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Spore-forming bacteria responsible for food spoilage

Stéphane André [a,*], Tatiana Vallaeys [b], Stella Planchon [a]

[a] Centre technique pour la Conservation des Produits agricoles (CTCPA), Unité Expertise dans la Maitrise du Risque industriel en Thermorésistants sporulés (EMaiRIT'S), 449 Avenue Clément Ader, 84911, Avignon, France
[b] Laboratoire ECOSYM-UMR 51119, Université de Montpellier, Montpellier, France

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Abstract

This review explores the main spore-forming bacteria involved in the spoilage of various processed foods. Bakery products are specifically spoiled by *Bacillus* species, the dominant one being *Bacillus amyloliquefaciens*, while different *Clostridium* species classically contaminate refrigerated vacuum-packed meats. These two genera have also been isolated from milk products, even when milk is pasteurized, sterilized, dehydrated or fermented, according to heat treatment and storage temperature. Finally, the most heat-resistant microorganisms are isolated in low-acid canned foods, the three predominant species being *Geobacillus stearothermophilus*, *Moorella thermoacetica* and *Thermoanaerobacterium* spp.

Keywords: Spoilage; *Bacillus*; *Clostridium*; *Geobacillus*; *Morella*; *Thermoanaerobacterium*

1. Introduction

Spore-forming bacteria are considered a major threat in heat-treated food plants. Spores show typical resistance to both chemicals such as disinfectants and physical treatments (thermal or non-thermal) used in the food processing industry [1]. Endospore formers may thrive in different parts of the food processing plant. Surviving spores can thus germinate and grow out in the product under suitable conditions (time and temperature during process, nutrients, and physical and chemical properties) [2].

Endospore-forming bacteria were long classified into two orders: Bacillales for aerobic rods and Clostridiales for strictly anaerobic bacteria, with *Bacillus* and *Clostridium* as representative genera, respectively [3]. The evolution of molecular tools, and particularly 16S-based molecular classification of bacterial species, has led to reconsideration of food spoilage flora diversity. Food spoilage bacteria are now classified into three distinct orders, adding the more recently described Thermoanaerobacterales [4]. Food spoilage Bacillales members are typically assigned to the *Bacillus, Geobacillus, Anoxybacillus, Alicyclobacillus,* and *Paenibacillus* genera, while for Clostridiales, cases of contamination by species of the *Clostridium* and *Desulfutomaculum* genera have been reported [5]. The vast majority of "flat sour" spoilage species belong to the *Bacillus* genus and its close relatives, with a few exceptions. These two genera also include several species with recognized pathogenic properties such as *Bacillus cereus* and *Clostridium botulinum*. As our purpose was specifically to address conventional industrial food spoilage flora, and given that a large number of reviews have been specifically dedicated to pathogenic species, we did not include these in this review.

The Thermoanaerobacterales order mainly includes thermophilic anaerobes, previously classified under Clostridiales, but also some now obsolete genera such as *Acetogenium* and *Thermobacteroides*. This order has wide diversity, with case reports of contaminations by *Moorella* (formerly known as *Clostridium* species), *Thermoanaerobacter, Thermoanaerobacterium, Caldanaerobacter, Caldanaerobius* and *Gelria* [6].

* Corresponding author.
E-mail address: sandre@ctcpa.org (S. André).

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Classification of food spoilage bacteria is, however, an emerging area, and more molecular work is required to investigate the potential uncultivable microbial diversity of this habitat.

Food spoilage is due to spore germination and outgrowth, key factors in the stability and non-stability of heat-treated foods. Food spoilage is characterized by changes in texture and odor, pH variation and gas production, and depends mainly on species and food matrix (plant or animal). This review illustrates the diversity of food spoiling microflora (bread-making, meat and milk products and different types of canned foods). Links between different domains can be established, and it is noteworthy that some species seem to be product-specific, while others are ubiquitous.

2. Bakery products and aerobic flora

In the early 1980s, various species of *Bacillus*, such as *Bacillus mesentericus* (now *Bacillus pumilus*) and *Bacillus subtilis*, were identified as responsible for spoilage of bakery products. This spoilage is generally characterized by a stringy crumb, discolored crust and a melon-like odor. It could be explained by decreased use of chemical preservatives in bakery product manufacturing [7]. Spoilage was often associated with the presence of *Bacillus* spp. in raw materials. Their spores withstand cooking temperatures close to 100 °C for several minutes. The decimal reduction time D, which expresses the resistance to moist heat, was studied for the three main species involved. Its values lay between 10 and 56 min at 100 °C [8]. In the 1990s, several studies showed the dominance of *B. subtilis* and *Bacillus licheniformis* as spoilage species. Given the low accuracy of identification in the 1990s, Valerio et al. (2012) conducted a new study on raw materials composing bakery products, using Fourier-transform near-infrared spectrometry (FT-NIRS) [9]. Among the thirteen species identified, three *Paenibacillus* and ten *Bacillus* species were present in more than 50% of the samples, with concentrations between 1 and 100 spores g^{-1}. The authors identified *Bacillus amyloliquefaciens* as the major species instead of *B. Subtilis,* as claimed in earlier studies. Special attention has been directed to antimicrobial substances in order to control these spoilage micro-organisms [10,11].

3. Refrigerated vacuum-packed meats: anaerobic niche bacteria

McBride in 1911, and later Sturges and Drake in 1926, identified *Clostridium* as the main genus responsible for spoilage of refrigerated meat called 'blown pack', and in particular, the species *Clostridium putrefaciens* [12]. New methods of molecular biology available in the 1990s identified further species: *Clostridium estertheticum*, *Clostridium algidicarnis*, *Clostridium frigidicarnis*, *Clostridium gasigenes*, *Clostridium algidixylanolyticum*, *Clostridium frigoris* and *Clostridium bowmanii*. These species are particularly associated with refrigerated and vacuum-packed meat products. Meanwhile, many studies regularly identified *C. botulinum* or other *Clostridium* species that have since been renamed (as described in the Introduction). In all cases, spoilage is characterized by an abundant production of gas, which causes the plastic packaging to swell, a strong smell, and loss of meat color and texture. This spoilage can result from very low initial contamination. Clemens et al. (2010) showed that as little as one spore of *C. estertheticum* per product could spoil beef and lamb during storage [13]. These species are classified according to their growth temperature into psychrotrophes (optimum at 12 °C and growth at 37 °C) and psychrophiles (optimum at 8–12 °C and no growth above 30 °C). Spoilage by *C. gasigenes* has sometimes been observed even at −1 °C or −2 °C [14]. Although many species are regularly isolated, the main ones (cited above) are usually found in the same sample, suggesting that there may be a synergistic effect between species [15]. The combination of psychrotolerant and anaerobic characteristics makes these species notoriously hard to isolate and handle. Many studies have been published that propose new isolation media [16]. In parallel, some work on defining methods of rapid identification have been published by Pichner et al. (2012) with a PCR for *C. estertheticum sensu stricto* or *C. estertheticum*-like [16]. In the CTCPA laboratory, *C. algidicarnis* has been predominantly found in recent years in spoiled pasteurized "*foie gras*" (fat duck liver). The years 2011–2012 were one period when *C. bowmanii* critically affected several industrial operators (personal data). In this particular matrix, the same defects were observed: strong smell and gas production.

Sources of contamination were sought by several authors. Broda et al. (2002) found that soil particles on carcass skins, together with feces, needed to be monitored, with particular care in health control procedures to reduce slaughterhouse contamination by *C. gasigenes* or *C. estertheticum* [17]. Moschonas et al. (2009) worked on 1680 samples collected in beef abattoirs in the course of a year, and tracked C. *estertheticum* and *C. gasigenes* in particular [18]. They found that up to 38% of the samples taken at the slaughterhouse in May were positive. Ten to fourteen percent of the soil samples analyzed contained both species. For example, Silva et al. (2011) identified only *C. gasigenes* and *C. algidicarnis* in both spoiled and compliant beef samples, and at the slaughterhouse on skin samples [19]. The specific localization of *Clostridium* spp. on meat surfaces could result from cross-contamination. This was confirmed in washing tests with both hot and cold water by Adam et al. (2013) [20]. Simple washing increased shelf life by 12 and 13 days relative to untreated samples. Feces were also characterized as a vector of contamination by psychrophilic *Clostridium*: high counts of *C. estertheticum* and *C. gasigenes* were found [18].

4. Dairy products: diversified spore-forming flora

4.1. Fresh milk: the basic source

Studies on the spoilage of fresh milk include spoilage of end-products such as dairy products and/or contamination of dairy processing lines.