscium* CNCM I-4031 inhibited the growth of *B. thermosphacta* by 3-4 Log (CFU. g^{-1}) in cooked and peeled shrimps (Fall et al., 2010a; 2012) and totally stopped its growth in CSS (Leroi et al., 2015). The inhibition of *B. thermosphacta* had been reported previously with some LAB such as *Lactobacillus* spp. in meat (Castellano and Vignolo, 2006; Russo et al., 2006) but not with some other such as *Carnobacterium* spp. in MAP shrimp (Laursen et al., 2006).

L. piscium is also able to inhibit the growth of pathogens or opportunistic pathogens such as *E. coli, Salmonella, Staphylococcus aureus, Clostridium sporogenes* and *L. monocytogenes* (Table 3). (Matamoros et al., 2009a; Fall et al., 2010b). The antagonist activity against *L. monocytogenes* is particularly relevant in meat and seafood since this pathogen is frequently isolated from these foodstuffs (Gambarin et al., 2012; Lomonaco et al., 2015). Its anti-listeria activity has been confirmed in VP and MAP cooked and peeled shrimp by Matamoros et al. (2009a) and Fall et al. (2010b).

The antimicrobial activities of LAB against food spoiling bacteria is generally associated with the production of (i) antimicrobial peptides, such as bacteriocins (Stiles, 1996; Brillet et al., 2005) or reuterin (El-Ziney et al., 1999); (ii) organic acids, such as acetic and lactic acid (Wong and Chen, 1988); (iii) hydrogen peroxide (Alomar et al., 2008) and with (iv) nutrient competition (Nilsson et al., 2005). In the case of *L. piscium* CNCM I-4031, the mechanism involved in its antimicrobial properties has not yet to be elucidated, remaining a challenge for researchers. Different tests performed on various culture media and food matrix demonstrated that the inhibition of *L.*

monocytogenes was not due to the production of extracellular antimicrobial compounds (Matamoros et al., 2009a; Saraoui et al., 2016). The strain MKFS47 has not been studied for its antimicrobial activity but its genome analysis has revealed the presence of some genes that could be involved in putative antimicrobial factors. This strain contains three gene clusters involved in the biosynthesis of putative bacteriocins. In addition, various enzymes putatively involved in H_2O_2 biosynthesis have been identified. However, these different gene clusters may not be activated in all environmental conditions. As an example, H_2O_2 was produced from glycerol under aerobic conditions but not from glucose under aerobic and anaerobic conditions (Andreevskaya et al., 2015). Nutrient depletion has also been reported to explain the competition between microbial populations (Hibbing et al., 2010). For instance, Juillard et al. (1998) showed a nutrient competition for non-protein nitrogenous substrates in milk between *Lactococcus* and *Leuconostoc* strains. Nilsson et al. (2005) showed that the inhibition of *L. monocytogenes* by *Carnobacterium piscicola* was due to the competition for glucose. Saraoui et al. (2016) demonstrated that the inhibition of *L. monocytogenes* by *L. piscium* was not due to nutrient competition (various compounds tested). The inhibition occurred in co-culture but not in a diffusion chamber, where bacteria were separated by a filter membrane, nor in medium pre-fermented by *L. piscium.* These results indicate that the inhibition of *L. monocytogenes* by *L. piscium* is through a non-uncharacterized, cell-to-cell contact-dependent that are never been reported in LAB.

Conclusion

L. piscium is gaining the interest of researchers because it is increasingly isolated from various meat, seafood, vegetable and dairy products. It is the only psychrotrophic species in the genus *Lactococcus* and thus can play an important role in minimally processed products stored at chilled temperatures. The spoiling activity depends on the strain, the food matrix and storage conditions. In most cases, *L. piscium* does not spoil seafood and even has a protective effect, whereas it strongly alters the quality of meat, with discoloration, acidification and the production of buttery, rancid and sour off-odors. In seafood, some strains can prevent the spoilage and extend the sensory shelf-life.

This has been attributed to the inhibition of *B. thermosphacta*, a major spoilage bacterium in refrigerated packed meat and fish. However, other mechanisms are probably involved, as a protective effect observed in one batch of CSS could not be attributed to the reduction of this species, nor other bacteria enumerated by classic and culture-independent methods (Leroi et al., 2015). An anti-listerial effect of *L. piscium* has also been observed and attributed to cell-to-cell contact, although the mechanism still remains unknown. This inhibitory mechanism has never been described in other LAB. Comparative genomic and transcriptomic analyses may help to answer many questions concerning the spoiling and protective potential of *L. piscium*.

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Conflict of interest

There is no conflict of interest.

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Figure 1

Phylogenetic relationship of species and subspecies of the genus *Lactococcus*. The 16S rRNA gene sequences of the eleven different *Lactococcus* species, including the four subspecies of *L. lactis* and two genome sequenced *L. piscium* (♦), were retrieved from GenBank (http://www.ncbi.nlm.nih.gov). After the trimming of the gaps and missing data, a total of 1330 positions were aligned using clustal W software. The construction of phylogenetic trees was performed with MEGA 6 Toolbar using the neighbor-joining method with bootstrap of 1000 replicates. *Lactobacillus curvatus* was used as an outgroup species.

Figure 2: Scanning Electron Microscopy of *Lactococcus piscium* CNCM I-4031 after 24 h of culture in Elliker medium (x 30000).

Table 1: Food sources of *Lactococcus piscium* and its sensory effect, when inoculated into fish and meat product.

Food and storage condition	Strain	Sensory effect	Reference
Fish and sellfish			
- Rainbow trout fish	GTC 552	Not examined	Williams et al., (1990)
- MAP fresh salmon-8°C	CNCM I 4031	Not spoiled (cold smoked salmon) Butter and smoke odors - 4 week of storage	Matamoros et al., (2009a), (2009b)
		Not spoiled (shrimp) Cheese and feet/no odors - 4 week of storage	Fall et al. (2010a), (2012); Matamoros et al., (2009a), (2009b)
	EU2229	Not spoiled/spoiled (cold smoked salmon) Butter and smoke odors - 2 week of storage Cheese/feet, amine, acid, and sour odors- 4 week of storage	(Matamoros et al. (2009a), (2009b)
		Not spoiled (shrimp) Cheese and feet - 4 week of storage	Matamoros et al., (2009a), (2009b)
- Raw salmon-MAP 4-8°C	MIP 2434, MIP 2450, MIP 2482, MIP 2484	Not spoiled/Lightly spoiled (raw salmon) Butter and fatty fish - 12 days of storage	Macé et al. (2013)
Meat			
- VP Fresh beef - 2°C	E2B2, A2T2, C2T11, C2T15	Not examined	Sakala et al. (2002b)
- VP beef	R-46592	Spoiler (sweet ball pepper simulation medium)	Pothakos et al.(2014a), (2014c)
- MAP meet product	LMT33-6	Lightly spoiled (pork) Buttery- 14 days	Rahkila et al.(2012)

		JL3-4	Lightly spoiled (pork) Sour – 16 days	Rahkila et al. (2012)
-	VP pork - 4°C	EU621998	Not examined	Jiang et al., (2010)
-	VP pork	R-46738	Lightly spoiler (sweet ball pepper simulation medium)	Pothakos et al.(2014a), (2014c)
-	MAP broiler filet strips	MKFS47	Spoiled (pork) Buttery	Andreevskaya et al. (2015)
Vegetable				
-	Sweet bell pepper salad (air)	R-46976	Lightly spoiler (sweet ball pepper simulation medium)	Pothakos et al.(2014b)

Table 2: Genome overview of some *Lactococcus* strains genome sequenced including the two *L. piscium* strains genome sequenced.

Strain	Genome deposit number (GenBank)	Genome size (kbp)	GC%	Plasmid(s)	CDS	Ribosomal RNA operons	tRNA	Reference
Lactococcus piscium CNCM I-4031	In progress	2257	39	1	2239	4	55	Marché et al. (2014)
Lactococcus piscium MKFS47	LN774769-LN77477	2394	38.79	2	2476	4	56	Andreevskaya et al., (2015)
Lactococcus lactis subsp. *lactis* IL1403	AE005176	2365	35.4	0	2310	6	62	Bolotin et al. (2001)
Lactococcus lactis subsp. *cremoris* SK11	CP000430	2641	35.8	5	2509	6	62	Makarova et al., (2006)
Lactococcus garvieae ATCC 49156	AP009332	1950	38.8	0	1947	5	62	Morita et al., (2011)
Lactococcus raffinolactis 4877	CALL00000000	2280	38.7	0	2418	-	48	Meslier et al. (2012)
Lactococcus fujiensis JCM 16395	BBAL00000000.1	2088	36.9	nd	2252	4	47	http://www.ncbi.nlm.nih.gov
Lactococcus chungangensis CAU 28	-	2243	40	nd	2194	3	47	Cho et al. (2008); https://www.patricbrc.org

Table 3: Antimicrobial activity of *Lactococcus piscium* strains against spoilage and pathogenic bacteria relevant in food

Methods	***L. piscium strains***	**Target strain**	**Growth inhibition**	**Reference**
Agar spot assay with *L. piscium* colony	EU2229	*Staphylococcus aureus*, *Brochothrix thermosphacta*, *Psychrobacter* sp., *Pseudomonas* sp., *Serratia liquefaciens*, *Photobacterium phosphoreum*, *Shewanella putrefaciens*, *Clostridium sporogenes*, *Lactobacillus farciminis* and *Listeria monocytogenes*	Inhibited	Matamoros et al. (2009a)
		Bacillus subtilis, *Staphylococcus xylosus*, *Escherichia coli*, *Salmonella enterica*	Not inhibited	Matamoros et al. (2009a)
	CNCM I-4031	*Brochothrix thermosphacta*, *Carnobacterium alterfunditum*, *C. divergens*, *C. maltaromaticum*, *Clostridium sporogenes*, *Escherichia coli*, *Lactobacillus farciminis*, *Listeria monocytogenes*, *Photobacterium phosphoreum*, *Psychrobacter* sp., *Pseudomonas* sp., *Salmonella enterica* serovar Typhimirium, *Serratia liquefaciens*, *Serratia* sp., *Shewanella putrefaciens*, *Staphylococcus aureus*, *Vagococcus fluvialis* and *Vagococcus carniphilus*	Inhibited	Fall et al. (2010a); Matamoros et al. (2009a)
		Bacillus subtilis, *Staphylococcus xylosus*, *Vibriosp*.	Not Inhibited	Fall et al. (2010a); Matamoros et al. (2009a)
Agar spot assay with *L. piscium* supernatant (MSMA medium)	CNCM I-4031	*Listeria monocytogenes*	Not Inhibited	Fall et al. (2010a); Saraoui et al. (2016)
Co-culture on MSMA medium	CNCM I-4031	*Listeria monocytogenes*	Inhibited	Saraoui et al. (2016)
Peeled and cooked shrimp	CNCM I-4031	*Brochothrix thermosphacta*	Inhibited	Fall et al. (2010a)
		Listeria monocytogenes	Inhibited	Fall et al. (2010b)
Cold smoked salmon	CNCM I-4031	*Brochotrix. thermosphacta*, *Serratia proteamaculans*	Inhibited	Leroi et al. (2015)
		Photobacterium phosphoreum	Not inhibited	Leroi et al. (2015)