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MINI REVIEW

A review: characteristics and prevalence of psychrotolerant food spoilage bacteria in chill-stored meat, milk and fish

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Abstract

As the human population increases globally, the demands for getting high-quality and nutritious food content are also getting higher. However, the emergence of food spoilage microorganisms is remained challenging to fulfil society's demands in the current food industry. Food spoilage microbes can be introduced into any point across the farm-to-fork supply chain to cause notable degradation in contaminated food, therefore making it unsuitable for human consumption. The majority of food spoilage microbes will not cause serious illness even when consumers have accidentally ingested the contaminated food. Chilling and freezing are commonly used to inhibit microbial proliferation on food quality. However, neither chilling nor freezing are ineffective for psychrotolerant and psychrophilic spoilage microbiota, respectively due to their good adaptation to survive in chilling or freezing temperatures (below 4°C or lower than 0°C) to cause spoilage in refrigerated food. In this article, the process of spoilage development by *Brochothrix thermosphacta*, *Pseudomonas fluorescens* and *Shewanella putrefaciens* in meat, milk and fish, respectively, was reviewed. Meat, milk and fish were chosen as predominance reservoirs for *B. thermosphacta*, *P. fluorescens* and *S. putrefaciens*, respectively. Microbiological descriptions and spoilage symptoms produced by these bacteria were also reviewed in this article. The review concluded that food spoilage would be difficult to control unless certain effective strategies were introduced in the current supply chain. Earlier identification is necessary to detect the prevalence of spoilage bacteria to prevent food wastage.

1. Introduction

Food spoilage is a naturally occurring phenomenon to cause an undesirable modification in organoleptic properties of food such as appearance, colour, shape, flavour, odour, and texture. Spoiled food is heavily influencing consumer acceptability, and is unable to be consumed. Perishable food with higher protein content (meat, poultry, seafood, and dairy product) are more susceptible to invasion of food spoilage bacteria, whereas yeast and mould are frequently involved in the spoilage of non-perishable food (such as fermented food). Several factors that influenced food spoilage are indigenous microbiota, temperature, pH, water activity, food handlers, transportation, and food processing condition (Iulietto *et al*., 2015; Qian *et al*., 2018; Odeyemi *et al*., 2020). Although spoiled food usually has been rejected due to alteration of sensory qualities, it might be still safe to consume in the absence of pathogenic microbes or toxins that cause foodborne illness (Bourdichon and Ronzeau, 2012; Rawat, 2015). Microbiological spoilage can be introduced during pre-harvesting or post-harvesting due to improper food handling and poor hygienic practices (Doyle and Beuchat, 2007; Kuan *et al*., 2017; Lorenzo *et al*., 2018).

Spoilage bacterium can grow on a particular food by production and accumulate by-products such as ammonia and organic acid to cause organoleptic rejection, indicating the food shelf life has come to an end (Amit *et al*., 2017; Boziaris and Parlapani, 2017). Chilling and freezing are the most common, effective and well-

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practised food preservation methods to inhibit and delay microbial invasion to prolong food shelf life (Qian *et al*., 2018). However, it is not reliable for psychrotolerant invasion since the minimum required temperature to grow is between 0 to 5°C (Walker, 2003). There is a possibility for psychrotolerant microbes' growth because they are known to survive in low temperatures to cause food quality deterioration during cold storage, known as specific spoilage microorganisms (SSO). SSO is always defined as the leading cause to cause quality deterioration in that food. Besides that, it can compete and dominate other types of spoilage microbiota. SSO might categorize into only one representative microbial genus or species compared to spoilage microbiota which might have more than one microbial genus or species (Walker, 2003; Qian *et al*., 2018). For example, *Brochothrix thermosphacta*, *Pseudomonas fluorescens* and *Shewanella putrefaciens* are predominantly involved in the degradation of chilled packed meat, milk and seafood, respectively (Vogel *et al*., 2005; Korber *et al*., 2009; Holley, 2014; Odeyemi *et al*., 2020).

Food spoilage is considered consumable waste and economic loss to consumers and producers. FAO and WHO claim that one-third of food is wasted every year (FAO, 2011; Odeyemi *et al*., 2020). According to the European Commission (EC), around 88 tons of food is wasted annually and costs 143 billion euros to be lost in Europe (EC, 2018). Despite increasing advanced technology in food processing and preservation, food spoilage remains a serious global issue. It has been estimated that around 30% of manufactured food products generally undergo microbial spoilage and around 25% of food globally is wasted during post-harvest or post-slaughter processes. However, the actual world economic waste from food spoilage is difficult to estimate accurately (Bourdichon and Ronzeau, 2012; Iulietto *et al*., 2015; Odeyemi *et al*., 2020). Without proper decontamination techniques, microbial contamination to induce food spoilage will not be solved effectively, particularly during food storage periods and food preparation (Scott, 2003; Doyle and Erickson, 2006). This review aimed to highlight the prevalence of *B. thermosphacta*, *P. fluorescens* and *S. putrefaciens* in different high-risk foods under chilling and freezing. The information can help consumers be aware of the ideal condition and target foods of aforementioned bacteria to cause their spoilage activity.

2. *Brochothrix thermosphacta*

2.1 Microbiological descriptions

Brochothrix thermosphacta is a Gram-positive, rod-shaped, non-motile, non-spore-forming, facultative anaerobes (Stanborough *et al*., 2017; Pellissery *et al*., 2020). The bacilli diameter is measured as 0.6 to 0.8 µm, with 1.0 to 2.0 µm long. *Brochothrix thermosphacta* is identified as a non-spore bearing, non-capsulated membrane and non-motile bacterium. *Brochothrix thermosphacta* has classified under *Listeriaceae* family, the same as *Listeria monocytogenes*. Different from *L. monocytogenes*, *B. thermosphacta* has been highlighted as a spoilage microbe, which is non-pathogenic and non-hemolytic to humans (Holley, 2014; Illikoud *et al*., 2018). As a psychotropic bacterium, it can grow at 0 to 30°C, provided with an optimum temperature of 20 to 25°C. The microbial growth seldom occurs beyond 30°C or after exposure to 63°C for 5 mins. A study related to the vacuum-packed spoiled lamb and beef was noticed after 13 to 16 and 124 storage days at refrigeration conditions of 8°C and 1.2°C, respectively. The predominant species was *B. thermosphacta* and it had outgrown other spoilage microbes (*Enterobacteriaceae*, *Pseudomonas* and lactic acid bacteria) in the same pieces of meat. (Holley, 2014; Odeyemi *et al*., 2020). The pH range to assist in the growth of *B. thermosphacta* is 5 to 9 (optimum pH of 7.0). All the strains of *B. thermosphacta* can tolerate 5%, and some of the strains can even go up to 10% of salt concentration, accompanied by a condition of low water activity (0.940 to 0.960). This will cause the application of traditional preservatives such as nitrite or sulphite to be ineffective in preserving meat products since it might facilitate the development of *B. thermosphacta*. As a facultative anaerobe, *B. thermosphacta* can grow in the depletion of oxygen or high concentration of carbon dioxide to conduct meat deterioration in modified atmosphere packaging (MAP) or vacuum packaging (Walkers and Betts, 2008). Besides that, it can withstand up to 50% of carbon dioxide. It has been categorized as a typical representative microbiological agent in meat spoilage, especially in pre-packed (in chilled storage), preserved cure meat, vacuum-packed, and MAP meat (Betts, 2006; Holley, 2014; Pellissery *et al*., 2020). *Brochothrix thermosphacta* becomes dominant over other meat spoilage bacteria. de Filippis *et al*. (2013) observed *B. thermosphacta* become the primary contaminant (75 to 79%) and outgrow other microbiota in beefsteaks at 4°C of aerobic storage. Bacterial counts of *B. thermosphacta* were found dramatically increased compared to the beginning of treatment.

The four main criteria to assist *B. thermosphacta* in achieving a high prevalence in meat products are able to survive in oxygen depletion and elevated carbon dioxide concentration, high tolerance in low pH conditions (5.5 to 6.5), high salt concentrations (approximately 10%), ability to survive in refrigeration temperature (4°C) and production of organoleptically unpleasant compounds. These four criteria are essential and inter-related during

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meat storage (Klicher *et al*., 2010; Gribble and Brightwell, 2013; Stanborough *et al*., 2017). For example, *B. thermosphacta* has been discovered to tolerate 100 ppm of nitrite at pH of more than 5.5 in the presence of 2 to 4% of salt at 4℃ during meat preservation. However, microbial growth can be inhibited when the concentration of nitrite has doubled to 200 ppm, accompanied by the absence of oxygen (Walkers and Betts, 2008; Holley, 2014).

Nevertheless, it can grow in low concentrations of oxygen or high concentration of carbon dioxide to conduct meat deterioration in MAP or vacuum packaging. Low oxygen concentration will not affect the survival of *B. thermosphacta* unless the oxygen level lowers below 0.2%. This is because survival growth for *B. thermosphacta* is highly dependent on the oxygen amount that remains in the meat package. In short, the spoilage activity of *B. thermosphacta* is determined by the concentration of oxygen residue present inside the meat packet, unless the meat package is stored in strictly anaerobic conditions. Generally, the residual oxygen is sufficient to support the spoilage activity to emit offensive odours (Walkers and Betts, 2008, Gribble and Brightwell, 2013; Holley, 2014). Despite a high prevalence of *B. thermosphacta* in aerobically stored chilled meat, a concentration between 50 to 60% of carbon dioxide is effective against the growth of *B. thermosphacta* (EFSA, 2016; Odeyemi *et al*., 2020; Pellissery *et al*., 2020).

2.2 Main reservoirs

Brochothrix thermosphacta was first discovered in sausage and pork trimmings in 1951 (Klicher *et al*., 2010). This type of microbe is usually associated with the spoilage of fresh and cured meat. The natural habitat of *B. thermosphacta* is found in ground soil (Walkers and Betts, 2008; Klicher *et al*., 2010). This could contribute to the transmission route for *B. thermosphacta* during farming to contaminate different varieties of meat products such as beef, chicken, lamb, pork, and sausage, no matter is raw or cooked. For example, 25% of *B. thermosphacta* has been isolated from spoiled meat, raw or cooked sausages using multiplex qPCRs (Bahlinger *et al*., 2021). Other than meat products, few works of literature research documented the occurrence of *B. thermosphacta* in seafood products such as salmon and shrimp. Also, *B. thermosphacta* can be isolated from the food processing environments such as animal carcasses, slaughterhouses, and animal processing equipment using 16S rRNA gene sequencing (de Filippis *et al*., 2013; Grible and Brightwell, 2013; Illikoud *et al*., 2018).

2.3 Development of spoilage activity and spoilage symptoms

Brochothrix thermosphacta is usually required a lower microbial load to initiate an alteration sensory attribute of meat than other meat spoilage microbes. However, the sensory defects of the meat can be observed when the microbial count is achieved at 10^5 to 10^6 CFU/g, *B. thermosphacta* can grow up to 10^8 CFU/g (Betts, 2006; EFSA, 2016). As a facultatively anaerobic meat colonizer, *B. thermosphacta* is capable to grow on meat during aerobiosis or anaerobiosis, in order to conduct its spoilage activity. Under gas mixture with 2% of oxygen, 20% of carbon dioxide and 78% of nitrogen, bacteria count increases (Russo *et al*., 2006; Pellissery *et al*., 2020). According to Russo *et al*. (2006), the microbial load of *B. thermosphacta* on beef was increased from below the detection limit to 10^4 CFU/g under the first MAP conditions (60% oxygen and 40% carbon dioxide), as well as the second MAP condition (20% oxygen, 40% oxygen and 40% nitrogen). In the same study, it was analysed that the increase of *B. thermosphacta* of after seven days of storage. This caused the sudden drop in oxygen level and increase in carbon dioxide level inside the beef package.

To extend the food shelf life and inhibit aerobic microbial growth, vacuum packed and MAP methods are used to preserve meat. However, the mixture of carbon dioxide and nitrogen promotes the prevalence of *B. thermosphacta* (Iulietto *et al*., 2015). Meat spoilage associated with *B. thermosphacta* can be categorized into aerobic and anaerobic spoilage. Under anaerobic conditions, *B. thermosphacta* can tolerate up to 50% concentration of carbon dioxide, inducing a shift from the domination of aerobic microbes (such as lactic acid bacteria) initially present and replacing them. The spoilage is characterized by meat souring rather than meat putrefaction (Betts, 2006; Gribble and Brightwell, 2013; EFSA, 2016). *B. thermosphacta* will produce ethanol and lactic acid as end products of primary metabolisms without producing acetoin. In aerobic conditions, *B. thermosphacta* is produced acetoin, acetic acid, diacetyl, 2- and 3-methylbutanoic acid, which is derived from precursors of valine, leucine, and isoleucine. Besides, spoilage of *B. thermosphacta* is attributed to the formation of volatile fatty acid (VOS) and arises typically from 2 to 6 carbon atoms as a result of amino acid degradation or oxidation of aldehydes ketones and esters but not from lipolysis. VOS originated from phospholipids and triglycerides act as the initial substrate of oxidation with the formation of odorous compounds. However, the information regarding the detailed roles of lipolysis and VOS formation in meat spoilage is still poorly unknown (Casaburi *et al*., 2015;

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Illikoud *et al*., 2018; Pellissery *et al*., 2020). Figure 1 summarizes the common characteristics to be observed in meat spoilage associated with *B. thermosphacta*.

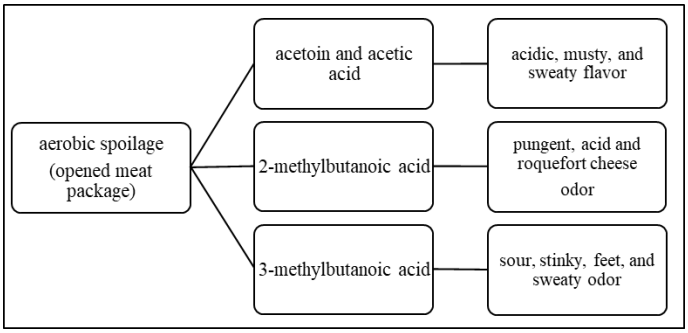

Figure 1. Formation routes of spoilage compound and types of odours released from aerobic spoiled meat (Borch *et al*., 1996; Betts, 2006; Casaburi *et al*., 2015; EFSA, 2016; Illikoud *et al*., 2018; Pellissery *et al*., 2020).

The aerobic condition is due to the remained oxygen present in the opened meat package from the initially sealed package being stored anaerobically. This may induce acetoin formation and give rise to acidic, musty, and sweaty flavours emitted from meat (Borch *et al*., 1996; Betts, 2006). The formation of off-flavours is due to the bacterial metabolism of *B. thermosphacta*. The meat off-odours will be found perceptible to consumers when the microbial load is between 10^7 to $10^{7.5}$ CFU/g. There are two major types of undesirable flavour reported from spoiled meat, foul-smelling odour, and cheesy odour. The foul-smelling smell is due to the aerobic metabolism of glucose to produce acetoin and acetic acid (Dave and Ghaly, 2011; Iulietto *et al*., 2015). The cheesy odour is due to the release of 2 and 3-methylbutanoic acids from spoiled meat (sweaty, stinky feet smell and sour) or Roquefort cheese-like flavour (acid and pungent) (EFSA, 2016; Pellissery *et al*., 2020).

3. *Pseudomonas fluorescens*

3.1 Microbiological description

Pseudomonas fluorescens is Gram-negative, motile bacilli, non-spore-forming, and primarily aerobic microbe. It can grow over a pH range of 4 to 8 and a temperature range of 4 to 42°C, provided with an optimum temperature above 20°C (Scales *et al*., 2014; Meng *et al*., 2017). *Pseudomonas* spp. accounts for 70 to 90% of the microbial population in chilled raw milk spoilage, while *P. fluorescens* is predominant species that is more often identified as post-pasteurized contaminants (PPC) frequently isolated from cold-stored raw milk. Its capability to produce heat-stable hydrolytic enzymes during heat treatment in raw milk makes *P. fluorescens* retain their enzymatic activity even though subjected to low-temperature conditions (Lafarge *et al*., 2004; Quigley *et al*., 2013; Machado *et al*., 2017). Moreover, *P. fluorescens* can outgrowth other genera of psychrotolerant spoilage bacteria found in raw milk such as *Enterococcus*, *Listeria*, *Aeromonas,* and *Staphylococcus*. It is suggested the storage time of raw milk should be reduced across the storage temperature (lower than 3.5°C). It is necessary to minimize the quality deterioration of the dairy supply chain that is altered by *P. fluorescens* during cool storage (De Jonghe *et al*., 2011).

3.2 Main reservoirs

The common strains involved in milk spoilage are *P. fluorescens* SIKW1, *P. fluorescens* AR11, *P. fluorescens* NCIMB 702085 or 701274, and *P. fluorescens* B1-B6, or BJ-10. Previous studies have proved that this bacterium can be isolated from different kinds of pre-heated or post -heated milk such as raw cow milk, goat milk, skim milk, pasteurized milk, and Ultra-High Temperature (UHT) milk (Rajmohan *et al*., 2002; Kumar *et al*., 2019) Polymerase chain reaction (PCR) and next-generation sequencing of 16S rDNA have confirmed *P. fluorescens* as the dominant isolate present in raw milk (Meng *et al*., 2017; Machado *et al*., 2017; Reichler *et al*., 2018). Other than direct raw milk contamination, *P. fluorescens* are detected on the surfaces of various milk processing-related equipment or tools in the dairy supply chain. De Jonghe *et al*. (2011) observed the bacterium had started growing in the farm tank during 8 hrs of transport and 24 hrs of storage. The bacterium growth was observed to increase with 1 log CFU/mL in the farm tank. A striking difference of 2 log CFU/mL has been detected between the optimal (4°C) and suboptimal storage (6°C) in the cold stored raw milk at the end of the study. According to Decimo *et al*. (2014), *P. fluorescens* categorizes as a milk contaminant in bulk milk tanks in Italy. Moreover, *P. fluorescens* has been discovered to form a strong biofilm on the surface of the raw milk tank. The fully established biofilm is difficult to remove from cleaning and sanitizing (Ksontini *et al*., 2013). The nutrient available in the pipeline provides an ideal condition for sustainable growth for *P. fluorescens.* The biofilm growth associated with *P. fluorescens* is temperature-dependent (±10°C) after 48 hrs incubation (Rossi *et al*., 2016; Kumar *et al*., 2019).

3.3 Development of spoilage activity and spoilage symptoms

As psychrotrophic bacteria, a low temperature and long periods of storage are essential criteria in the proliferation of *P. fluorescens* to cause a severe quality issue in preserving raw milk during chilled storage. *Pseudomonas fluorescens* produces two main heat-stable enzymes; protease (AprX) and lipase (LipM) which are able to survive pasteurization or UHT treatment and retain their activity during refrigeration (de Oliveira *et*

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al., 2015; Martins *et al*., 2015). The enzymatic activity of spoilage bacteria depends on initial psychotropic loads and milk storage temperature before undergoing milk processing (Odeyemi *et al*., 2020). *Pseudomonas fluorescens* will start producing protease and lipase to cause spoilage when the bacterial population has reached or exceeded approximately 10^6 CFU/mL or g. The flavour deflection in milk is detected when the bacterial count has been found beyond 10^7 CFU/mL or g. Both enzymes are generally produced during early stationary growth or late log phase with a high density of cells. A bitter and rancid smell is released from the stale milk due to protein degradation and lipid breakdown (de Oliveira *et al*., 2015; Pazdzior, 2016). At first, lecithinase degrades lipid droplets present in milk and increases the lipase action towards milk fat. The lipase can hydrolyse milk fat to induce lipid deterioration via the generation of free short chains of free fatty acids (rancid flavour) and medium chains of free fatty acids (bitter, foamy, and unclean flavour) (De Jonghe *et al*., 2011; Machado *et al*., 2017). It can demonstrate that lipase activity depends on the surrounding temperature and level of lipase activity. Based on the study of Rajmohan *et al*. (2002), the lipase activity had increased during cold storage (10°C) due to the declination of protease level. Another research found that the lipase activity will be optimum at 25°C and pH range from 7 to 10 (Martins *et al*., 2015). Figure 2 lists out the spoilage routes associated with proteolytic and lipolytic enzymes produced *by P. fluorescens* in milk.

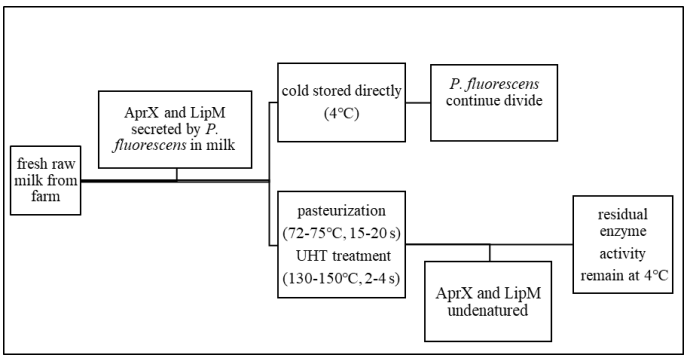

Figure 2. Spoilage routes associated with proteolytic and lipolytic enzymes produced by *P. fluorescens* in milk (De Jonghe *et al*., 2011; de Oliveira *et al*., 2015; Martins *et al*., 2015; Pazdzior, 2016; Machado *et al*., 2017; Odeyemi *et al*., 2020).
AprX: Thermostable protease, LipM: Thermostable lipase

Metalloprotease is a common peptidase produced by *P. fluorescens* which displays thermostability properties and grow optimally between the temperature of 30 to 45°C and at a neutral pH range. This enables *P. fluorescens* to survive during pasteurization (72 to 75°C for 15 to 20 s) and during UHT treatment (130 to 150°C for 2 to 4 s). Consequently, the residual activity is still significant at 4°C during storage (de Oliveira *et al*., 2015; Martins *et al*., 2015). In the study of Meng *et al*. (2017), most of the isolate secreted thermostable peptidase at a temperature of 25°C, following the isolation at a temperature of 7, 10, 4, and 2°C. The most crucial peptidase, namely alkaline metalloprotease (AprX), belongs to the serralysin family and is characterized by different *Pseudomonas* spp., including *P. fluorescens*. *AprX* gene is frequently involved in nutrient utilization, causing the degradation of extracellular milk protein. Due to the specificity of *AprX* in milk casein, protease conducts casein degradation into peptides and amino acids to form grey colour and bitter flavour in UHT milk. Heat treatment is insufficient and impractical to inactivate the enzymatic activity of including *P. fluorescens*. The enzyme activity is found persistent to cause an undesirable change in milk or other dairy products (Raj Mohan *et al*., 2002; De Jonghe *et al*., 2011; Martins *et al*., 2015). The roles of both thermostable enzymes in the process of milk spoilage have been recorded in Figure 3.

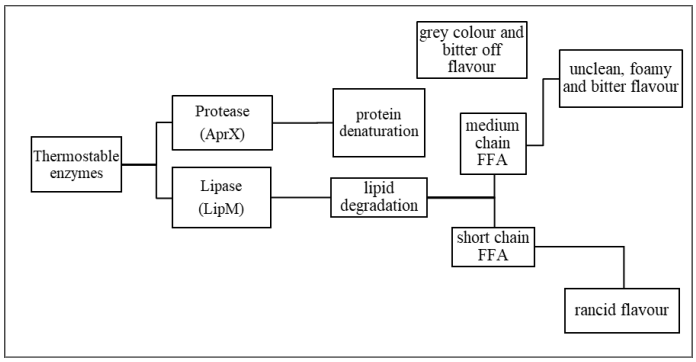

Figure 3. Schematic diagram in the roles of thermostable proteolytic and lipolytic enzymes in the process of milk spoilage associated with *P. fluorescens* (Raj Mohan *et al*., 2002; De Jonghe *et al*., 2011; de Oliveira *et al*., 2015; Martins *et al*., 2015; Meng *et al*., 2017). FFA: free fatty acid

4. *Shewanella putrefaciens*

4.1 Microbiological description

In the food industry, *Shewanella putrefaciens* has been frequently categorized as the main spoilage bacteria in cold or ice stored fish. *Shewanella putrefaciens* is a gram-negative, non-spore forming and curve or rod-shaped (0.5 to 2.0 μm) facultative anaerobes that are categorized under the family of Shewanellaceae (Padzior, 2006; Odeyemi *et al*., 2018). All strains of *S. putrefaciens* are psychrotolerant since they can grow at 0 to 4°C but unable to grow beyond a temperature of 41°C (Liao, 2006; Satomi, 2014). The motility of *S. putrefaciens* is contributed by single, unsheathed, and polar flagellum (Korber *et al*., 2009). It is recognized as a non-pathogenic bacterium and constitutes 1 to 10% of natural marine microbiota in commercial marine fish flesh or bodies surfaces. Thus, it is easily isolated from fresh-caught marine fishes, especially from temperate marine water (Lunestad and Rones, 2008; Wright *et al*., 2016). However, this bacterium will multiply and

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dominate at 10^7 to 10^8 CFU/g to cause problems in marine fishes during chilled or ice cube storage (Korber *et al*., 2009; Satomi, 2014). Since *Shewanella* spp. require the presence of salt to grow and multiply, *S. putrefaciens* has a high prevalence in marine coasters environments, particularly in coasters in warm climates countries. It is well-adapted and survived in marine environments with a salinity of 15 to 20%. A study conducted in Denmark shows that the frequency of *S. putrefaciens* occurrence is highly correlated with seawater temperature. *Shewanella putrefaciens* are high prevalence from July to October when the water temperature is beyond 13℃ (Holt *et al*., 2005; Françoise and Jean-Jacques, 2011). Al-Harbi *et al.* (2004) and Qin *et al.* (2014) reported the isolation of *S. putrefaciens* from infected tilapia was found highest in summer (21.56%; 23 to 39), compared to autumn (17.14%; 12 to 31℃) and winter (15.75%; 7 to 12℃). The temperature of seawater ranged between 7 to 31℃ in these three seasons. The minimum water activity required for *S. putrefaciens* to grow is ranging from 0.950 to 0.970, depending on the concentration of sugar and salt present within the food matrix. Furthermore, *S. putrefaciens* is very sensitive to acidic pH conditions since it is supported by minimum pH of 5.3. For example, *S. putrefaciens* can conduct spoilage on chicken legs and chicken breasts in pH 6.4 to 6.7 and pH 5.7 to 5.9, respectively (Liao, 2006).

4.2 Main reservoirs

Shewanella putrefaciens is ubiquitously distributed in marine water, sewage, soil and natural gas and oil wells. It is occasionally discovered in freshwater sources. It can be tracked in different types of fish such as codfish, cold-cooked salmon, raw salmon, fish fillet, sea bass, and sea bream (Vogel *et al*., 2005; Korber *et al*., 2009; Odeyemi *et al.*, 2018). Other than marine fishes, Beaz-Hidalgo *et al.* (2015) and Paździor *et al.* (2016) have isolated *S. putrefaciens* from brown trout, silver trout, common carp, and silver carp in freshwater sources. Among these identified samples, most of the clusters belonged to *S. putrefaciens* based on the 16S-rDNA phylogenetic tree. Other than ice-stored or vacuum packed marine fishes, *S. putrefaciens* can be isolated from other food sources such as broiler chickens, ground beef and white shrimp based on previous literature studies by using the method of 16S-rRNA genetic sequencing (Vogel *et al*., 2005; Korber *et al*., 2009; Yang *et al*., 2017).

4.3 Development of spoilage activity and spoilage symptoms

The offensive sensory change in ice store marine fish could be noticed when the microbial load of *S. putrefaciens* has been achieved to 10^8 CFU/g. Several foul odours such as putrid, rotten, and sulphurous will begin to emit between 10^8 to 10^9 CFU/g of microbial loads. Furthermore, a slimy appearance on the fish surface could be observed by the naked eye in 10^8 CFU/g. The fish matrix contains lots of non-protein and low molecular weight compounds favouring microbial development for *S. putrefaciens*, including free amino acids, urea, trimethylamine oxide, and a low percentage of carbohydrates (0.2 to 1.5%) which can rapidly be metabolized by *S. putrefaciens*. During anaerobic respiration, *S. putrefaciens* is capable to reduce trimethylamine oxide (TMAO) into abundant amounts of trimethylamine (TMA) rather than aerobic respiration. TMAO is an important pungent molecule that is responsible for the formation of strong amine and fishy odours from spoiled fish. The concentration of TMAO is highly dependent on the types of fish, mainly attributed to marine fish but occasionally discovered in freshwater fish (Vogel *et al*., 2005; Françoise and Jean-Jacques, 2011; Satomi, 2014).

Shewanella putrefaciens can degrade sulphur-containing amino acids to produce volatile hydrogen sulphides (H_2S) and dimethyl-disulphide (CH_3SH) from cysteine and methionine, respectively. The previous analysis discovered these H_2S producing species constitute only minor microbiota in newly caught fish and it will become more dominant during iced storage, multiply into high levels of 10^7 to 10^9 CFU/g (Françoise and Jean-Jacques, 2011; Pazdzior, 2016). H_2S plays a prominent role as an important marker for detecting seafood spoilage such as raw fish stored with ice, raw or cooked shrimps, cold-smoked salmon, and oysters in low storage temperature which indicates the end of the product's shelf life. The emitting of a putrid smell indicates the presence of H_2S. Besides, *S. putrefaciens* can rely on utilizing lactate as a carbon source to survive. It is still able to cause spoilage although the food has been packed under conditions of MAP and vacuum packaging (Yang *et al*., 2017; Odeyemi *et al*., 2018).

In addition, the formation of biogenic amine (BA) from *S. putrefaciens* is important to indicate the quality degradation of seafood (Wright *et al*., 2016). BA accumulation results in proteolytic and dicarboxylic activities by spoilage microorganisms. Cadaverine is identified as the main BA accumulated by *S. putrefaciens.* BA index is an indicator of fish freshness, and it should be in the range of 15 to 20 μg/g. Unlike TMA, the biogenic amine is odourless and will not cause any adverse effects on public health (Liao, 2006, Satomi, 2014; Al-Harbi *et al*., 2004; Visciano *et al.*, 2012). Despite negligible health effects on consumers, biogenic amine spoilage is considered product waste and increases

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the economic burden globally. Furthermore, hypoxanthine is produced by inosine-5' monophosphate. It is known as another index for autolytic deterioration for many kinds of fish, such as codfish. The content of hypoxanthine is represented as low nuclease activity posed by spoilage bacteria in fish (van Spreekens, 1977; Wright *et al*., 2016). Figure 4 illustrates the formation of different compounds associated with the spoilage of fish.

4. Conclusion

It is necessary to ensure food safety along the food supply chain. The presence of food spoilage microbes in food means the food source is unfit for human consumption. It lowers food quality and badly affects food shelf life, leading to food waste. Therefore, several criteria such as temperature, pH, water activity and environmental factors should be carefully monitored by using an established food safety system (ISO 220000 Food Safety Management System, Good Manufacturing Practises; GMP and Hazard Analysis and Critical Control Point; HACCP) in order to minimize and prevent cross-contamination of food spoilage microbes, especially SSOs which specifically cause food deterioration. Hence, the public is responsible for understanding and identifying properties for several food spoilage microbes, including microbiological description, spoilage development, and spoilage signs on targeted and more vulnerable food types towards deterioration.

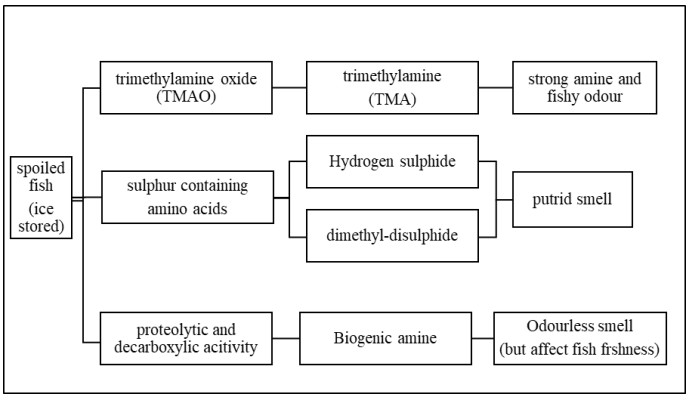

Figure 4. The process of ice-stored fish spoilage and respective flavour types derived associated with *S. putrefaciens* (van Spreekens, 1977; Liao, 2006, Satomi, 2014; Al-Harbi *et al*., 2004; Visciano *et al.*, 2012; Wright *et al*., 2016; Yang *et al*., 2017; Odeyemi *et al*., 2018).

Conflicts of interest

The authors declare that they have no competing interests.

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References

Al-Harbi, A.H. and Uddin, M.N. (2004). Seasonal variation in the intestinal bacterial flora of hybrid tilapia (*Oreochromis niloticus* x *Oreochromis aureus*) cultured in earthen ponds in Saudi Arabia. *Aquaculture*, 229, 37-44. https://doi.org/10.1016/S0044-8486(03)00388-0

Amit, S.K., Uddin, M.M., Rahman, R., Islam, S.M.R. and Khan, M.S. (2017). A review on mechanisms and commercial aspects of food preservation and processing. *Agriculture and Food Security*, 6, 51. https://doi.org/10.1186/s40066-017-0130-8

Bahlinger, E., Dorn-In, S., Beindorf, P.M., Mang, S., Kaltner, F., Gottschalk, C., Gareis, M. and Schwaiger, K. (2021). Development of two specific multiplex qPCRs to determine amounts of *Pseudomonas*, *Enterobacteriaceae*, *Brochothrix thermosphacta,* and *Staphylococcus* in meat and heat -treated meat products. *International Journal of Food Microbiology*, 337, 108932. https://doi.org/10.1016/j.ijfoodmicro.2020.108932.

Beaz-Hidalgo, R., Agüeria, D., Latif-Eugenín, F., Yeannes, M.I. and Figueras, M.J. (2015). Molecular characterization of *Shewanella* and *Aeromonas* isolates associated with spoilage of common carp (*Cyprinus carpio*). *FEMS Microbiol Letters*, 362(1), 1-8. https://doi.org/10.1093/femsle/fnu029

Betts, G. (2006.) Food Spoilage Microorganisms. Cambridge: Woodhead Publishing.

Borch, E., Marie-Lousie, K.M. and Blixt, Y. (1996). Bacteria spoilage of meat and cured meat products. *International Journal of Food Microbiology*, 33(1), 103-120. https://doi.org/10.1016/0168-1605(96)01135-x

Bourdichon, F. and Ronzeau, K. (2012). Microbial food spoilage: A major concern for food business operators. Brasted, Westerham, Great Britain: Russell Publishing Limited.

Boziaris, I.S. and Parlapani, F.F. (2017). The microbiological quality of food. Amsterdam: Elsevier Ltd.

Casaburi, A., Piombino, P., Nychas, G.J., Villani, F. and Ercolini, D. (2015). Bacterial populations and the volatilome associated to meat spoilage. *Food Microbiology*, 45(A), 83-102. https://doi.org/10.1016/j.fm.2014.02.002

Dave, D. and Ghaly, A.E. (2011). Meat spoilage

mechanisms and preservation techniques: A critical review. American *Journal of Agricultural and Biological Science*, 6(4), 485-510. https://doi.org/10.4081/ijas.2015.4011

de Filippis, F., La Storia, A., Villani, F. and Ercolini, D. (2013). Exploring the sources of bacterial spoilers in beefsteaks by culture-independent high-throughput sequencing. *PLoS ONE*, 8(7), e70222. https://doi.org/10.1371/journal.pone.0070222

De Jonghe, V., Coorevits, A., Van Hoorde, K., Messens, W., Van Landschoot, A., De Vos, P. and Heyndrickx, M. (2011). Influence of storage conditions on the growth of Pseudomonas species in refrigerated raw milk. *Applied Environmental Microbiology*, 77(2), 460–470. https://doi.org/10.1128/AEM.00521-10

de Oliveira, G.B., Favarin, L., Luchese, R.H. and McIntosh, D. (2015). Psychrotrophic bacteria in milk: How much do we really know? *Brazilian Journal of Microbiology*, 46(2), 313-321. https://doi.org/10.1590/S1517-838246220130963

Decimo, M., Moran, D.S, Silvetti, T. and Brasca, M. (2014). Characterization of gram-negative psychrotrophic bacteria isolated from Italian bulk tank milk. *Journal of Food Science*, 79(10), 2081–2090. https://doi.org/10.1111/1750-3841.12645

Doyle, M. and Erickson, M. (2006). Emerging microbiological food safety issues related to meat. *Meat Science*, 74(1), 98-112. https://doi.org/10.1016/j.meatsci.2006.04.009

Doyle, M.P. and Beuchat, L.R. (2007). Food Microbiology: Fundamentals and Frontiers. Washington, DC, USA: ASM Press. https://doi.org/10.1128/9781555815912

European Commission (EC). (2018). Food waste. European Union: DG Health and Food Safety. Retrieved on April 1, 2020 from European Commission Website: https://ec.europa.eu/food/safety/food_waste_en.

European Food Safety Authority (EFSA). (2016). Growth of spoilage bacteria during storage and transport of meat. *EFSA Journal*, 14(6), e04523. https://doi.org/10.2903/j.efsa.2016.4523

Food and Agriculture Organization (FAO). (2011). Global Food Losses and Food Waste: Extent, Causes and Prevention. Rome: Food and Agriculture Organization Publication

Françoise, L. and Jean-Jacques, J. (2011). Microbial degradation of seafood. *Aquaculture Microbiology and Biotechnology*, 2, 42-72.

Gribble, A. and Brightwell, G. (2013). Spoilage characteristics of *Brochothrix thermosphacta* and *campestris* in chilled vacuum packaged lamb, and their detection and identification by real time PCR. *Meat Science*, 94(1), 361-368. https://doi.org/10.1016/j.meatsci.2013.03.016

Holley, R.A. (2014). Encyclopaedia of Food Microbiology. Cambridge, Great Britain: Elsevier Ltd.

Holt, H.M., Gahrn-Hansen, B. and Bruun, B. (2005). *Shewanella algae* and *Shewanella putrefaciens*: Clinical and microbiological characteristics. *Clinical Microbiology Infection*, 11(5), 347-352. https://doi.org/10.1111/j.1469-0691.2005.01108.x

Illikoud, N., Klopp, C., Roulet, A., Bouchez, O., Marsaud, N., Jaffres, E. and Zagorec, M. (2018). One complete and three draft genome sequences of four *Brochothrix thermosphacta* strains, CD 337, TAP 175, BSAS1 and EBP 3070. *Environmental Microbiome*, 13(22), 1-12. https://doi.org/10.1186/s40793-018-0333-z

Iulietto, M.F., Sechi, P., Borgogni, E. and Cenci-Goga, B.T. (2015). Meat spoilage: A critical review of a neglected alteration due to ropy slime producing bacteria. *Italian Journal of Animal Science*, 14(3), 316-326. https://doi.org/10.4081/ijas.2015.4011

Klicher S, Loessner M.J. and Klumpp, J. (2010). *Brochothrix thermosphacta* bacteriophages feature heterogeneous and highly mosaic genomes and utilize unique prophage insertion sites. *Journal of Bacteriology*, 192(20), 5441-5453. https://doi.org/10.1128/JB.00709-10

Korber, D.R., Mangalappalli-Illathu, A.K. and Vidović, S. (2009). Biofilm in the Food and Beverage Industries. Cambridge, Great Britain: Woodhead Publishing.

Ksontini, H., Kachouri, F. and Hamdi, M. (2013). Dairy biofilm: Impact of microbial community on raw milk quality. *Journal of Food Quality*, 36(4), 282-290. https://doi.org/10.1111/jfq.12036

Kuan, C.H., Rukayadi, Y., Ahmad, S.H., Wan Mohamed Radzi, C.W.J., Thung, T.Y., Premarathne, J.M.K.J.K., Chang, W.S., Loo, Y.Y., Tan, C.W., Ramzi, O. B., Mohd Fadzil, S. N., Kuan, C.S., Yeo, S.K., Nishibuchi, M. and Radu, S. (2017). Comparison of the microbiological quality and safety between conventional and organic vegetables sold in Malaysia. *Frontiers in Microbiology*, 8, 1433. https://doi.org/10.3389/fmicb.2017.01433

Kumar, H., Franzetti, L., Kaushal, A. and Kumar D (2019). *Pseudomonas fluorescens*: a potential food spoiler and challenges and advances in its detection. *Annals of Microbiology*, 69, 873–883. https://doi.org/10.1007/s13213-019-01501-7

Lafarge, V., Ogier, J.C., Girard, V., Maladen, V., Leveau, J.Y., Gruss, A. and Delacroix-Buchet, A. (2004). Raw cow milk bacterial population shifts attributable to refrigeration. *Applied and Environmental Microbiology*, 70(9), 5644-5650. https://doi.org/10.1128/AEM.70.9.5644-5650.2004

Liao, C.H. (2006). Food Spoilage Microorganism. Cambridge, Great Britain: Woodhead Publishing.

Lorenzo, J.M., Munekata, P.E., Dominguez, R., Pateiro, M., Saraiva, J.A. and Franco, D. (2018). Main groups of microorganisms of relevance for food safety and stability. In Barba, F.J., Sant'Ana, A.S., Orlien, V. and Koubaa, M. (Eds.) Innovative Technologies for Food Preservation, p. 53-107. USA: Academic Press. https://doi.org/10.1016/B978-0-12-811031-7.00003-0

Lunestad, B.T. and Rones, J.T. (2008). Improving Farmed Fish Quality and Safety. Cambridge, Great Britain: Woodhead Publishing Series in Food Science, Technology and Nutrition.

Machado, S.G., Baglinière, F., Marchand, S., Coillie, E., Vanetti, M.C.D., Block, J.D. and Heyndrickx, M. (2017). The biodiversity of the microbiota producing heat-resistant enzymes responsible for spoilage in processed bovine milk and dairy products. *Frontiers in Microbiology*, 8, 302. https://doi.org/10.3389/fmicb.2017.00302

Martins, M.L., Pinto, U.M., Riedel, K. and Vanetti, M.C.D. (2015). Milk-deteriorating exoenzymes from *Pseudomonas fluorescens* 041 isolated from refrigerated raw milk. *Brazilian. Journal of Microbiology*, 46(1), 207–217. https://doi.org/10.1590/S1517-838246120130859

Meng, L., Zhang, Y., Liu, H., Zhao, S., Wang, J. and Zheng, N. (2017). Characterization of *Pseudomonas* spp. and associated proteolytic properties in raw milk stored at low temperatures. *Frontiers in Microbiology*, 8, 2158. https://doi.org/10.3389/fmicb.2017.02158

Odeyemi, O.A., Alegbeleye, O.O., Strateva, M. and Stratev, D. (2020). Understanding spoilage microbial community and spoilage mechanisms in foods of animal origin. *Comprehensive Reviews in Food Science and Food Safety*, 19(2), 311-331. https://doi.org/10.1111/1541-4337.12526

Odeyemi, O.A., Burke, C.M., Bolch, C.C.J. and Stanley, R. (2018). Seafood spoilage microbiota and associated volatile organic compounds at different storage temperatures and packaging conditions. *International Journal of Food Microbiology*, 280(2), 87-99. https://doi.org/10.1016/j.ijfoodmicro.2017.12.029

Paździor, E., Pękala-Safińska, A. and Wasyl, D. (2019). Phenotypic diversity and potential virulence factors of the *Shewanella putrefaciens* group isolated from freshwater fish. *Journal of Veterinary Research*, 63, 321-332. https://doi.org/10.2478/jvetres-2019-0046

Pazdzior, E. (2016). *Shewanella putrefaciens*-a new opportunistic pathogen of freshwater fish. *Journal of Veterinary Research*, 60(4), 429-434. https://doi.org/10.1515/jvetres-2016-0064

Pellissery, A.H., Vinayamohan, P.G., Amalaradjou, M.A.R. and Venkitanarayanan, K. (2020). Meat Quality Analysis: Advanced Evaluation Methods, Techniques, and Technologies. Massachusetts, USA: Academic Press.

Qian, Y. F., Ye, J.X., Yang, S.P., Lin, Z.Q., Cao, W. and Xie, J. (2018). Evaluation of the spoilage potential of *Shewanella putrefaciens*, *Aeromonas hydrophila*, and *Aeromonas sobria* isolated from spoiled Pacific white shrimp (*Litopenaeus vannamei*) during cold storage. *Journal of Food Safety*, 38(6), 1-12. https://doi.org/10.1111/jfs.12550

Qin, L., Zhu, M. and Xu, J. (2014). First report of *Shewanella* sp. and *Listonella* sp. infection in freshwater cultured loach, Misgurnus anguillicaudatus. Aquaculture Research, 45(4), 602–608. https://doi.org/10.1111/j.1365-2109.2012.03260.x

Quigley, L., O'Sullivan, O., Stanton, C., Beresford, T.P., Ross, R.P., Fitzgerald, G.F. and Cotter, P.D. (2013). The complex microbiota of raw milk. *FEMS Microbiology Reviews*, 37(5), 664-698. https://doi.org/10.1111/1574-6976.12030

Rajmohan, S., Dodd, C.E.R. and Waites, W.M. (2002). Enzymes from isolates of *Pseudomonas fluorescens* involved in food spoilage. *Journal of Applied Microbiology*, 93(2), 205-213. https://doi.org/10.1046/j.1365-2672.2002.01674.x

Rawat, S. (2015). Food spoilage: Microorganisms and their prevention. *Asian Journal of Plant Science and Research*, 5(4), 47-56.

Reichler, S.J., Trmčić, A., Martin, N.H., Boor, K.J. and Wiedmann, M. (2018). *Pseudomonas fluorescens* group bacterial strains are responsible for repeat and sporadic post pasteurization contamination and reduced fluid milk shelf life. *Journal of Dairy Science*, 101(9), 7780-7800. https://doi.org/10.3168/jds.2018-14438

Rossi, C., Chaves-Lopez, C., Serio, A., Goffredo, E., Goga, B.T.C. and Paparella, A. (2016). Influence of incubation conditions on biofilm formation by *Pseudomonas fluorescens* isolated from dairy products and diary manufacturing plants. *Italian*

Journal of Food Safety, 5(3), 154–157. https://doi.org/10.4081/ijfs.2016.5793

Russo, F., Ercolini, D., Torrieri, E., Masi, P. and Villani, F. (2006). Changes in the spoilage related microbiota of beef during refrigerated storage under different packaging conditions. *Applied and Environmental Microbiology*, 72(7), 4667-4671. https://doi.org/10.1128/AEM.00468-06

Satomi, M. (2014). Encyclopaedia of Food Microbiology. Amsterdam: Elsevier Ltd.

Scales, B.S., Dickson, R.P., Li-Puma, J.J., Huffnagle, G.B. (2014). Microbiology, genomics, and clinical significance of the *Pseudomonas fluorescens* species complex, an unappreciated colonizer of humans. *Clinical Microbiology Reviews*, 27(4), 927-948. https://doi.org/ 10.1128/CMR.00044-14

Scott, E. (2003) Food safety and foodborne disease in 21st century homes. *The Canadian Journal of Infectious Disease*, 14(5), 277-280. https://doi.org/10.1155/2003/363984

Stanborough, T., Fegan, N., Powell, S.M. and Tamplin, M. (2017). Insight into the genome of *Brochothrix thermosphacta*, a problematic meat spoilage bacterium. *Applied and Environmental Microbiology*, 83(5), 1-20. https://doi.org/10.1128/AEM.02786-16

van Spreekens, K.J.A. (1977) Characterization of some fish and shrimp spoiling bacteria. *Antonie Van Leeuwenhoek*, 43(3-4), 283-303. https://doi.org/10.1007/BF02313756

Visciano, P., Schirone, M., Tofalo, R. and Suzzi, G. (2012). Biogenic amines in raw and processed seafood. *Frontiers in Microbiology*, 3, 188. https://doi.org/10.3389/fmicb.2012.00188

Vogel, B.F., Venkateswaran, K., Satomi, M. and Gram, L. (2005). Identification of *Shewanella baltica* as the most important H2S-producing species during iced storage of Danish marine fish. *Applied and Environmental Microbiology*, 71(11), 6689-6697. https://doi.org/10.1128/AEM.71.11.6689-6697.2005

Walker, S.J. (2003). Chilled Storage. Amsterdam: Elsevier Science Ltd.

Walker, S.J. and Betts, G. (2008). Chilled Foods. Cambridge: Woodhead Publishing.

Wright, M.H., Farooqui, S.M., White, A.R. and Greenea, A.C. (2016). Production of manganese oxide nanoparticles by *Shewanella* species. *Applied and Environmental Microbiology*, 82(17), 5402-5409. https://doi.org/10.1128/AEM.00663-16

Yang, S.P., Xie, J. and Qian, Y.F. (2017) Determination of spoilage microbiota of Pacific white shrimp during ambient and cold storage using next-generation sequencing and culture-dependent method. *Journal of Food Science*, 82(5), 1178–1183. https://doi.org/10.1111/1750-3841.13705

