Ropiness in Bread - A Re-Emerging Spoilage Phenomenon

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As bread is a very important staple food, its spoilage threatens global food security. Ropy bread spoilage manifests in sticky and stringy degradation of the crumb, slime formation, discoloration, and an odor reminiscent of rotting fruit. Increasing consumer demand for preservative-free products and global warming may increase the occurrence of ropy spoilage. *Bacillus amyloliquefaciens*, *B. subtilis*, *B. licheniformis*, the *B. cereus* group, *B. pumilus*, *B. sonorensis*, *Cytobacillus firmus*, *Niallia circulans*, *Paenibacillus polymyxa*, and *Priestia megaterium* were reported to cause ropiness in bread. To date, the underlying mechanisms behind ropy bread spoilage remain unclear, high-throughput screening tools to identify rope-forming bacteria are missing, and only a limited number of strategies to reduce rope spoilage were described.

Bacillus spp. bread rope spoilage wheat

1. Introduction

Spoilage of bread and other bakery products can manifest as inanimate physical and chemical spoilage with moisture loss or rancidity, or in the form of animate spoilage due to growth of molds or bacteria [1]. The spoilage potential of bakery products is dependent on their acidity (high: pH < 4.6; low: pH 4.6–7; non-acidic: pH > 7) and water activity (high: a_w > 0.85; intermediate: a_w = 0.6 to 0.85; low: a_w < 0.6), with high moisture and low acidity products being the most susceptible to microbiological spoilage [1]. By the end of the 19th century, spore-forming bacilli were identified as causative agents of ropy bread spoilage [2]. Besides molds, *Bacillus* spp., originating from raw materials or bakery equipment, are among the most important spoilage agents of non-acidified white and wholemeal bread [3]. At the beginning of the 20th century, research efforts focused on the control of rope-spoilage organisms and the inhibition of their germination and outgrowth through preservatives, such as lactic acid, acetic acid, or propionic acid [4][5]. The bakery industry is now heavily dependent on preservatives to control ropey spoilage. Generally, sodium, potassium or calcium salts of propionic and sorbic acid are used as chemical preservatives in bakery products [6]. Recently, however, increased demand for preservative-free “clean label” products and the inclusion of whole-grain flours [6][7], in combination with global warming and rising ambient temperatures, may lead to more frequent occurrence of bread spoilage by rope-forming bacilli.

Bacillus spp. is a gram-positive, rod-shaped, aerobic or facultative anaerobic, motile, and endospore-forming bacterium [8]. *Bacillus* endospores are not only heat stable and able to survive baking in the center of the bread crumb, but they are also highly resistant to desiccation, radiation, and chemical agents [5][9]. Intracellular endospore formation is triggered by nutrient starvation, with subsequent cell lysis and the release of spores [10]. If conditions are favorable, for instance temperatures above 25 °C in combination with an $a_w \geq 0.95$ and pH > 5, spore germination and growth can lead to spoilage [11]. Rope formation may occur in localized high-moisture areas inside the bread loaf [12]. Ropiness is associated with a patchy discoloration and a stringy bread crumb, and characterized by an unpleasant sweetish odor resembling rotting melons or pineapples that is caused by the release of volatile compounds including diacetyl, acetoin, acetaldehyde, and isovaleraldehyde (**Figure 1**) [12][13][14]. In advanced stages, the bread crumb can be almost liquefied and forms long, silky, web-like strands when pulled apart, as depicted in **Figure 1**, giving rise to the designation of rope spoilage [12][15][16]. This phenomenon mostly occurs in high-moisture bakery products during summer months or countries with moist and hot climates [17][18]. The production of extracellular slimy polysaccharides and proteins, as well as the production of proteolytic and amylolytic enzymes that degrade the bread crumb, is characteristic of rope-forming bacterial species (**Figure 1**) [3][18][19][20].

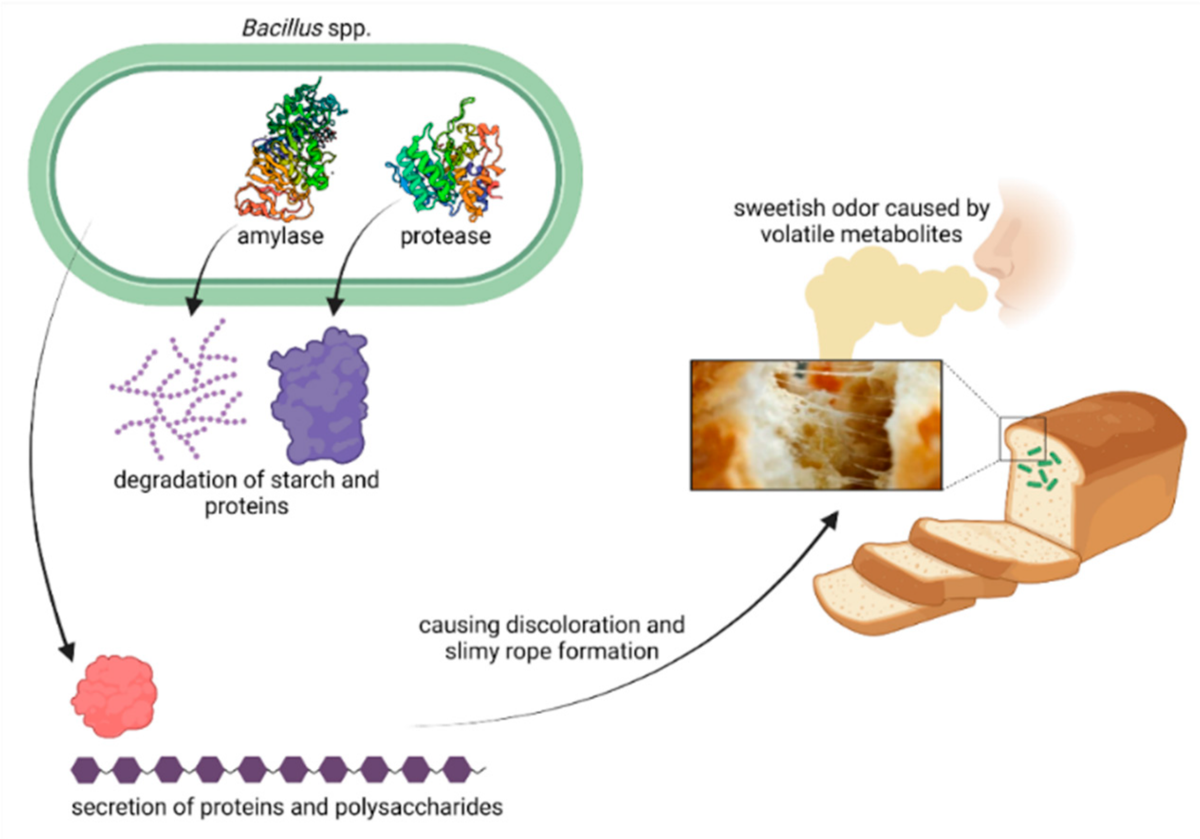

Figure 1. Mechanisms of rope formation, discoloration, odor development, and changes in bread structure. Created with BioRender.com.

2. The Route of Endospores into the Bakery Environment

Bacillus spp. are ubiquitous in nature and form symbiotic communities with different types of plants [21]. Soil is considered as the primary endospore reservoir with concentrations of up to 10^6 spores/g, thus representing a major route of entry for *Bacillus* spp. into the food chain (**Figure 2**A) [22]. Contamination levels of cereal grains and successive products may vary because of the influence of the cereal microbiota, which is dependent on growth conditions, including the location of crop growth and atmospheric conditions, such as precipitation levels and relative humidity [23][24][25][26]. Studies suggest that wheat originating from hotter, wetter areas generally carries higher microbial loads, as wheat grown under dry and warm wheatear conditions exhibited bacterial counts of 5.7 log CFU/g in contrast to 8.1 log CFU/g for wheat grown under unusually wet conditions [25][27]. Further, Sabillón et al. [28] observed lower microbial loads on wheat in areas where relative humidity levels were below 55%, and the temperatures were lower than 13.7 °C and higher than 31.5 °C.

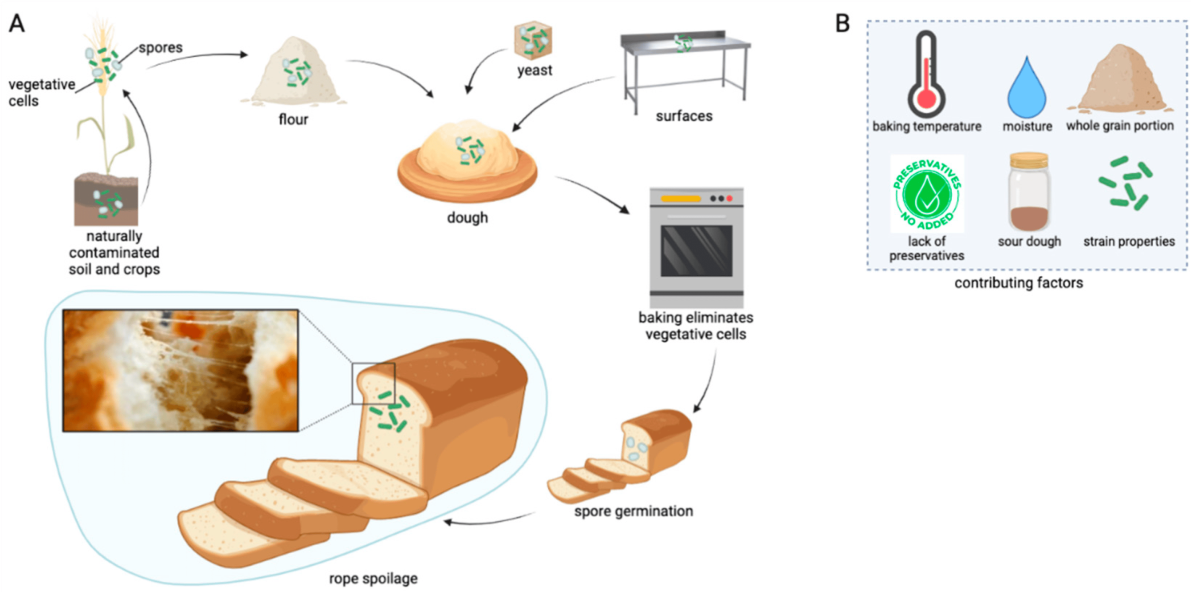

Figure 2. The route of endospores into bread (**A**) and influencing factors for the development of rope spoilage (**B**). Created with BioRender.com.

Due to its low a_w (<0.60), flour does not support bacterial growth and is regarded as a microbiologically safe product [27]. Nevertheless, bacterial spore concentrations in flours can exceed 10^3 spores/g and spores remain dormant for long periods of time [22]. Consequently, high loads of bacterial spores prominently endanger the quality and shelf life of bread [22][29].

Baker's yeasts are also known to be sources of contamination with spoilage bacteria, as studies showed that it is almost impossible to obtain bacteria-free commercial yeast [15][30]. Consequently, baker's yeast is considered an important introduction vector of vegetative bacteria and spores into the baking environment (**Figure 2**A) [31]. Viljoen and coworkers suggested that compressed yeast might represent the main source of bacterial contamination in bread doughs, as total aerobic counts of up to 10^8 CFU/g in baker's yeast were observed [32]. Several studies reported the presence of different rope-associated spore formers in raw materials, with baker's yeast showing high *Bacillus* spp. spore counts of 5.15 log spores/g [33].

Besides their occurrence in raw materials, rope-associated spores may also be present on equipment surfaces (**Figure 2**A), as well as in the bakery atmosphere [20][34]. Rope spoilage can result from inadequately cleaned and sanitized equipment, as spores may contaminate mixers, dough bowls, pipelines, filters, water tanks, conveyor belts, slicing blades, and other equipment [5]. Bailey and Holy [33] showed that *Bacillus* spore contamination of pre-baking food contact surfaces was not higher than 2.5 log CFU/swab, concluding that equipment surface contamination did not contribute significantly to the overall spore contamination of the bakery environment. In 1997, Viljoen and Holy investigated the microbial ecology of a commercial bread production line. According to their data, the APCs of equipment surfaces ranged between 7 and 5 log CFU/swab with high species variability. From 316 bacterial isolates, 50% were identified as *Bacillus* spp. [32]. While most of the studies focus on the occurrence of rope-forming bacteria in the bakery environment, few studies quantified bacilli in bread. Rosenkvist [18] sampled white and wholemeal wheat loaves baked without preservatives from different retail bakers. *Bacillus* spp. counts above 10^6 CFU/g after two days of storage at ambient summer temperatures of 25–30 °C were consistently enumerated.

3. The Diversity of Species Inducing Rope Spoilage in Bread

Considering recent changes in taxonomy and the microbiological and molecular biological methodologies used, attribution of strains to some of the species might not be consistent with current taxonomic frameworks and must be interpreted with caution. Table 1 provides an overview over the different bacterial species associated with rope spoilage.

Table 1. Bacterial species that were suggested to cause rope spoilage in bread. The table summarizes data on growth characteristics, metabolism, and spore survival.

	Growth					Metabolism						Survival		
Taxonomy	Optimum Growth Temperature [°C]	Minimum Growth Temperature [°C]	Maximum Growth Temperature [°C]	Growth at pH	NaCl Tolerance	Anaerobic Growth	Urease	Nitrate Reduction	Hydrolysis of Starch	Citrate	Propionate	Egg Yolk Reaction	Spore D_{100} Value [min]	References
B. amyloliquefaciens	30–40	15	50	5.7	5–10%	−	+	d	+	+	n.a.	+	23–44	[11][20][34][35][36][37][38][39]
***B. cereus* group**	37	5	50	4.9–9.3	n.a.	+	d	+	+	d	n.a	+	<10	[18][20][29][30][31][32][33][34][35][36][40][41][42][43][44][45][46]
B. licheniformis	37	15	50–55	5.7	7%	+	+	+	+	+	+	−	56	[11][18][20][34][35][36][39][41][45][47][48][49]
B. pumilus	30	15	50–55	5.7	7%	+	+	+	+	+	+	−	56	[11][18][20][34][35][36]

Taxonomy	Optimum Growth Temperature [°C]	Minimum Growth Temperature [°C]	Maximum Growth Temperature [°C]	Growth at pH	NaCl Tolerance	Anaerobic Growth	Urease	Nitrate Reduction	Hydrolysis of Starch	Citrate	Propionate	Egg Yolk Reaction	Spore D_{100} Value [min]	References
	Growth							Metabolism				Survival		
														[39][41][45][47]
B. sonorensis	30	15	55	n.a.	<5%	+	+	+	+	+	+ [5][53]	−	n.a.	[41][45]
B. subtilis[a]	28–30	5-20	45–55	5.5–8.5	7–10%	Facultative	−	+	+	+	[5]	−	14	[2][8][18][19][20][34][35][36][41][45][48][50][51][52] [54]
C. firmus [b]	30–37	n.a.	n.a.	n.a.	n.a.	n.a.	−	+	+	−	n.a.	n.a.	n.a.	[34][35][36]
N. circulans [c]	30–37	n.a.	n.a.	n.a.	n.a.	n.a.	−	n.a.	+	−	n.a.	n.a.	n.a.	[34][35][36]
P. polymyxa [d]	30	n.a.	n.a.	n.a.	n.a.	n.a.	−	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	[20][41][45]
Pr. megaterium[e]	30	3-15	35–45	n.a.	7%	−	+	d	+	+	n.a.	−	n.a.	[20][36][47]

The symbol + denotes positive, − negative, and d diverse results in the respective tests/compound utilization. When no data were available, this is indicated by n.a. [a] *B. inaquosorum*, *B. spizizenii*, and *B. stercoris* formerly subsumed under the species *B. subtilis*, were recently designated as separate species, according to Dunlap et al. (2020) [51]. *B. subtilis* isolates that were involved in rope spoilage, could potentially belong to these new species. However, there is no direct evidence of their involvement in rope spoilage. Metabolic characteristics may differ from *B. subtilis*. [b] formerly designated as *B. firmus*. [c] formerly designated as *B. circulans*. [d] formerly designated as *B. polymyxa*. [e] formerly designated as *B. megaterium*.

...shes the ... mers are ... such as ... assessed ... *ubtilis*, *B.* ... dia. The ... pH was ... efficacies decreased but were still as effective as calcium propionate, a common preservative in the baking industry [54]. Consistent with these findings, Pereira et al. [41] demonstrated that the addition of calcium propionate, together with low aw and pH, inhibited rope development in laboratory-baked bread inoculated with a *B. licheniformis* strain isolated from wholemeal flour and stored at 37 °C throughout a shelf life of seven days [41].

A frequently used natural preservative of bread is the addition of sourdough (**Figure 2**B), whose microbiota is dominated by lactic acid bacteria (LAB) [55]. Due to the production of lactic and acetic acids by LAB, the bread pH drops drastically, thus mostly inhibiting the growth of the *Bacillus* genera. In addition, some LAB secrete other antimicrobial compounds, such as bacteriocins, ethanol, hydrogen peroxide and fatty acids, that suppress *Bacillus* growth in the dough [56][57][58][59]. Hence, numerous studies assessing the inhibiting properties of LAB against *Bacillus* spp. and consequent rope formation were conducted [53][59][60][61][62][63][64]. The addition of 20% sourdough with pH > 4 to bread dough prevented the formation of visual rope by *B. subtilis* and *B. licheniformis* [65]. When the added sourdough had a pH of between 3.5 and 4, just 15% of sourdough was needed to prevent visible rope formation [64]. Interestingly, breads prepared with the same amounts of lactic acid as the dough containing 20% sourdough did not prevent *Bacillus* growth and subsequent rope development, suggesting that rope formation is prevented by the combination of reduced pH and low-molecular-mass compounds produced by LAB [65][66]. Mantzourani et al. [67] studied sourdough breads prepared with kefir grains, which showed good results against rope spoilage by *Bacillus* spp. It is believed that the organic acids play a synergetic role together with the antimicrobial compounds produced by LAB [63]. Another type of antagonistic bacteria used as a natural alternative to chemical food additives are propionic acid bacteria. As the name suggests, these bacteria have the innate ability to produce considerable quantities of propionic acid, thereby inhibiting *Bacillus* spp. based on pH reduction due to the synthesis of propionic acid [3][68].

Sudha et al. [69] reported the use of carambola-pomace powder in wheat bread to control the growth of rope-forming bacteria. The authors reported an equal minimum inhibitory concentration and minimum bactericidal concentration of 1.25 mg/mL for *B. spizizenii* ATCC 6633 and *B. cereus* ATCC 11,778 [69]. However, the addition of carambola fruit pomace powder is not suitable for the production of conventional white wheat bread, as the bread quality is significantly reduced. Breads baked with pomace powder have a decreased loaf volume, denser and more compact crumb of a brownish color, and a fruity to sour taste [69]. It is, therefore, not likely to be widely accepted by consumers.

5. Considerations for the Future

The lack of systematic approaches allowing the characterization and quantification of rope formation makes assessment of rope development in bread extremely difficult. Furthermore, a combination of methods will likely reveal meaningful information about the actual behavior of *Bacillus* spp. in bread. For instance, Pereira et al. [41] used direct inoculation of bread slices for pre-screening potential rope-forming strains and baking trials of one selected isolate to investigate its rope-forming potential in different dough formulations. Li et al. [70] also applied a combination of baking and direct inoculation of bread slices with *Bacillus* spores. The authors relied on baking trials to determine the survival of *Bacillus* spp. with different copy numbers of the $spoVA^{2mob}$ operons. Bread slices were afterwards directly inoculated with spores to determine the spoilage phenotype of different *Bacillus* strains [70]. Both studies acknowledge the importance of this combination, but the characterization and quantification of rope formation was still conducted by somewhat subjective parameters, given the development of patchy discoloration, bread-crumb deterioration, and the characteristic smell produced by rope-forming strains. Thus, more objective parameters are needed to improve the characterization of rope-forming bacilli. Because the attributes of bread-crumb discoloration and deterioration are considered strain dependent, an objective quantification of parameters, such as texture and color, would be of great importance.

The lack of standard protocols and interpretation methods for the analysis of rope spoilage ultimately leads to the absence of clear boundaries for a proper definition of ropiness in bread, and the identification of rope-forming strains and non-rope forming strains, respectively. For instance, hard evidence of enhanced extracellular amylase and protease expression in rope-forming strains compared to non-rope forming strains is still to be found. Furthermore, genetic data on rope formers are scarce and, therefore, the genetic basis of rope formation is still poorly understood. The development of standard protocols and the generation of whole-genome sequence data of rope formers and strains not able to cause rope formation could enable the identification of biomarkers useful for the prediction of RIP. Detection procedures for quick identification of rope-forming bacilli could subsequently be developed to enhance quality control within the baking industry.

As software development and computational power increased rapidly over the last years, many mathematical models for quantitative microbial risk assessment were rendered [71][72]. These models assess, for example, the growth kinetics of foodborne pathogens, such as *Listeria monocytogenes*, in ready-to-eat foods, *Salmonella enterica* in eggs, and *B. cereus* in fried rice [71][73]. Models to optimize baking processes and assess the relationship of raw ingredients and physicochemical properties of baked bread, such as its volume or crumb texture, were also created [71][74][75][76]. The design of predictive models to estimate rope development in bread, as created for the assessment of the spoilage potential of *Clostridium tyrobutyricum* during cheese ripening, would be of great value [77]. As a prerequisite for predictive models, a deeper understanding of rope spoilage and comprehensive data are required.

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