Bacterial spoilers of food: Behavior, fitness and functional properties

Benoît Remenant [a,b], Emmanuel Jaffrès [a,b], Xavier Dousset [a,b], Marie-France Pilet [a,b], Monique Zagorec [a,b,*]

[a] INRA, UMR 1014 Secalim, F-44307 Nantes, France
[b] LUNAM Université, Oniris, F-44307 Nantes, France

ARTICLE INFO

Article history:
Available online 24 March 2014

Keywords:
Bacterial genomes
Meat products
Seafood products
Dairy products

ABSTRACT

Most food products are highly perishable as they constitute a rich nutrient source for microbial development. Among the microorganisms contaminating food, some present metabolic activities leading to spoilage. In addition to hygienic rules to reduce contamination, various treatments are applied during production and storage to avoid the growth of unwanted microbes. The nature and appearance of spoilage therefore depend on the physiological state of spoilers and on their ability to resist the processing/storage conditions and flourish on the food matrix. Spoilage also relies on the interactions between the microorganisms composing the ecosystems encountered in food. The recent rapid increase in publicly available bacterial genome sequences, as well as the access to high-throughput methods, should lead to a better understanding of spoiler behavior and to the possibility of decreasing food spoilage. This review lists the main bacterial species identified as food spoilers, their ability to develop during storage and/or processing, and the functions potentially involved in spoilage. We have also compiled an inventory of the available genome sequences of species encompassing spoilage strains. Combining *in silico* analysis of genome sequences with experimental data is proposed in order to understand and thus control the bacterial spoilage of food better.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Food spoilage caused by microorganisms results from microbial metabolism leading to the production of molecules that alter the sensory quality of the products, in particular the aspect (including texture and color) and odor (Gram et al., 2002). There is thus a large diversity of spoilage: from volatile compound production, which provokes off-odors and/or blown-pack spoilage of vacuum-packed foods, to slime or liquid production, or various color deteriorations (Borch et al., 1996; Brightwell et al., 2007; Dainty et al., 1989; Nychas et al., 2008). In most studies, spoilage characteristics are assessed by sensory analyses and/or by measurement of various physicochemical parameters such as the amount or nature of different metabolites or gases, color, pH, etc., which are then correlated to the presence of microorganisms (Diez et al., 2009; Jaffrès et al., 2011; Macé et al., 2013; Paarup et al., 2002). It is

often accepted that the bacteria responsible for spoilage are those belonging to the major population in the spoiled product. A large majority of studies reported in the literature first used classical plating and phenotyping methods to characterize the bacterial population of spoiled products (Dainty and Mackey, 1992; Rattanasomboon et al., 1999). More recently, an increasing number of articles have reported species identification through DNA extraction, followed by various types of molecular identification such as Denaturing Gradient Gel Electrophoresis (DGGE) or Temporal Temperature Gel Electrophoresis (TTGE) performed on 16S rRNA gene portions amplified by PCR (Doulgeraki and Nychas, 2013; Ercolini et al., 2006; Jaffrès et al., 2009). The use of high-throughput 16S rDNA pyrosequencing or metagenomics relying on the whole bacterial food ecosystem, including spoilers, is still scarce. By such approaches, many different bacterial species have been reported as specific spoiler organisms (Nieminen et al., 2012). The involvement of these putative spoilers has sometimes been proven by re-inoculation of candidate strains into food model systems to reproduce spoilage. Yet, only a few studies have successfully proved the exact nature of spoilage and the bacterial functions involved in producing the spoiling molecules (Jääskeläinen et al., 2013).

* Corresponding author. INRA, Oniris, UMR 1014 Secalim, rue de la Géraudière, CS82225, F-44322 Nantes cedex 3, France. Tel.: +33 2 51 78 55 42; fax: +33 2 51 78 55 20.

E-mail address: monique.zagorec@oniris-nantes.fr (M. Zagorec).

2. The main bacterial species known as food spoilers, their spoiling effects and fitness

Many studies have reported the main bacterial species associated with various types of spoilage from a large range of foodstuffs. The ability of some species to grow under or resist the harsh conditions encountered during processing and storage has been described. For various foods of animal origin, Gram-positive bacteria, and especially Firmicutes, have often been reported as spoilers (Bron and Kleerebezem, 2011). In particular, lactic acid bacteria (LAB), encompassing *Lactococci*, *Lactobacilli*, *Leuconostoc*, *Weissella*, and *Carnobacteria* species, as well as *Enterococci* are frequently associated with spoilage. In fact, although LAB are generally beneficial for food and are used for the fermentation of a variety of food and raw materials, where they contribute to flavor, texture and shelf-life (Bron and Kleerebezem, 2011), some species can play a significant role in food spoilage and decay. This is the case, for instance, of *Lactobacillus alimentarius*, known as a specific spoilage organism in marinated herring (Lyhs et al., 2001), and of *Lactobacillus sakei* and *Lactobacillus curvatus*, also found in the spoilage microbiota of herring (Lyhs and Bjorkroth, 2008). *L. sakei* was reported as predominant in the spoilage microbiota of sliced, vacuum-packed, smoked, oven-cooked turkey breast fillets, which developed sour spoilage flavors (Samelis et al., 2000). *L. curvatus* was also found to be one of the specific spoilers in cold-smoked salmon (Jørgensen et al., 2000), as was *L. sakei* (Stohr et al., 2001; Joffraud et al., 2006). The latter was also isolated in the spoilage microbiota of brined and drained shrimp stored under modified atmosphere packaging (Mejlholm et al., 2012, 2008). *Carnobacterium* is another LAB genus commonly involved in the spoilage process of food, with notably *Carnobacterium divergens* and *Carnobacterium maltaromaticum* known to dominate the spoilage microbiota of different meat and seafood products, particularly those packed under vacuum or modified atmosphere (Laursen et al., 2005; Leisner et al., 2007; Vasilopoulos et al., 2008). *Carnobacterium* sp. was shown to be an important contributor to the spoilage-related microbiota of freshly prepared chicken products stored aerobically under refrigeration (Liang et al., 2012). *C. maltaromaticum* is considered a strong spoiler of raw salmon (*Salmo salar*) fillets stored under modified atmosphere packaging (Macé et al., 2013), and also of cooked and peeled shrimps, where it can be responsible for cheese/feet, sour/fermented and milky-boiled off-odors (Jaffrès et al., 2011). Other LAB genera and species have also been incriminated in the spoilage process of various food products. *Enterococcus faecalis* and *Enterococcus faecium* can be involved in the greening of meat products (Foulquié-Moreno et al., 2006) while *E. faecalis* is also a spoilage species of artisan-type cooked ham under modified atmosphere packaging (Vasilopoulos et al., 2008). *Leuconostoc* species like *Leuconostoc gelidum* and *Leuconostoc gasicomitatum* are known to produce slime on marinated herring (Lyhs et al., 2004). The role of *L. gasicomitatum* in gas and slime formation, the development of pungent acidic and buttery off-odors, and the greening of beef have been proven by challenge tests (Johansson et al., 2011). The species *Leuconostoc mesenteroides* was recovered as the predominant spoiler from the microbial community of blown packs of vacuum-packaged beef (Yang et al., 2009). Vihavainen et al. (2008) reported the spoilage potential of several *Leuconostoc* species on vegetable sausage products. They demonstrated that *L. gelidum*, *L. gasicomitatum*, and *L. mesenteroides* were the predominant LAB in commercial vegetable sausages. The inoculation of these species into vegetable sausages resulted in the formation of gas, slime, and a sour off-odor. The *Weissella* genus, notably *Weissella confusa*, was also found to be one of the major spoilers of oyster gill stored at 10 °C (Chen et al., 2013). Diez et al. (2009) highlighted that the

sensory and volatile changes during the anaerobic cold storage of morcilla de Burgos, a typical blood sausage from Spain, could be induced to a great extent by *Weissella viridescens* and *L. mesenteroides*. Both species have also been associated together in the spoilage of other food products such as sliced vacuum-packed cooked ham after high-pressure treatment (Han et al., 2011).

In addition to LAB species, other Gram-positive bacteria can play a significant role in food spoilage. One of the most prominent is the psychrotrophic species *Brochothrix thermosphacta*, known as an important spoiler bacterium of various food matrixes (Rattanasomboon et al., 1999; Russo et al., 2006). *B. thermosphacta* is an ubiquitous microorganism throughout the meat production chain, from animal to food. It has been isolated from beef carcasses during boning, dressing and chilling. Moreover, lairage slurry, cattle hair, rumen content, walls of slaughterhouses, hands of workers, air in the chill room, neck and skin of the animals as well as the cut muscle surfaces have all been shown to be contaminated by this organism (Nychas et al., 2008). In meat products packed under vacuum or modified atmosphere, *B. thermosphacta* can dominate the spoilage microbiota at the expense of other genera, such as *Carnobacterium*, *Lactobacillus* or *Leuconostoc* (Borch et al., 1996; Ercolini et al., 2006). *B. thermosphacta* was also found in the spoilage microbiota of raw turkey breasts (Samelis et al., 2000), pork meat (Bohaychuk and Greer, 2003) and cooked ham (Samelis et al., 1998). It was identified as the dominant spoiler in freshly prepared chicken products (Liang et al., 2012) and was responsible for cheesy/dairy off-odor and discoloration of chilled vacuum-packaged lamb meat (Gribble and Brightwell, 2013). Seafood products also provide a favorable niche for *B. thermosphacta* development, i.e. neutral pH, high water activity (A_w) and high content of low molecular weight compounds such as free amino acids and nucleotides (Jeyasekaran et al., 2006). Nickelson et al. (1980) isolated this species from minced fish flesh, at various production stages. It was also isolated from fish (*Archosargus probatocephalus*) packed under CO_2 (Lannelongue et al., 1982) and frozen fish such as cod (Gardner, 1981). On refrigerated tuna (*Thunnus alalunga*) steaks, packed under modified atmosphere, the growth of *B. thermosphacta* was not affected by CO_2, as long as O_2 was also present at a concentration above 0.2% (Lopez-Galves et al., 1995). More recently, *B. thermosphacta* was found to be predominant in meat and coral of king scallops (*Pecten maximus*), during storage under air at low temperature (Coton et al., 2013). The growth of *B. thermosphacta* in vacuum-packed meat products depends largely on the amount of available oxygen remaining in the package. *B. thermosphacta* may become the dominant spoilage species when oxygen is present, but is displaced by *Lactobacillus* species under anaerobic conditions. The spoilage potential of *B. thermosphacta* in vacuum-packed meat products is thus influenced by factors that control the level of oxygen in the pack like, for example, the film permeability, the residual gas composition and the pack integrity (Gribble and Brightwell, 2013). In vacuum packs, there is often enough residual oxygen for *B. thermosphacta* to metabolize offensive off-odors. The anaerobic growth of *B. thermosphacta* at chill temperatures is also reduced by meat pH values of less than 5.8; consequently *B. thermosphacta* is most often associated with the early spoilage of high pH meat cuts of lamb and beef (Bell, 2001; McClure et al., 1993).

Several species have been reported as responsible for the so-called blown-pack defect of vacuum-packed food due to gas production. This defect is mostly associated with chilled fresh meat particularly beef, lamb and venison, but was also reported for cooked meat products (Broda et al., 1996). The causative agents include psychrotrophic *Enterobacteriaceae* or *Clostridium* species such as *Clostridium estherteticum* (Brightwell et al., 2007; Hernandez-Macedo et al., 2012; Yang and Badoni, 2013). Other