Characterization and Antibiotic Susceptibility of Lactobacilli, Pathogenic and Spoilage Bacteria Isolated from Meats

ABDI Akila[(1;2)*] ; BENHADJ Mabrouka[(1)] ; ALIOUA Souad[(1;2)] ; GACEMI-KIRANE Djamila[(1)]

1*-Departement of Biochemistry, Faculty of Sciences, University BADJI Mokhtar Annaba
Annaba B.P. 12 ALGERIA
2*-Laboratory of Applied Biochemistry and Microbiology, University BADJI Mokhtar Annaba
Annaba B.P. 12 ALGERIA

akila.abdi@yahoo.com

Abstract: The meat very rich on nutritive elements constitutes an excellent culture medium, which offers a wide spectrum of microbial contamination. This microflora of contamination is very heterogeneous constituted by pathogenic, spoilage, and protective (lactic acid bacteria) microorganisms. Our investigation is about the choice of specific cultures medium in order to isolate some group of bacteria (Lactobacillus, Staphylococcus, Pseudomonas and Enterobacteria) in detriment of others. Those bacteria were isolated from 19 samples of red meat (beef and sheep) under different conditionings. Their phenotypical characterization based on morphological, physiological, biochemical characters and their susceptibility to the antibiotic has been determined. The 29 strains of isolated lactobacilli were attributed to group II and III of Kandler and Weiss. The strains of Staphylococcus, Pseudomonas and Enterobacteria have been also identified until species by Api identification systems. The 29 strains of isolated lactobacilli present resisting to the majority of antibiotics used. The other bacteria (pathogenic and spoilage) have variable profiles according to the antibiotics.

Keywords: red meat - different conditionings – lactobacilli - pathogenic and spoilage bacteria – identification - antibiogram.

Introduction

Meat and in general meat products because of their nutrient richness (Bourgeois, 1996) are media of choice for different microbial species. Among which bacterial pathogens should be avoided because dangerous to health; spoilage bacteria which are to be avoided because if they do not present a health hazard, they can cause significant economic loss (Jacotot, 1999) and lactic acid bacteria that are part of the microflora of fresh meat, but are predominant in meat vacuum packaged (Sutra, 1998; Sakala, 2002). Fermented meat products are processed through the development of lactic acid bacteria involved in their processing, conservation and stabilization of their organoleptic quality. Many studies have shown that lactic acid bacteria have properties that could be used to better preservation of fresh meat, as they offer activities that allow them to antagonists inhibit undesirable microflora (Dembele et al., 1998). Meat lactobacilli have original properties to adapt to this biotope complex that they confer a role bioprotectant, which can extend the shelf life and thus ensure safety of meat (Schillinger and Luke, 1989; Marceau et al., 2004).

Lactic acid bacteria used in the transformation and improvement of meat products are not yet used in Algeria in the meat industry and red meat, which is a field for innovation and research development.

The interest is to isolate lactic acid bacteria of meat sold in our markets, to determine their antagonistic properties.

This first part of our study focuses on the isolation of lactobacilli and other bacteria in the presence (pathogens and spoilage) from red meat (beef and sheep) fresh or packaged in different ways, it is clearly established that packaging under vacuum or modified atmosphere promote lactic acid bacteria that have become the majority.

Collection strains of lactobacilli identified will be subject to further research which will focus on the identification of antibacterial activity and characterization of the molecules involved. Character search technology interesting for diversification and proper preservation of fermented meat products is also considered.

Materials and Methods

Nineteen (19) samples of red meat (beef and sheep) from local markets in the region of Annaba (Algeria-East) were collected and placed in sterile bags stomachers (4°C). These samples were packaged in various forms: fresh meat , ached meat, and meat packaged under vacuum or CO_2 (packaging done in the laboratory or in the conditioning-center) stored at 4°C, dried and salted meat traditionally "Guedid" kept at room temperature and meat frozen at -20°C.

25 g of meat were ground aseptically and homogenized with 225 ml of saline-peptone water (NaCl 8.5g/l ; bactopeptone 1g/l), and the mixture was incubated for 24 hours at room temperature according to the method of Najjari and al. (2008). From this stock solution, serial dilutions were then made in saline-peptone water (Bonnefoy et al., 2002). The isolation of lactobacilli was carried out on two agar media: (i) MRS (De Man, Rogosa and Sharpe, 1960) containing bromocresol green (25mg/l), the pH is adjusted to 6.4 (Bonnefoy et al., 2002). 0.2 ml of dilutions (10^{-5} to 10^{-9}) was plated on the surface of the medium in Petri dishes, which were incubated at 30°C for 24 to 48 hours under anaerobic conditions; (ii) LAMVAB (Lactobacillus Anaerobic MRS with Vanconmycin Bromocresol green), the pH is adjusted to 5 ± 0.5 (Hartemink et al., 1997) is a selective medium for the isolation of lactobacilli. Gram negative bacteria are inhibited by the acidity of the medium, and other Gram-positive bacteria that no lactobacilli are inhibited by vancomycin (20mg/l); this medium also contains a reducing agent cysteine (0.5g/l) increasing the anaerobic conditions and bromocresol green as an indicator of pH. 0.2 ml of dilutions (10^{-1} to 10^{-4}) was plated on the surface of the medium in Petri dishes, which were incubated at 37°C for 24 to 72 hours under anaerobic conditions.

The isolation of other pathogens and spoilage microorganisms is done on different media and under different conditions specific bacterial type to sought (Larpent, 2000). 0.2 ml of the 10^{-1} dilution was spread on different media respectively specific bacteria investigated.

Staphylococci were isolated in two media: (i) on Chapman agar, after incubation 24h-48h at 37°C; pigmented colonies appear yellow and/or pink; (ii) on Baird Parker agar, colonies of *S. aureus* have a characteristic appearance after 24 hours of incubation at 37°C: shiny black colonies surrounded by a clear halo 2 to 5 mm in diameter, most other species are inhibited or produce no characteristic colonies after 24 hours.

Enterobacteria are isolated on Hectoen agar, after incubation 24-48h at 37°C; by fermentation of at least three sugars (salicin, saccharose, lactose) and production of hydrogen sulfide, which guides to genus or species bacteria.

Pseudomonas: are isolated on agar media with cetrimide, after incubation for 24h at 30°C, the presence of blue-green colonies moving towards *Pseudomonas aeruginosa*, the presence of green fluorescent colonies moving towards *Pseudomonas fluorescens*.

The purified isolates were differentiated by Gram stain, the search for catalase and oxidase activity according to the protocols described by Prescott et al. (2003).

Purified isolates of media MRS and LAMVAB represented by Gram positive, catalase and oxidase negative were considered as potential lactic acid bacteria and were stored at -30°C in MRS broth and in skim milk (reconstituted 10%) to 20% glycerol and on MRS solid tube at 4°C (Accolas et al., 1972).

Other isolates purified of specific media for pathogenic and spoilage bacteria are seeded with bite in the central conservation agar, after 18h at 37°C, the cultures were stored at 4°C. Conservation at -30°C in liquid medium specific added glycerol is also possible.

Characterization of the isolates was performed by testing physiological, biochemical and identification systems API. Identification is established based on morphological characters of colonies and cells and the type of Gram, and various physiological and biochemical specific characters to genus and/or species (Larpent, 2000) such as catalase, oxidase, growth at different temperatures (10°C, 15°C, 45°C), carbon dioxide production by fermentation of glucose, fermentation of various sugars, growth at different pH, different levels of NaCl, looking for specific enzymes and highlighting pigments.

The bacilli Gram positive, catalase and oxidase negative, that other test results meet to the Lactobacillus's characteristics have been a biochemical analysis using a range of sugars provided in the API 50CHL (Bio Merieux sa: in Leyral and Joffin , 1998).

The identification of other bacteria was complemented by systems API Staph, API 20E and API 20 NE (Bio Merieux sa: in Leyral and Joffin 1998). Seeding and reading galleries were performed according to the manufacturer's instructions (Bio Merieux, France). Biochemical profiles obtained are read using software API Plus (Bio Merieux, France) and identification charts (Leyral and Joffin, 1998).

The antibiogram can keep a record of the characters of sensitivity or resistance to add at identification, is a real identity card microorganism. The method used is that of Kirby-Bauer in Qin et al. (2004), recommended by the NCCLS (Nationnal Commitee For Clinical Laboratory Standard). The disk diffusion method on agar is described in the release of the 2007 Antibiogram Committee of the French Society for Microbiology (CASFM) Cavallo et al. (2007). The media used are MRS for lactobacilli (Aniewska-Moroz et *al.*, 2001; Ammor et *al.*, 2007) and Muller Hinton (MH) for other bacteria (Cavallo et *al.*, 2007). Antibiotics are used in the form of discs (Oxoid) impregnated with pure antibiotic at a level defined Cavallo et al. (2007).

RESULTS:

Different contaminating bacteria were isolated from 19 samples of meat.

Selective media were chosen to target specific pathogens and spoilage bacteria at the expense of others. Lactobacilli were isolated on two selective media MRS medium supplemented with bromocresol green and LAMVAB medium.

The results of the cultivation, of Gram stain, catalase and oxidase allowed us to divide the different bacterial groups for 19 samples of all types of meat analyzed (Figure 1).The results of identify tests physiological, biochemical, systems Api, compared with bibliographic data allowed us to bring in different genus and species.

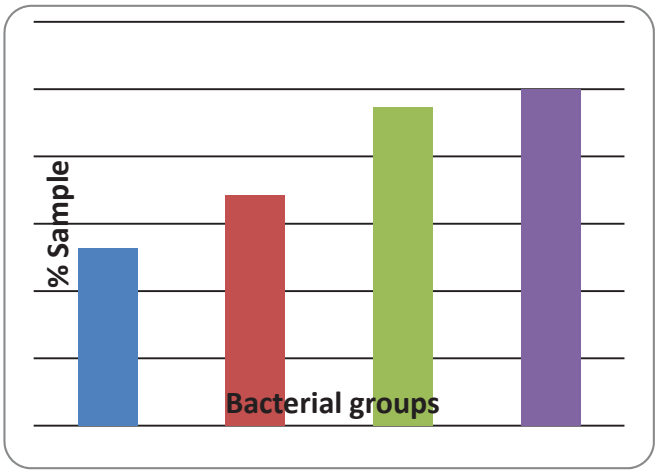


Figure 1: Distribution of different bacterial groups isolated from different types of meat.
C: cocci; **B**: bacilli; **NE**: Non enterobacteria; **E**: Enterobacteria ; **+** : positive ; **-** : negative

Distribution and identification of pathogenic and / or spoilage bacteria:

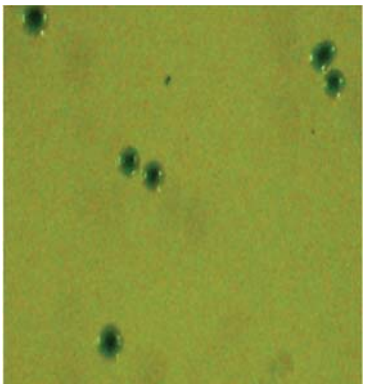
The cocci Gram positive are represented in 52.63% of samples. The absence of this group in three samples packaged under vacuum shows that the vacuum exerts an inhibitory effect on the development of the aerobic bacterial flora. Cultures in the Chapman medium have different aspects: small and large colonies to smooth contour, color is yellow due to the fermentation of mannitol or pink due to non mannitol fermentation. The results of preliminary tests (catalase and coagulase-free) and identification by the API Staph shows that all Gram positive cocci were identified to the genus Staphylococcus: *Staphylococcus aureus* (50%), *Staphylococcus xylosus* (30%) and *Staphylococcus epidermidis* (20%).

The bacilli non-Enterobacteria Gram negative are represented in 68.42% of the samples, macroscopic observation on Cetrimide agar show whitish colonies and pigmented colonies. The results of preliminary tests and API 20 NE show that these Gram negative non-Enterobacteria were identified and divided into two genera: the genus Chromobacterium represented by single species *Chromobacterium violaceum*, the genus Pseudomonas represented by *Pseudomonas fluorescens* (61.53%) and *Pseudomonas putida* (30.76%). Pseudomonas is the main agent responsible for the spoilage of the meat. We note its absence in two samples packaged under vacuum, the absence of oxygen resulted in the inhibition of the growth of Pseudomonas (strict aerobic bacteria).

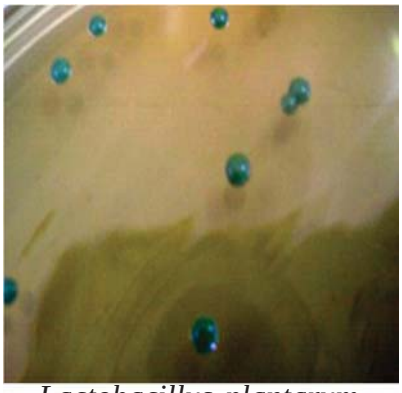
The bacilli Enterobacteria Gram negative represented in 94.73% of the samples, macroscopic observation of cultures in the Hectoen medium show colorless colonies are lactose negative, colonies salmon-colored are lactose positive, colorless colonies with black center that are lactose negative but produce H_2S and salmon-colored colonies with black center are lactose positive and H_2S positive. The results of the API 20 E and other tests show the presence of different species. The species *Hafnia alvei*1 (26.31%), *Serratia liquifasciens* (15.78%), *Escherichia coli* (10.52%), *Enterobacter cloacae* (10.52%) and other species such as *Escherichia fergusonii*, *Klebsiella pneumoniae*, *Enterobacter intermedius*, *Enterobacter asburiae*, *Proteus vulgaris*, *Salmonella arizonae* and *Edwarsiella hoshinae*, each representing 5.26% of Enterobacteriaceae identified. The diversity of species isolated in this family confirms that contamination of meat is due to several causes. This group includes species causing spoilage such as *Hafnia alvei*1 and pathogenic species such as *Klebsiella pneumoniae* and *Salmonella arizonae*.

Distribution and identification of lactobacilli

The bacilli Gram positive was isolated from all samples. Macroscopic observation of the cultures on two media: MRS with bromocresol green and LAMVAB shows different types of colony (colonies dark green, bright green colonies, transparent colonies green center, green colonies dark center and gray colonies) small or large sizes, these correspond to different aspect colonies of different species of lactobacilli and can guide the identification of the strains (Figure 2). The use of LAMVAB media highly selective for the genus Lactobacillus has allowed us to avoid the excesses of subcultures which can lead to mutations.



Lactobacillus sakei



Lactobacillus plantarum

Lactobacillus rhamnosus

Lactobacillus divergens

Figure 2: Appearance of colonies of Lactobacillus species on the medium LAMVAB.
(LAMVAB : Lactobacillus Anaerobic MRS with Vanconmycin Bromocresol and green).

The preliminary test results show that these are Gram positive, catalase and cytochrome oxidase negatives, motionless, non spore-forming, which are characteristic of the genus Lactobacillus (Axelsson, 1998). The results of tests of differentiation in groups, compared with bibliographic data (Kandler and Weiss, 1986; Schillinger and Luck, 1987; Larpent, 2000) allowed us to divide the 29 strains of lactobacilli between only two groups: 65.51% belong to group II and 34.49% belong to the group III, against any strain was identified to belong to group I. All strains can grow at 4°C and 15°C but not at 45°C.

The results also show that the majority of these bacteria tolerate a concentration of 7% NaCl five strains tolerate up to 10% NaCl, where their presence in salted meats. All strains grow well on MRS at pH 3.9 ; highlighting an arginine deaminase in all strains of lactobacilli, show their ability to degrade arginine what they confer an advantage, it is to grow on media without or with small quantities of glucose, hydrolysis of this amino acid allows them to synthesize ATP and compete with undesirable bacteria.

Strains of lactobacilli pre-identified on the basis of morphological, physiological and biochemical to data as reference (Kandler and Weiss, 1986; Schillinger and Luck, 1987), and those given by Holt et al. (1994) in Bergey's Manual of Determinative Bacteriology 9th edition). According to the identification criteria given by Leyral and Joffin (1998) and to data from Brossard et al. (2008), these strains were attributed only between groups II and III of Kandler and Weiss (1986). The approximation to species for all these strains is given through their fermentation profiles of the 26

sugars tested (present in the system Api 50CHL). The results for some sugars have been used as key comparison of profiles between species identified.
Nineteen strains of lactobacilli were assigned to group II which are optional heterofermentative, all these strains do not produce CO_2 by fermentation of glucose, except *L. plantarum* $14_{(4)}$; ferment pentose (mainly ribose) do not grow at 45°C, are mesophilic, except *L. casei* $19_{(2)}$. These strains were close to the following species:
Two *Lactobacillus casei*: do not ferment rhamnose and xylose, but ferment mannitol, sorbitol and.amygdalin
Four *Lactobacillus rhamnosus*: ferment rhamnose, mannitol, sorbitol and amygdalin, do not ferment xylose.
Four *Lactobacillus sakei*: do not ferment rhamnose, mannitol, sorbitol, and xylose. These strains were isolated mainly meat samples packaged under vacuum or CO_2.
Two *Lactobacillus curvatus*: which are a closely related to *L. sakei*, but do not ferment amygdalin and arabinose.
One *Lactobacillus alimentarus*: does not ferment rhamnose, mannitol, sorbitol, and xylose, it is a closely related to *Lactobacillus curvatus*, but does not ferment lactose.
Five *Lactobacillus plantarum*: do not ferment rhamnose but ferment mannitol, sorbitol and amygdalin.
One *Lactobacillus murinus*: This is close to *L. rhamnosus*, but does not ferment rhamnose.
Ten strains of lactobacilli were assigned to group III, are strict heterofermentative, CO_2 produced by fermentation of glucose, ferment pentose (mainly ribose), and are for the vast majority of mesophilic. These strains were close to the following species:
Two *Lactobacillus bifermentans*: ferment fructose, galactose, glucose, maltose, mannitol, mannose and rhamnose.
One *Lactobacillus fermentum*, near *L. bifermentans* ferments more gluconate and lactose, does not ferment mannitol and rhamnose.
Two *Lactobacillus fructivorans*: ferment fructose, glucose, maltose, saccharose and gluconate.
One *Lactobacillus sanfrancisco*, near *L. fructivorans*, ferments more galactose, but not fructose and ribose.
Three *Lactobacillus divergens*: ferment amygdalin cellobiose and have profile which brings them closer to or L. bifermentans, or L. fermentum respectively.
One *Lactobacillus confusus*: ferment xylose, esculine, this is close to *L. divergens*, but does not ferment trehalose.

Characteristics of pathogenic and spoilage bacteria against antibiotics
The susceptibility of staphylococci was determined for all strains identified.
The results show a significant sensitivity for 100% of strains to penicillin, 90% to Vancomycin (VA) and Rifampicin (RA), 80% to Lincomycin (L) and Oxaciline (OX) ; resistance for 50% of strains to tetracycline (TE).
Strain *S. aureus* (12) has a resistance to four antibiotics (VA, RA, L, and OX).
Strains of the same species *S. aureus* (4) and *S. aureus* (11) from different samples have the same sensitivity profile.
Strains of different species *S. aureus* (11) and *S. epidermidis* (11) from the same sample have the same sensitivity profile.
The susceptibility of Pseudomonas has been established for some strains identified.
All strains tested were resistant to trimethoprim (TM), amoxicillin + clavulanic acid (AMC),
ampicillin (AM) and Imipenem (IMI) by cons they are susceptible to amikacin (AN). 66.6% of the strains showed an intermediate profile for pipemidic acid (PI).
The susceptibility of Enterobacteriaceae has been established for some strains identified. All strains were sensitive to chloramphenicol (C), cefotaxime (CTX) and 83.3% to Cotrimoxazole (CO) by cons 83.3% are resistant to amoxicillin (AMX), to ampicillin (AM) and colistin (CT) profiles vary from one strain to another.

Characteristics of lactobacilli towards antibiotics.
The results of susceptibility testing of lactobacilli (Figure 3), showed that 100% of strains were resistant to Kanamycin (K) and Streptomycin (S), 96.55% of the strains were resistant to Gentamicin (G) against 65.51 % are sensitive to Trimethoprim (TM), and 79.31% of the strains have an intermediate profile for the Rifampicin (RA).

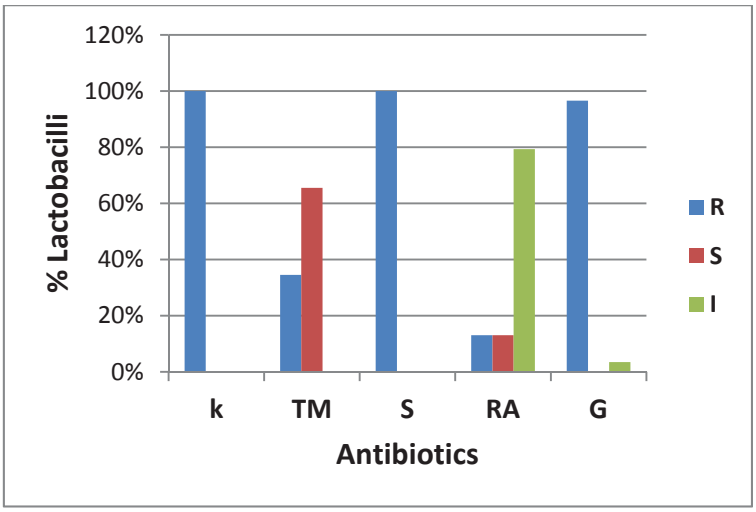


Figure 3: Profile of Lactobacillus strains against antibiotics.
K: Kanamycin, TM: Trimethoprim, S: Streptomycin, RA: Rifampicin, G: Gentamicin.
R (resistant), S (susceptible), I (intermediate).

Discussion

Characteristics and identification of lactobacilli.

The results found in this work are similar to those found by Gancel et al. (1997), who isolated lactobacilli herring fillet (fresh, salted, smoked, packaged under vacuum and CO_2), they found that lactobacilli belong only to groups II and III of Kandler and Weiss(1986), and are resistant to conditions hostile (NaCl, bile salts, smoke, pH 3.3) and grown at 5°C and 20°C ; Kacem et al. (2003) found that the Lactobacillus's species isolated from sheep and cows milk grown at 15°C, but not at 45°C, and in media with 6.5% NaCl.

Marceau et al. (2004), by a proteomic study to determine the N-terminal sequence by the technique of two-dimensional electrophoresis coupled to mass spectrometry showed that six proteins of *Lactobacillus sakei* are involved in the mechanism of adaptation at low temperature and high level of NaCl. They showed that two of these proteins are involved in the general metabolism of carbohydrates and other proteins called "stress proteins" are induced during hostile conditions, so they play a protective role.

Low temperature and salinity are two conditions often used in meat preservation, these lactic acid bacteria are therefore an adaptation to these conditions.

The strains identified have the general characteristics of the genus Lactobacillus, they grow under anaerobic and aerobic because it is facultative anaerobes, Champomier et al. (2002) found that *Lactobacillus sakei* which represents the original species of meat and meat products can grow aerobically and anaerobically. This confirms our results that these bacteria are present in meat vacuum packed or not.

Lactobacillus sakei is one of the most important lactic acid bacteria of meat and fermented meat products. She produced from arginine, ammonia, CO_2 and ATP through a chain of degradation arginine deiminase (ADI). This chain is composed of three catabolic enzymes: arginine deiminase, ornithine transcarbamoylase and carbamate kinase, and a transport system for arginine (Manca and Presce 1982; Montel and Champomier, 1987).

All lactobacilli strains isolated in this work degrade arginine, these results are consistent with those found by Gancel et al. (1997) and Gacem et al. (2003).

Susceptibility of lactobacilli

Lactobacilli is very beneficial microorganisms to humans, considered not involved in infections is why very little research is available on their susceptibility to antibiotics. Nevertheless the work of Charteris et al. (1998) carried out on 46 strains of Lactobacillus isolated from milk and human origin, were tested for their susceptibility to 44 antibiotics. The results showed that all strains were resistant to 14 antibiotics including Gentamicin, Kanamycin, Streptomycin, and Trimethoprim, antibiotics tested in our work, where we found the same results except for Trimethoprim which 65.5% strains were susceptible.

They also found that all strains were sensitive to Tetracyclin, Chloramphenicol, and Rifampicin for the latter we found 79.31% of susceptible strains.

For the remaining antibiotics tested they found mixed results, explained by the fact that the resistance observed is dependent on the origin of strains or are acquired in vivo exposure to antibiotics. Their work has been done to find consensus therapeutic antibiotics / probiotics to prevent and / or cure digestive infections, urogenital, and endocarditis. Aniewska-Moroz et al. (2001) determined the level of resistance to antibiotics of different strains of lactic acid bacteria isolated from naturally pickled vegetables. The results show that the resistance of lactobacilli depends on the strain and its origin. They found that all strains of *L. plantarum* and *L. brevis* are resistant to nalidixic acid, 41.3% to kanamycin and for neomycin 3.4%.
All strains of lactic acid bacteria are susceptible to antibiotics studied β-lactams, Rifamycins, Furan derivatives, and chloramphenicol.

Conclusions

Lactobacilli are naturally present in meat products are mainly represented by *L. casei*, *L. sakei*, *L. curvatus*, *L. brevis*, *L. plantarum*, *Carnobacterium pisicola*, but other species of various origins may be present as contaminants. These species are adapted to meat (low glucose, rich of glycogen, ribose and arginine) and at different packaging. They are characterized by a broad spectrum of fermentation. The heterofermentative represent the dominant microflora of meat packaged under modified atmospheres due to their resistance to CO_2, growth at low temperatures, and the production of inhibitory substances (organic acids and bacteriocins) Lebert al. (2006a).
Strains isolated in this work shown these characteristics of adaptation: they tolerate concentrations of NaCl at 7% or even 10% (e.g. *L. divergens* isolated from a salt meat) they grow at low temperatures, and degrade arginine and ribose. This work was continued by other research (by the same team), the results show that the identified strains of lactobacilli produce substances inhibitory (H_2O_2, acids, bacteriocins) against pathogenic and spoilage bacteria isolated from the same habitat.
The long-term challenge, especially for agric-alimentary is to select strains or species most successful in these properties, possibly in order to use as starter in processed meat products or as natural preservatives for fresh meat products.

References

Accola JP, Auclair J, Angelier N (1972). Conservation à l'état congelé de suspension concentrée de bactéries lactiques destinées à l'industrie laitière. Revue générale de froid. pp.21-24.

Ammor MS, Florz AB, Mayo B (2007). Antibiotic resistance in non enterococcal lactic acid bacteria and bifidobacteria. Food Microbiol. 24: 559-570.

Aniewska-Moroz Ł, Warminska-Radyko I, Gradzka J, Dajnowiec M (2001). Resistance of lactic fermentation bacteria Lactobacillus isolated from natural environments to antibiotics. Polish J. Food Nutr. Sci. 10 (3): 51-54.

Axelsson L (1998). Lactic acid bacteria: classification, physiology and functional aspects. 2ème éd: Marcel dekker Inc. New York. pp. 1-72.

Bio-Merieux : Catalogue et fiches techniques des systèmes Api (20E, 20NE, Staph, et 50CHL). 69280 Marcy, l'étoile, France, *in* Leyral G, Joffin JN (1998). Microbiologie technique. 2. Documentation Technique. Biologie Technique. 2èm édition CRDP. Bordeaux, France. pp. 37-42 ; 61-73 ; 137-145 ; 183-193.

Bonnefoy C, Guollet F, Guy L, Verne-Bourdais E (2002). Microbiologie et qualité dans les industries agroalimentaires. Sciences des aliments. Edition DOIN, 45 : p. 245.

Brossard H, Leyral G, Terry O (2008). Activités technologiques en microbiologie. Bactériologie systématique. Collection Biologie Technique. Ed. CRDP. Aquitaine. 2 : pp.139-144.

Bourgeois CM, Mescle JF, Zucca J (1996). Microbiologie alimentaire tome 1. Aspect microbiologique de la sécurité et de la qualité des aliments. Collection sciences et techniques agroalimentaire. Edition Tec & Doc. Lavoisier, Paris. pp. 62 ; 94 ; 117 ; 332-344 ; 596 ; 608 - 609.

Cavallo JD, Chardon H, Soussy CJ et *al.* (2007). Communiqué du comité de l'antibiogramme de la société Française de microbiologie.

Champomier MC, Marceau A, Zagorec M (2002). The *pepR* Gene of *Lactobacillus sakei* is Positively Regulated by Anaerobiosis at the Transcriptional Level. Appl. Environ. Microbiol. 68 (8): 3873–3877.

Charteris WP, Kelly PM, Morelli L, Collins JK (1998). Antibiotic susceptibility of potentially probiotic Lactobacillus species. J. Food Protect. 61: 1636-1643.

De Man JC, Rogosa M, Sharpe ME (1960). A medium for the cultivation of lactobacilli. J. Appl. Bacteriol. 23: 130-135.

Dembele T, Obdrzalek V, Votava M (1998). Inhibition of bacterial pathogens by lactobacilli. *Zentralbl Bakteriol.* 288 (3): 395-401.

Gancel F, Dzierszinski F, Tailliez R (1997). Identification and characterization of lactobacillus species isolated from fillets of vacuum-packed smoked and salted herring (*Clupea harengus*). J. Appl. Microbiol. 82: 722-728.

Hartemink R, Domenech VR, Rombouts FM (1997*).* LAMVAB: a new selective medium for the isolation of lactobacilli from faeces. J. Microbiol. Methods. 29: 77-84.

Holt JG, Krieg NR, Sneath P H A, Stanley JT, Williams ST **(**1994) : In: Bergey 's manual of determinative bacteriology. **1** (Snaeth PHA, Mair NS, Sharpe ME, Holt JG, Ed.). Ninth Edition William and Wilkins. Baltimore. pp. 527-557.

Jacotot B, Le Parco JC (1999). Nutrition et alimentation 2ème édition. Edition Masson pp. 120-121.

Kacem M, Zadi-Karam H, Karam N (2003). Identification of lactic acid bacteria isolated from milk and fermented olive oil in Western Algeria. Acts Agron.Vet. Maroco. 23(2-4): 135-141.

Kandler O, Weiss N (1986). Genus *Lactobacillus* in: Bergey's manual of systematic bacteriology. (Sneath PHA Ed.) Williams and Wilkins Co., Baltimore. MD 2, pp.1209-1234.

Larpent JP. (2000). Introduction à la nouvelle classification bactérienne. Les principaux groupes bactériens. Edition Tec.et Doc. Lavoisier, Paris. p. 182.

Lebert I, Leroy S, Giammarinaro P, Chacornac JP, Talon R (2006a). Saucissons secs fermiers du Massif central. Ecosystèmes microbiens des saucissons et de l'environnement. Viandes et Produits Carnés. 25**(**5) : 165-170.

Leyral G, Joffin JN (1998). Microbiologie technique. 2. Documentation technique. Biologie Technique. 2ème édition CRDP. Bordeaux, France. pp. 37-42 ; 61-73 ; 137-145 ; 183-193.

Manca De Nadra MC, Presce De Ruiz Holgado AA (1982). Arginine dihydrolase activity in lactic acid bacteria.Milchenwissenschaft. 37: 669-670.

Marceau A, Zagorec M, Chaillou S, MERA T, Champomier-Verges MC (2004). Evidence for Involvement of at Least Six Proteins in Adaptation of *Lactobacillus sakei* to Cold Temperatures and Addition of NaCl. Appl. Environ. Microbiol. 70(12): 7260-7268.

Marchal N, Bourdou JC, Richard D (1987). Les milieux de cultures pour l'isolement et l'identification biochimique des bactéries. 3ème édition, Doin éditeurs. pp.350-355.

Montel MC, Champomier MC (1987). Arginine catabolism in *Lactobacillus sakei* isolated from meat. Appl. Environ. Microbiol. 53(11): 2683–2685.

Najjari A, Ouzari H, Boudabous A, Zagorec, M (2008). Method for reliable isolation of *Lactobacillus sakei* strains originating from Tunisian seafood and meat products. Int. J. Food Microbiol. 121: 342-351.

Prescott LM, Harley JP. Donald, A (2003). Microbiologie, De boeck université, 2ème édition Française. 128: 28-29.

Qin X, Weissman SJ, Chesnut MF, Zhang B, Shen L (2004). Kirby-Bauer disc approximation to detect inducible third-generation cephalosporin resistance in *Enterobacteriaceae*. Annals of Clinical Microbiology and Antimicrobials. 3:13.

Sakala R, Kato Y, Hayashidani H, Murakami M, Kaneuchi C, Ogawa M (2002. *Lactobacillus fuchensis* sp. nov. isolated from vacuum-packaged refrigerated beef. Int. J. Syst. Evol. Microbiol.. 52: 1151-1154.

Schillinger U, LUCK FK (1987). Identification of lactobacilli from meat and meat products. Food Microbiol., 4: 199-208.

Schillinger U, Luke FK (1989). Antibacterial activity of *Lactobacillus sakei* isolated from meat. Appl. Environ. Microbiol. 55: 1901–1906.

Sutra L, Federighi M, Jouve JL (1998). Manuel de bactériologie alimentaire. Edition Polytachnica. pp. 53, 56,57, 82, 114, 115, 235,236, 253.