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REVIEW

Microbiological analysis and microbiota in oyster: a review

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Abstract

Oyster, is a popular shellfish consumed globally. As a bivalve filter-feeding invertebrate mollusk, oyster harbors many microorganisms, which could eventually cause potential health risks of human. Microorganisms were correlated to oyster mortality, shelf life, spoilage, and foodborne pathogenic bacteria. Meanwhile, they could be adjusted by the preservative technologies in order to prolong the shelf life. With the development of molecular biological techniques, such as 16S Polymerase Chain Reaction (PCR), Real-time PCR, Temperature Gradient Gel Electrophoresis (TGGE), Denaturing Gradient Gel Electrophoresis (DGGE), Restriction Fragment Length Polymorphism (RFLP), Fluorescent in situ Hybridization (FISH), etc., microbiological diversity and spoilage mechanism of oyster can be further investigated. The spoilage microbiota belongs to *Vibrio*, *Pseudomonas*, *Aeromonas, Bacillus*, *Enterobacteriaceae*, Lactic Acid Bacteria (LAB), and *Micrococcus*, etc., and the main pathogens are *Vibrio*, *Salmonella*, *Escherichia coli*, *Listeria*, *Staphylococcus*, *Photobacterium*, and *Shewanella* according to current studies. However, little information is available for the spoilage mechanism of entire oyster and different tissues under different preservation conditions. This article reviews the oyster microbiota analysis methods, the impacts of aquaculture and pathogenic bacteria on oyster mortality and food safety, as well as initial and spoilage microbiotas in whole oyster and separated tissues during preservation.

Key Words: oyster; microbiota; pathogen; spoilage mechanism; molecular analysis; preservation

Background

Oyster, a bivalve mollusk, is a nutritious marine food resource that high in protein, vitamin A, vitamin B_{12} and zinc, but low in calories. Many researchers analyzed various nutritional components from oyster and verified that they have functional activities (Achour *et al.*, 1997; Shiozaki *et al.*, 2010; Anderson and Beaven, 2001). With the increase of consumption, oyster farming grows fast, and it is the most popular mollusk aquaculture around the world. The top 6 countries contributing to oyster production are China, Japan, Korea, USA, France and Mexico (Heinonen, 2014). Since 1970, the aquaculture of shellfish doubled every decades worldwide, and the demand is still increasing (Dégremont *et al.*, 2015). There are approximately 4 million tons of oysters consumed annually and half of them are eaten raw (Fang *et al.*, 2015). China produces over 2 million tons of oyster per year, which is mainly used to make oyster sauce (Heinonen, 2014).

In view of the fast growth of the oyster aquaculture, the impacts of disease and mortality on the yield of oysters attracted prompt attentions by government, farmers, and researchers. However, the research on oyster pathogenic bacteria is challenging due to a wide variety of oysters and aquaculture location worldwide. In previous studies,

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List of abbreviations:
Automated Ribosomal Intergenic Spacer Analysis, ARISA; Denaturing Gradient Gel Electrophoresis, DGGE; Fluorescent *in situ* Hybridization, FISH; Lactic Acid Bacteria, LAB; Polymerase Chain Reaction, PCR; Restriction Fragment Length Polymorphism, RELP; Temperature Gradient Gel Electrophoresis, TGGE; Terminal Restriction Fragment Length Polymorphism, T-RFLP

Vibro aestuarianus and *Vibro splendidus* were reported to cause the summer mortality of *C. gigas* oysters in France (Le Roux *et al.*, 2002; Gay *et al.*, 2004; Garnier *et al.*, 2007). Furthermore, the introduction of nonnative oyster may lead to disease outbreak (Beck *et al*., 2011). The observation of oyster mortality is the main sign of diseases in aquaculture oyster. Preventing contamination and keeping pathogen-free environment is of vital importance in oyster farming (Dégremont *et al*., 2015). On the other hand, the bacteria from fresh oyster were attracted more attentions because some of the bacteria can bring about the outbreak of human diseases. For example, *Vibrio parahaemolyticus* is a pathogenic bacterium for oyster, which is also well-documented foodborne bacteria responsible for the outbreaks of shellfish-associated gastroenteritis and diarrhea correlated to seafood consumption in the United States (Dalsgaard, 1998; Liu *et al*., 2009).

Perishable oyster could cause serious foodborne problems in processing and distribution. Microbial activity is mainly responsible for the changes in flavor, texture, and odor (Cao *et al.*, 2009; Prapaiwong *et al.*, 2009a; Montanhini and Neto, 2015). Compared to terrestrial foods, oyster has shorter shelf life due to relatively higher levels of free ammonia nitrogen and high diversity of microbiota (Madigan *et al.*, 2014). The shelf-life of oyster could be affected by many factors, such as extrinsic factor (temperature, atmosphere), intrinsic factors (species, size, age, health and composition) and microbial flora load (Linton *et al*., 2003; Cao *et al*., 2010; Chen *et al*., 2016). Among those factors, microbiota in oyster plays critical roles on oyster diseases, food safety, and spoilage. This article summarized the oyster microbiota, including the analysis approaches, environmental impacts, pathogenic bacteria, and the microbiota in different oyster tissues.

Analysis approaches for oyster microbiota

Conventional cultivation method was widely used to analyze the bacterial population, and to isolate them through streak plate method. It plays an important role to obtain the bacterial strains. Cultivation method was used to investigate bacterial microbiota and dominant species in oyster, among which *Pseudomonas* were accounted for one third of 321 isolates and reported as dominant bacteria (Kueh and Chan, 1985). This method has been widely utilized for oyster microbiota analysis to reveal the bacterial population and community in details (Colburn *et al*., 1990; Cao *et al*., 2009, 2010; Liu *et al.*, 2009; Song *et al.*, 2009; Fang *et al*., 2015). However, cultivation and following isolation for microbiota analysis was time and resource consuming with poor reproducibility (Cao *et al.*, 2009; Prapaiwong *et al*., 2009a).

The phylogenetic analyses of rRNA genes from laboratory culture and isolates were applied to evaluate the microbiota, of which the efficiency were highly improved in oyster bacterial analysis and many species were identified by sequencing (Prapaiwong *et al*., 2009a; Green and Barnes, 2010; Lee *et al.*, 2010; Thupila *et al.*, 2011). Conventional cultivation method could result in overestimation or underestimation of the microbiological community, because many bacteria are naturally uncultivable and unsuitable media may lead to biased results (Randazzo *et al.,* 2002; Chen *et al.*, 2013). Molecular approach shows more abundant of bacterial microbiota than cultivation method in oysters (Romero *et al.*, 2002).

In the past decades, culture-independent methods of finger print profile were introduced to oyster analysis for bacterial microbiota and diversity, such as TGGE (Fernández *et al*., 2014) and DGGE (Chen *et al.*, 2013; Wood and Arias, 2015), Terminal Restriction Fragment Length Polymorphism (T-RFLP) (Garnier *et al*., 2007; Fernandez‐Piquer *et al.*, 2012), which revealed that oyster had high diversity in the bacteria. DGGE was widely utilized in the characterization of the bacterial communities from farmed, retailed, and storied oysters. The fingerprints of DGGE gel intuitively reflect the microbiota variation by the band changes, of which the band corresponding to the bacteria (more than 1 %) can be clearly profiled (Chen *et al.*, 2013; Wood and Arias, 2015). However, DGGE method may also subjected to the inaccuracy on bacterial diversity evaluations resulted from DNA extraction, PCR amplification, and sequencing errors from environmental samples (Wintzingerode et al., 1997). This bias was also observed in Wood's study (Wood and Arias, 2015) when they applied DGGE to reveal the bacteria in oyster, few bands from DGGE couldn't be amplified and identified.

Compared to DGGE, T-RFLP technique is more reproducible and accurate, but more expensive. Both of them provide overview of the bacterial communities and the variation of dominant bacteria in oyster. Real-Time PCR and Multiplex Real-Time PCR were also introduced to identify and track the target bacteria with higher efficiency and accuracy for the bacteria with lower abundant, especially pathogen community (Ward and Bej, 2006; Nordstrom *et al.*, 2007; Kim *et al*., 2008a). The FISH on the basis of the designed probe were used in different organ microbiota in oyster and the high abundance of the bacteria were observed (Hernández-Zárate and Olmos-Soto, 2006). The ARISA approach also showed the high diversity of oyster gill microbiota effectively (Zurel *et al*., 2011). However, because of the high cost the new technologies, such as metagenome and transcriptome, were not commonly used in previous oyster microbiota studies.

Environmental impacts on oyster microbiota

The diversity and community of bacteria in raw oysters were affected by many factors. Oyster is normally eaten by whole body, thus all tissues with its original microbiota are eventually consumed by human. The impacts of aquaculture environmental are of vital importance to original microbiota, because all attached initial bacteria from environment were closely correlated to the microbiota in the growing stages of oyster, harvest, sale, storage, and consumption. These factors include the location of the sea (Cao *et al*., 2009; King *et al.*, 2012; Madigan *et al*., 2014; Wood and Arias, 2015), harvest season (Parveen *et al*., 2008), water temperature (Gonzalez-Acosta *et al*., 2006; Shen *et*

Table 1 Food borne pathogen in oyster aquaculture, sale and storage

Species/ Location	Pathogen/ Food borne pathogen*	Analysis Method	Reference
C. virginica oyster/ Mobile Bay, US	*V. parahaemolyticus**	Alkaline phosphatase-labeled DNA probe procedures	(Kaufman *et al*., 2003)
Pacific oysters (*C. gigas*)/ Arcata Bay, US	*Listeria* sp.*, *L. monocytogenes**	Culture and isolates	(Colburn *et al.*, 1990)
Ostrea rivularis	*Salmonella* spp.	Culture	(Fang *et al*., 2015)
Oyster/ Washington, US	*V. parahaemolyticus**	Culture	(Liu *et al*., 2009)
Oyster (*C. gigas*)/ France	*Vibrio splendidus*, *Vibrio aestuarianus*, *Vibrio harveyirelated*, *Shewanella colwelliana*	Isolates genotyping by the 16S rRNA and gyvB genes	(Saulnier, Decker *et al*., 2009)
Pacific oyster (*C.gigas*)/ France	*V. aestuarianus*	Real-time PCR	(Saulnier, De Decker *et al*., 2009)
Commercial oyster/ New Jersey coast, US	*Shewanella algae*, *S. putrefaciens*, *Photobacterium damselae* subsp. *damselae*,	16S rRNA genes sequencing	(Richards *et al*., 2008)
Raw oyster	*V. parahaemolyticus**	Real-time PCR	(Kim *et al*., 2008b)
Oyster/ Louisiana, US	*Salmonella*, *V. parahaemolyticus**	MICRO-IS and API-20E systems	(Abeyta *et al*., 1986)
Retailed oyster/ Shanghai,China	*V. parahaemolyticus**	Polymerase chain reaction (PCR)	(Yu *et al*., 2016)
Salted oyster (*Jeotkal*)/ Korea	*L. monocytogenes**, *Staphylococcus aureus**, *V. parahaemolyticus**	Culture	(Song *et al*., 2009)
C. gigas oyster/ Mediterranean, France	*Escherichia coli**	Culture	(Derolez *et al*., 2013)
Commercial oyster/ US	*V. vulnificus**, *V. parahaemolyticus**, *V. alginolyticus**, *A. hydrophila**	Culture isolates with 16S rDNA identification	(Prapaiwong *et al*., 2009a)
C. virginica oyster/ Dauphin Island, US	*V. parahaemolyticus**, *V. mimicus*, *V. vulnificus*	Total Bacteria and *Vibrio*-Specific Denaturing Gradient Gel Electrophoresis	(Wood and Arias, 2015)
Oyster tissues	*V. parahaemolyticus**	PCR detection	(Wang *et al*., 2010)
Pacific Oyster (*C. giga*)/ Atlantic coast, France	*V. aestuarianus*, members of the *V. splendidus* group, *V. natriegens*, *V. parahaemolyticus**, *Pseudoalteromonas* sp.	The dominant colonies were identified by phenotypic and genotypic characters (RFLP)	(Garnier *et al*., 2007)
C. virginica oyster/ Mobile Bay, US	*V. parahaemolyticus**	Direct plating method involving an alkaline-phosphatase-labeled DNA probe	(Gooch *et al*., 2002)
Oyster	*L. innocua*	Isolation and biochemical tests	(Colburn *et al.*, 1990)
Oyster (*Crassostrea belcheri*)/ Thailand	*Salmonella**, *V. parahaemolyticus**, *V. vulnificus**	16S rRNA gene sequencing	(Thupila *et al*., 2011)
Raw oyster/ Korean	*V. parahaemolyticus**	Real-time PCR	(Kim *et al*., 2008a)
Pacific oysters (*C. gigas*)	*V. parahaemolyticus**	Culture	(Ma and Su, 2011)

Zhe oyster (*Crassostrea plicatula*)/ Zhejiang, China	*V. parahaemolyticus**	Culture	(Shen *et al*., 2009)
Alaskan oysters/ US	*V. parahaemolyticus**	Multiplex Real-Time PCR	(Nordstrom *et al*., 2007)
Oyster (*C. virginica*)/ Chesapeake Bay, US	*V. parahaemolyticus**	Quantitative direct-plating method followed by DNA colony hybridization	(Parveen *et al*., 2008)
Oyster/ Mandinga Grande Lagoon, US	*V. parahaemolyticus**	Culture	(Flores-Primo *et al*., 2014)
Raw oyster/ Alaska, US	*V. parahaemolyticus**	Isolates identified by PCR	(McLaughlin *et al*., 2005)
Live oysters	*Vibrio* spp.	Multiplex PCR and DNA microarrays	(Panicker *et al*., 2004)
Oyster/ Washington, US	*V. parahaemolyticus**	Multiplexed Real-Time PCR	(Ward and Bej, 2006)
Oyster/ Dauphin Island Bay, Alabama, US	*V. vulnificus*, *V. parahaemolyticus**	Quantitative PCR	(Givens *et al*., 2014)
Raw Pacific oysters	*V. parahaemolyticus**	Culture	(Liu *et al*., 2009)

* Food borne pathogen.

al., 2009), aquatic environment (La Valley *et al*., 2009; Shen *et al*., 2009; Azandégbé *et al*., 2012; King *et al*., 2012), and environmental stress (Paillard *et al*., 2004; Green and Barnes, 2010).

The initial bacterial communities from different areas are different. Cruz-Romero (2008b) reported that the initial bacterial communities in raw oyster (*C. gigas*) from Cork harbor were dominant by *Aeromonas, Vibrio,* and *Pseudomonas*. The results were similar to the reported bacterial communities of the oysters from Yellow Sea in China (Cao *et al*., 2009), in which *Pseudomonas*, *Vibrio* were presented as the dominant bacteria. Except *Pseudomonas* and *Flavobacterium,* Ortigosa *et al.* (1995) reported that *Alteromonas*, *Shewanella*, *Deleya*, and *Oceanospirillum* were detected in the oysters from Mediterranean Coast. Despite the location, the microbiota were different under controlled and natural environments (Colwell and Liston, 1960). In our previous study (Chen *et al*., 2013), the dominant microbiota in the raw oyster gills were *Lactococcus*, *Lactobacillus*, *Enterobacter*, and *Aeromonas*.

Harvest season was one of the main factors responsible for different varieties of the oyster microbiota (Parveen *et al.*, 2008; Wang *et al.*, 2014b; Roterman *et al*., 2015), which has been well demonstrated by molecular methods. Prapaiwong *et al.* (2009a) observed that more *Vibrio vulnificus* could be isolated from raw oysters living in relatively higher water temperature. In addition, the bacterial communities were correlated to oyster species (Roterman *et al*., 2015). The water temperature can affect the bacteria loads in oyster. The correlation between seawater and mictobiota in oyster were revealed through isolates and rDNA hybridization with phylogenetic probes, and most isolates unidentified corresponded to α-*Proteobacteria* (Pujalte *et al.*, 1999).

Pathogenic bacteria in oyster

The pathogenic bacteria related to oyster diseases and mortality, as well as human pathogens associated with aquaculture oyster were summarized as shown in Table 1. Among main human pathogenic bacteria, *Vibrio*, *Aeromonas*, *Salmonella*, *E. coli*, *Listeria*, *Staphylococcus*, *Photobacterium*, and *Shewanella* have been extensively investigated in oyster aquaculture and storage (Table 1). *Vibrio* and *Aeromonas* were the main genus of bacterial pathogenic for oyster. The traditional cultivation and identification, 16S PCR sequencing, Real-time PCR, DGGE, RFLP, Multiplex Real-Time PCR, and quantitative PCR were used to investigate the pathogenic bacteria and microbiota in oyster. Real-time PCR and quantitative PCR were regard as the effective way in *Vibrio* inspection, which were designed to reveal the existence of the target pathogen in oyster (Nordstrom *et al*., 2007; Kim *et al.*, 2008b; Saulnier *et al*., 2009). In view of the difficulty of identification, polyphasic approaches have been developed to identify potential pathogens associated with oyster diseases (Paillard *et al.,* 2004).

Vibrio species were reported as the main pathogenic species in the oyster leading to 8,000 illnesses per year in the United States *(Kaufman et al*., 2003), which has been extensive studied regarding oyster diseases and mortality, and food safety (Kaufman *et al*., 2003; Panicker *et al.*, 2004; Nordstrom *et al*., 2007; Liu *et al.*, 2009; Saulnier *et al.*, 2009; Yu *et al.*, 2016). The pathogenic bacteria associated with public health are *V. vulnificus*, *V. parahaemolyticus*, *Vibrio alginolyticus*, and *Aeromonas hydrophila* in raw oysters (Lorca *et al.*, 200; Prapaiwong *et al*., 2009a). The proliferation of *V. vulnificus* during storage at temperature abuse conditions (*e.g*., 7, 13, and 21 °C) makes the oyster unsafe (Lorca *et al.*, 2001).

The risk of raw and uncooked oysters resulting in gastroenteritis in consumers has been well described (Kueh and Chan, 1985; Green and Barnes, 2010). Using *Vibrio*-Specific DGGE and RFLP approaches, the profiles of *Vibrio* were clearly demonstrated (Garnier *et al*., 2007; Wood and Arias, 2015). More *V. parahaemolyticus* have been found in the gills and digestive glands than those in other portions of the oysters (Wang *et al*., 2010). Prapaiwong *et al.* (2009a) showed that *Shewanella*, *Vibrio*, *Psychrobacter* and *A. hydrophila* were also identified in raw oysters, quick frozen oysters, and high pressure processed oysters, whereas *V. vulnificus* was only detected in raw oysters. The potential risk of *V. parahaemolyticus* infection might increase, and recently Yu *et al.* (2016) demonstrated that 33 out of 96 isolates showed resistance to two or more antimicrobial agents in Shanghai, China.

Salmonella are regarded as one of the most common human pathogenic bacteria in shellfish; however, they were not detected in oyster either under high pressure treatment or other controlled storage conditions (Jones *et al.*, 1993; Bej *et al.*, 1994; López-Caballero *et al.*, 2000). *E. coli* found in raw oyster by culture-dependent DGGE method illustrated that they may have potential hazard for the ingestion of fresh oyster (Chen *et al.*, 2013). *Listeria monocytogenes* were reported to be associated with foodborne outbreaks (Colburn *et al.*, 1990). *L. monocytogenes* and *Staphylococcus aureus* have been presented to be killed using electron beam irradiation in salted oyster (Song *et al.*, 2009).

The pathogenic bacteria for oyster can also lead to the death of oysters, which cause big losses in oyster farming and related industry. *V. aestuarianus* and *V. splendidus* were reported to be related to the summer mortality of the *C. gigas* in the sea in France. While in North America, *V. tubiashii* were found to be associated with the mortalities of hatchery-reared *Crassostrea virginica* oysters and *C. gigas* (Saulnier *et al*., 2009). Garnier *et al*. (2007) demonstrated similar results in their study as *V. aestuarianus* was detected in 56 % of isolates while 25% of isolates contains *V. splendidus* group.

Microbiota in different oyster tissues

Bacterial microbiota in aquaculture, processing and preservation were studied in the past decades. The predominant bacterial communities were diverse in raw oysters. As list in Table 2, the microbiota in oyster mainly included *Pseudomonas*, *Vibrio*, *Aeromonas*, *Moraxella*, *Shewanella*, *Flavobacterium*, *Acinetobacter*, *Enterobacteriaceae*,

Table 2 Microbiota and analysis methods for oyster storied at different condition

Oyster species/ Location	Treatment methods	Initial dominant microbiota	Spoilage or Survival microbiota	Treatment conditions & duration	Analyzing method	Reference
Pacific oyster (*C. gigas*)	Natural flora	*Pseudomonas*, *Vibrio*, *Achromobacter*, *Flavobacterium*, *Corynebacterium*, *Alcaligenes*, *Micrococcus*, *Bacillus* sp., Enterococci	NA	NA	Culture and isolates	(Colwell and Liston, 1960)
Pacific oyster (*C. gigas*)/ Yellow sea, China	Refrigeration	*Pseudomonas**, *Vibrionaceae**, *Shewanella*, *Alcaligenes*, Enterobacteriaceae, *Moraxella*, *Acinetobacter*, *Flavobacterium*, *Corynebacterium*, *Staphylococcus*, *Micrococcus*, Lactic acid bacteria, *Bacillus* sp.	*Pseudomonas**, *Vibrionaceae**, *Moraxella*, *Flavobacterium*, *Micrococcus*, *Bacillus* sp.	Storage at 5 ±1 °C for 12d	Culture and isolates	(Cao, Xue and Liu, 2009)
Pacific oyster (*C. gigas*)/ Yellow sea, China	Ozonated water treated	*Pseudomonas*, *Vibrionaceae*, *Shewanella*, *Alcaligenes*, Enterobacteriaceae, *Moraxella*, *Acinetobacter*, *Flavobacterium*, *Corynebacterium*, *Staphylococcus*, *Micrococcus*, Lactic acid bacteria, *Bacillus* sp.	*Pseudomonas**, *Vibrionaceae**, Enterobacteriaceae, *Moraxella*, *Flavobacterium*, *Micrococcus*, *Bacillus* sp.	Ozonated water (5.0×10^{-6} g/L for 2 min)	Culture and isolates	(Cao *et al*., 2010)

Pacific oyster (*C. gigas*)/ Yellow sea, China	Refrigeration	*Pseudomonas*, *Vibrionaceae*, *Shewanella*, *Alcaligenes*, Enterobacteriaceae, *Moraxella*, *Acinetobacter*, *Flavobacterium*, *Corynebacterium*	*Pseudomonas**, *Vibrionaceae**, *Moraxella*, *Flavobacterium*, *Micrococcus*, *Bacillus* sp.	Storage at 0 °C	Culture and isolates	(Cao, Xue, Liu *et al.*, 2009)
		Staphylococcus, *Micrococcus*, Lactic acid bacteria, *Bacillus* sp.	*Pseudomonas*, *Vibrionaceae*, *Alcaligenes*, Enterobacteriaceae, *Moraxella*, *Flavobacterium*, *Micrococcus*, Lactic acid bacteria, *Bacillus* sp.	Storage at 10 °C		
C. gigas oyster	High hydrostatic pressure	*Bacillus*, *Moraxella*, *Acinetobacter*, *Pseudomonas*, *Micrococcus*, *Coryneforms*,	*Bacillus*	Control: 300 Mpa for 2 min at 20 °C , 0 d	Isolated from agar plates incubated at 7 °C	(Linton *et al.*, 2003)
		Flavobacterium, *Cytophaga*, *Alcaligenes*, *Agrobacterium*	*Moraxella*, *Acinetobacter*, *Flavobacterium*, *Cytophaga*	Storage at 2 °C 14 d		
			*Bacillus**, *Moraxella*, *Acinetobacter*	Storage at 2 °C, 28 d		
C. gigas oyster	High hydrostatic pressure	*Bacillus*, *Moraxella*, *Acinetobacter*, *Micrococcus*, *Coryneforms*, *Flavobacterium*, *Cytophaga*,	*Bacillus*, *Micrococcus*, *Alcaligenes*, *Agrobacterium*, *Staphylococcus*	Control: 500 Mpa for 2 min at 20 °C , 0 d	Isolated from agar plates incubated at 30 °C	(Linton *et al.*, 2003)
		Enterobacteriaceae, *Staphylococcus*	*Bacillus*, *Moraxella*, *Acinetobacter*, *Pseudomonas*, *Micrococcus*, *Flavobacterium*, *Cytophaga*, *Alcaligenes*, *Agrobacterium* , *Staphylococcus*	Storage at 2 °C, 14 d		
			*Moraxella**, *Acinetobacter**	Storage at 2 °C, 28 d		
Pacific oyster/Coffin Bay, Australia	Refrigeration	*Prosthecomicrobium*, *Mycoplasma*, *Helicobacter*, *Terasakiella*	*Vibrio*, *Arcobacter*, *Pseudoalteromonas*	Storage at 4 °C, 7 d	16S rRNA pyro-sequencing	(Madigan *et al.*, 2014)
Sydney rock oysters/ Australia	Refrigeration	*Mycoplasma*, *Spirochaeta*, *Haloplasma*	*Pseudoalteromonas*, *Vibrio*, *Colwellia*	Storage at 4 °C, 7 d		(Madigan *et al.*, 2014)
Pacific oysters (*C. gigas*)	High Pressure	*Aeromonas*, *Vibrio*, *Pseudomonas*, *Maraxella*, *Acitenobacter*, *Micrococcus*,	*Shewanella*, *putrifaciens*, *Pseudomonas*, *fluorescens*	260 MPa for 3 min, stored at 2 °C, 14 d	API identification system	(Cruz-Romer *et al.*, 2008a)
		Coryneforms, *Lactobacillus*, *Leuconostoc*, *Enterobacteriaceae*, *Bacillus*	*Pseudomonas* spp.*	500 or 800 MPa for 5 min stored at 2 °C, 14 d		
Commercial oyster/ US	High Pressure	Gammaproteobacteria, Alphaproteobacteria,	*Shewanella*, *Vibrio*, *Psychrobacter*	High pressures of	Culture isolates with	(Prapaiwong *et al.*, 2009a)

		Flavobacteria, Bacilli, Actinobacteria, Sphingobacteria		250 to 400 MPa for 1 to 3 min	16S rDNA identificaiton	
Commercial oyster/ US	Quick Frozen	NA	*Shewanella** (in winter); *Shewanella**, *Vibrio**, and *Psychrobacter ** (in summer); *Psychrobacter** and *Vibrio* (dominant in fall)	Quick Frozen oysters were kept at -20 °C	Culture isolates with 16S rDNA identificaiton	(Prapaiwong *et al.*, 2009a)
Pacific oyster (*C. gigas*)/ Tasmania	Refrigeration	*Proteobacteria** *Spirochaetes*, *Planctomycetes*, *Verrucomicrobia*, *Fusobacteria*, *Firmicutes*, *Tenericutes*, *Cyanobacteria*, *Bacteroidetes*	*Psychrilyobacter* spp.* (phylum Fusobacteria), Fusobacteria, Spirochaetes	4 °C	T-RFLP	(Fernandez-Piquer *et al.*, 2012)
			*Bacteroidetes**	15 °C & 30 °C		
Oyster (*C. plicatula*) gill/ Fujian, China	Refrigeration	*L. raffinolactis*, *Weissella cibaria*, *Lactococcus* sp., *Lactococcus lactis* subsp. *lactis*, *E. mundtii*, *E. coli*, *Aeromonas*, *Lactococcus garvieae*, *A. hydrophila* subsp. *hydrophila*	*Lactococcus**, *Lactobacillus**, *Weissella confusa*, *C. difficile*	10 °C, 4 & 8 d	DGGE	(Chen *et al.*, 2013)
			Lactococcus, *Weissella*, *Enterobacter*, *Aeromonas*	4 °C, 6 & 12 d		(Chen *et al.*, 2013)
Eastern Oyster (*C. virginica*)/ Dauphin Island, US	Refrigeration	*V. parahaemolyticus, V. shiloi*, *V. vulnificus*	*V. diazotrophicus*, *Listonella anguillarum*, *V. vulnificus*	Refrigeration at 6 ± 2 °C	Total Bacteria and *Vibrio*-Specific DGGE	(Wood and Arias, 2015)
Oysters (*Tiostrea Chilensis*)/ Chile	Room temperature	NA	*Pseudoalteromonas* species	Room temperature (18 °C) at 4, 25, and 100 h after harvest	PCR 16S-23S rDNA	(Romero, González *et al.*, 2002)
C. gigas oyster/ South Korea	Only for raw oyster test	*Lactobacillus* spp., *V. alginolyticus*, *V. proteolyticus*	NA	NA	16S rRNA gene sequencing	(Lee *et al.*, 2010)
Pacific oysters(*C. gigas*), Deep Bay, Hong Kong	Raw oyster	*Pseudomonas* spp.*, *Vibrio, Acinetobacter, Coliforms, Aeromonas* spp., *Flavohacterium*, *Cytophaga, Coryneforms, Alcaligenes, Micrococcus*	NA	NA	Culture Isolation and identification	(Kueh and Chan, 1985)
Oysters (*C.corteziensis*, *C. gigas* and *C. sikamea*)	Commercial production	Proteobacteria, Bacteroidetes, Actinobacteria, Firmicutes	NA	NA	Pyro-sequencing approach of the 16S rRNA gene	(Trabal *et al.*, 2012)
Commercial oysters (*C. corteziensis*)	Different growth phases (post-larvae, juvenile, and adult)	ß-Proteobacteria (post-larvae, juvenile, and adult), Spirochaetes (juvenile), Actinobacteria (juvenile)	NA	Different growth phases	PCR, RFLP, TGGE	(Fernández *et al.*, 2014)

Commercial oysters (*C.gigas*)		α-Proteobacteria (post-larvae, juvenile, and adult) β-Proteobacteria (post-larvae, juvenile, and adult) γ-Proteobacteria (adult), Bacilli (post-larvae, juvenile, and adult)	NA			
Mangrove oysters/ Gbolokiri creek, Nigeria	Depuration of oysters	Bacteria: *Bacillus* spp., *Pseudomonas aeruginosa*, *Proteus* spp., *Vibrio* spp., *E. coli*, *S. aureus,* *Acinetobacter* sp., *Micrococcus* sp., *Corynebacterium* sp., *Lactobacillus* spp. Fungi: *Aspergillus niger*, *A. flavus*, *A. nidulans*, *Penicillium* spp., *Fusarium* sp., *Rhodotorula* sp.	Bacteria: *Bacillus*, *Pseudomonas aeruginosa*, *Proteus* spp., *Vibrio* spp., *Streptococcus* spp., *S. aureus* Fungi: ND	Brackish water treatment	Culture isolated bacterial	(Amadi, 2015)
	Raw oyster		Bacteria: *Bacillus**, *Pseudomonas**, *Vibrio*, *Streptococcus*, *Proteus*, *Lactobacillus*, *Micrococcus*, *Corynebacterium* Fungi: *Aspergillus*, *Penicillium*, *Fusarium*	Storage at (30 ± 2°C) ambient temperature for 24 h		
Oyster (*Crassostrea plicatula*)/ Fujian, China	Gill	*Lactococcus**, *Photobacterium*, *Weissella*, *Lactobacillus**, *Enterococcus*, *Enterobacter*, *Leclercia*, *Escherichia*, *Spirochaeta*, *Aeromonas*, *Citrobacter*	*Lactobacillus**, *Lactococcus**	Modified Atmosphere Package	DGGE	(Chen *et al.*, 2016)

* Dominant bacteria; ND: not detected; NA: not available.

Photobacterium, *Alcaligenes*, *Micrococcus*, *Staphylcoccus*, *Lactococcus*, *Lactobacillus*, *Corynebacetrium*, and *Bacillus Mycoplasma.* In addition, fungi *of Aspergillus*, *Penicillium*, *Fusarium* and *Rhodotorula* were obtained in oyster. The cultivation sites, life stages (e.g. post-larvae at the hatchery, juvenile, and adult) and the oyster species (*Crassostrea corteziensis*, *C. gigas*, and *Crassostrea sikamea*) have an impact on the microbiological communities in oyster (Trabal *et al.,* 2012; Fernández *et al.*, 2014). In addition to aforementioned microbiota, *Shewanella* and *Photobacterium* were identified in spoilage oysters (Richards *et al.*, 2008). *Pseudomonas* and *Vibrionaceae* were frequently detected as dominant spoilage bacteria in oyster storage. Cao *et al.* (2009) studied the *C. gigas* from Yellow Sea in China, and results showed that *Pseudomonas* and *Vibrionaceae* were dominant bacteria in raw oyster which accounted for 22% and 20 % of the total bacteria, respectively. Whereas, Madigan *et al.* (2014) pointed out that two genera causing the spoilage of *Saccostrea glomerata* and *C. gigas* oysters were *Pseudoalteromonas* and *Vibrio*.

Seasonal difference affects the microbiota in fresh oysters, thus it also determines the dominant micobiotas in spoilage oyster. *Psychrobacter* appears to be predominant only in fall. Quick frozen oysters primarily contained *Shewanella* in winter, *Shewanella*, *Vibrio*, and *Psychrobacter* in summer, and *Psychrobacter* and *Vibrio* in fall, and most common dominant genera of high pressure treated oyster were *Shewanella* (15.7 - 23.9 %) and *Vibrio* (21.4 - 22.6 %) from all seasons (Prapaiwong *et al.,* 2009a).

The initial bacterial communities have decisive effect on dominant spoiled bacteria microbiotas in oyster, because spoiled bacteria were demonstrated to be main bacteria detected in fresh oyster in the previous studies. For instance, Cao *et al.* (2009) found that *Pseudomonas* and *Vibrionaceae* in fresh oyster were growing to be dominant bacteria after treatment and chilling storage. In addition, the dominant spoilage bacterial microbiota (*e.g., Bacillus*, *Moraxella* and *Acinetobacter*) after high hydrostatic pressure treatment and storage were also found in fresh oyster (Linton *et al*., 2003).

It is worth noting that not all dominant bacteria in fresh oyster are eventually growing competitive and became dominant spoiled bacteria after storage. Wood and Arias (2015) found that *C. virginica* oyster were dominated by *V. parahaemolyticus* (44 %), followed by *V. shiloi* (21 %) and *V. vulnificus* (13 %), whereas *V. parahaemolyticus* was replaced by other nonpathogenic Vibrio species (*e.g., Vibrio species*, *V. diazotrophicus*, *Listonella anguillarum*, *V. vulnificus*, and unidentified uncultured bacteria) after two weeks storage at 6 ± 2 °C (Amadi (2015) found that the dominant bacteria are *Bacillus* (20.8 %) and *Pseudomonas* (16.7 %), whereas those of fungal species are *Penicillium* species (45.4 %) and *Aspergillus flavus* (34.1 %). The role of fungi in oyster deterioration and spoilage should be assessed in the future investigation.

In oyster, the initial microbiota in different tissues was studied in previous reports as summarized in Table 3. The oyster tissues including gill, stomach, gut, digestive glands and gonads, body fluid, rectal area, crystalline, lower intestine, digestive diverticulum, pallial fluid were detected by culture or molecular approaches. From Table 3, the micriobiotas in different tissues of oyster harvested from different locations were different. Early in 1960, Colwell and Liston (Colwell and Liston, 1960) analyzed microbiota in gill, stomach, and body fluid in the Pacific oysters using cultivation and subsequent biochemical identification. In the past two decades, with the development of molecular analysis techniques for microbiology, the studies in microbiotas from different oyster tissues were gradually increased (Table 3)

Table 3 Microbiota in different oyster tissues

Tissues	Oyster species/ Location	Microbiota	Analysis methods	Reference
Glands	Sydney rock oysters (*Saccostrea glomerata)*/ Australia	α-Proteobacteria, γ-Proteobacteria, Fusobacteria, Firmicute, Spirochaetes, Chlorophyta, Cyanobacteria, Actinobacteria	RFLP	(Green and Barnes, 2010)
Digestive glands and gonads	*C. gigas* oyster/ Todos Santos Bay, Mexico	γ-Proteobacteria, Gram-positive bacteria with a low G+C	FISH	(Hernández-Zárate and Olmos-Soto, 2006)
Stomach and gut	*C. virginica* oyster/ Louisiana, US	Mycoplasma, Planctomyctes Phylotypes closely related to *Shewanella* and *Chloroflexi*	Roche 454 pyrosequencing platform	(King *et al*., 2012)
Gill and stomach	*C. gigas* oyster/ Japanese	*Pseudomonas*,*Vibrio*, *Flavobacterium*, *Pseudomonas*,*Vibrio*, *Achromobacter*, *Flavobacterium*, *Micrococcus*, *Bacillus*	Culture and isolates	(Colwell and Liston, 1960)
Body fluid		*Pseudomonas*,*Vibrio*, *Achromobacter*, *Flavobacterium*, *Corynebacterium*, *Micrococcus*, *Bacillus*, Enterococci	Culture and isolates	
Rectum		*Pseudomonas*/*Vibrio*, *Achromobacter*, *Alcaligenes*, *Flavobacterium*, *Micrococcus*, *Bacillus*	Culture and isolates	
Gill	*C. plicatua* oyster/ Fujian, China	*Lactococcus raffinolactis*, *Weissella cibaria*, *Lactococcus* sp., *Lactococcus lactis* subsp. *lactis*, *Enterococcus mundtii*, *E. coli*, *Aeromonas aquariorum*, *Aeromonas jandaei*, *Lactococcus garvieae*, *A. hydrophila* subsp. *hydrophila*	DGGE	(Chen *et al*., 2013)
Gill	*C. gigas* oyster/ Todos Santos Bay, Mexico	*Cytophaga*, *Flavobacterium*, γ-Proteobacteria	FISH	(Hernández-Zárate and Olmos-Soto, 2006)
		α- and β-*Proteobacterias*, *Pseudomonas* spp. and *Bacillus* spp.	PCR	(Hernández-Zárate and Olmos-Soto, 2006)

Gill	C. *pacifica*	*Methanobrevibacter, Corynebacterium, Macrococcus, Streptococcus, Prosthecochloris, Flavobacterium, Sphingomonas, Paracoccus, Maritalea, Nevskia, Schlegelella, Paramoritella, Shewanella, Vibrio*, *Moraxella, Acinetobacter, Endozocomonas, Spongiobacter*	Automated ribosomal intergenic spacer analysis (ARISA)	(Zurel *et al*., 2011)
	C. *savignyi*	*Methanobrevibacter*, *Thalassobacter*, *Endozoicomonas*, *Spongiobacter*, *Acinetobacter*, *Moraxella*, *Limnobacter*, *Schlegelella*, *Neisseria*, *Stenotrophomonas*, *Nevskia*, *Vibrio*, *Prosthecochloris*, *Staphylococcus*, *Flavobacterium*, *Eudoria*, *Corynebacterium*, *Actinomyces*		
Stomach	*C. gigas* oyster/ Deep Bay, Hong Kong, China	*Pseudomonas* spp., *Vibrio*, *Acinetobacter*, *Coliforms*, *Aeromonas* spp., *Flavohacterium*, *Cytophaga*, *Coryneforms*, *Alcaligenes*	Culture and isolation	(Kueh and Chan, 1985)
Crystalline		*Vibrio*, *Acinetobacter*, *Coliforms*, *Aeromonas* spp., *Alcaligenes*		
Digestive diverticulum		*Pseudomonas* spp., *Vibrio*, *Acinetobacter*, *Coliforms*, *Aeromonas* spp., *Flavohacterium*, *Cytophaga*,, *Coryneforms*, *Alcaligenes*		
Lower intestine		*Pseudomonas* spp., *Vibrio*, *Acinetobacter*, *Coliforms*, *Bacillus Aeromonas* spp., *Coryneforms*, *Alcali genes*, *Micrococcus*		
Gut and pallial fluid	Eastern oyster (*C. virginica*)	Bacterial groups include Bacteria (EUB338 I, II, & III), Bacteroidetes (CF319a), and Pseudomonas Group I (Pseudo120)	T-RFLP	(Pierce *et al*., 2016)
Gills	*C. gigas* oyster/ Shanghai, China	*Vibrio*, *Aeromonas*, *Photobacterium*, *Pseudoalteromonas*, *Dokdonella*, *Microbacterium*, *Micrococcus*, *Flavobacterium*, *Psychrilyobacter*, *Bacillus*, *Granulicella*, *Firmicutes*, *Verrucomicrobia*	Culture-independent DGGE	(Wang *et al*., 2014a).
Digestive glands		*Vibrio*, *Aeromonas*, *Photobacterium*, *Pseudoalteromonas*, *Pseudomonas*, *Dokdonella*, *Microbacterium*, *Micrococcus*, *Flectobacillus*, *Flavobacterium*, *Bacillus*, *Granulicella*, *Verrucomicrobia*		
Residual tissues		*Vibrio*, *Aeromonas*, *Photobacterium*, *Dokdonella*, *Microbacterium*, *Micrococcus*, *Flavobacterium*, *Fusobacterium*, *Bacillus*, *Granulicella*, *Verrucomicrobia*		

As filter-feeding shellfish, oysters ingest nutrients and microbiology by gills. Thus, the gills of oysters accumulate different types of microorganisms, including *Pseudomonas*, *Vibrio*, *Flavobacterium*, *Lactococcus, Aeromonas, Leuconostoc, Lactobacillus, Bacillus, Weissella*, *Enterobacter, Pseudoalteromonas and Enterococcus*, *Photobacterium, Dokdonella, Microbacterium, Micrococcus, Psychrilyobacter, Granulicella, Firmicutes,Verrucomicrobia* (Colwell and Liston, 1960; Hernández-Zárate and Olmos-Soto, 2006; Zurel *et al*., 2011; Chen *et al.*, 2013; Wang *et al.*, 2014a). Colwell and Liston (1960) separated the different part of the Pacific oyster to

study the original microbiotas, which showed that *Pseudomonas*, *Vibrio* and *Flavobacterium* were dominant bacteria by traditional cultivation methods. Spoiled micriobiotas of oyster gill under 4 °C, 10 °C , and 20 °C storage could be clearly characterized by DGGE, through which *Lactobacillus* and *Lactococcus* were found to be the dominant bacteria at various investigating temperatures (Chen *et al.*, 2013). Other methods, including FISH, Automated Ribosomal Intergenic Spacer Analysis (ARISA), PCR, were also used to investigate the oyster gill microbiotas (Hernández-Zárate and Olmos-Soto, 2006; Zurel *et al*., 2011).

The microbiotas in oyster stomach included *Pseudomonas*, *Vibrio*, *Achromobacter*, *Flavobacterium*, *Micrococcus*, *Bacillus*, *Miscellaneous*, *Acinetobacter, Coliforms, Aeromonas*, *Flavohacterium*, *Cytophaga, Coryneforms,* and *Alcaligenes* (Colwell and Liston, 1960; Kueh and Chan, 1985; Hernández-Zárate and Olmos-Soto, 2006). More microbiota information were obtained through Roche 454 pyrosequencing platform by King (King *et al*., 2012). Kueh and Chan (1985) indicated that the microbiota communities in stomach, crystalline, digestive diverticulum, and lower intestine were different when studying the inner parts of Pacific oysters (*C. gigas*). Among those microbiotas, *Vibrio, Acinetobacter, Coliforms, Aeromonas* were detected in all analyzing parts. However, *Pseudomonas* was previous regarded as main spoilage bacteria found in stomach, digestive diverticulum, and lower intestine (Kueh and Chan, 1985; Cao *et al.,* 2009).

The bacteria in the parts of digestive diverticulum and glands were also studied to demonstrate the relationship among the digestive system and original microbiota. RFLP was used and results showed that those microbiota were belonged to α-*Proteobacteria*, γ-Proteobacteria, Fusobacteria, Firmicute, Spirochaetes, Chlorophyta, Cyanobacteria, Actinobacteria (Green and Barnes, 2010). FISH revealed that γ-Proteobacteria and Gram-positive bacteria with a low G+C were dominant (Hernández-Zárate and Olmos-Soto, 2006).

Kueh and Chan reported that the isolates from glands mainly belong to *Pseudomonas*, *Vibrio, Coliforms, Aeromonas* (Kueh and Chan, 1985). Through culture-independent DGGE technology, the dominant communities were clearly profiled (Wang *et al*., 2014a). These bacteria were considered as the most commonly reported microbiotas in shellfish (Xuyama and Qusi, 1987). The microbiota were complex in whole oyster, because the high diversity in oyster gill, gland, stomach, body fluid, rectal area, and gut were all included in above microbiota studies.

Microbiota in oyster preservation

Oyster spoilage resulting in quality losses during preservation was investigated by many researchers (Cruz-Romero *et al*., 2008b; Cao *et al*., 2010; Xi *et al*., 2012; Bunruk *et al*., 2013; Chen *et al.*, 2014). The shelf life and quality changes of raw and treated oysters were well documented. Preservative methods, such as high-pressure treatment *(López-Caballero et al.*, 2000; Prapaiwong *et al*., 2009b), chitosan coating (Cao *et al*., 2009), and ozone treatment (Cao *et al*., 2010; Chen *et al*., 2014), have been proven to effectively slow down the reproduction of spoilage bacteria. In these studies, the spoilage bacteria were investigated by a culture-dependent method followed by traditional oyster isolate identification. The microbiotas in oyster were mainly affected by the preservation technologies as below.

Refrigeration

Temperature is the major impact factor for the microbiota in oyster during storage. Different bacterial communities of spoiled oyster under various storage temperatures were summarized in Table 2, which showed that storage temperature affects the dominant bacteria in the oyster microbiota. After stored at 0 °C, 5 °C and 10 °C, *Pseudomonas* became the major species and took up to 42 % - 66 % of detected microbiotas, and *Vibrionaceae* was around 20 % (Cao *et al*., 2009). Abundant *Pseudomonas* was also found in sampled oysters (*Tiostrea chilensis*) stored at room temperature (18 °C) (Romero *et al*., 2002). Except *Pseudomonas*, *Bacillus* became dominant bacteria in the oysters if the storage temperature is up to 30 ± 2 °C (Amadi, 2015). At phylum level, *Bacteroidetes* became the dominant bacteria under 15 °C and 30 °C storage (Fernandez-Piquer *et al*., 2012).

The spoilage bacteria in the different species of oysters could be different at the same storage temperature. After the storage at 4 °C for 7 days, the spoilage bacteria were *Vibrio*, *Arcobacter* for Pacific oysters, and *Pseudoalteromonas*, while the spoilage bacteria were *Pseudoalteromonas*, *Vibrio* and *Colwellia* for Sydney rock oysters (Madigan *et al*., 2014). The spoilage bacterial microbiota of Pacific oyster (*C. gigas*) after 4 °C storage were *Psychrilyobacter* spp., Fusobacteria, Spirochaetes (Fernandez-Piquer *et al*., 2012).

Chen *et al.* (2013) revealed that the main spoilage microbiotas in the gill of oyster were *Lactococcus*, *Lactobacillus, Weissella confusa* and *C. difficile* under 10 °C storage, while the main spoilage microbiota were *Lactococcus*, *Weissella*, *Enterobacter* and *Aeromonas* under 4 °C storage. Furthermore, the impact of modified atmosphere packaging (MAP) on gill microbiotas suggested that the investigation on the mechanism of oyster spoilage microbiotas during preservation requires to be focused on different tissues as well (Chen *et al*., 2016).

High pressure treatment

Cruz-Romero *et al.* (2008a) demonstrated that the dominant spoilage microbiotas in oyster were *Shewanella putrifaciens* and *Pseudomonas fluorescens* after 260 MPa treatment for 3 min and stored at 2 °C for 14 days, while the dominant spoilage bacteria was *Pseudomonas* spp. after 500 or 800 MPa treatment for 5 min and stored at 2 °C for 14 days. High pressure can inactivate *Vibrio* effectively in oyster. The *Vibrio* spp. accounted for 44 % of the microbiotas in untreated oysters, while they were not detected in all high pressure treated oysters after storage at 2 °C for 14 days (Cruz-Romero *et al*., 2008a). However, Prapaiwong *et al*. (2009a) demonstrated that the predominant

bacteria were *Shewanella*, *Vibrio* and *Psychrobacter* (only in the fall) after treated by high pressures of 250 to 400 MPa for 1 to 3 min, in which *Vibrio* were survived and became dominant bacteria. High hydrostatic pressures were also utilized in oyster treatment. After 300 Mpa treatment for 2 min, the dominant bacteria were *Moraxella*, *Acinetobacter*, *Flavobacterium*, and *Cytophaga* after 14d of storage at 2 °C. After 500 Mpa treatment for 2 min, the dominant bacteria were *Bacillus* (90%), *Moraxella*, *Acinetobacter* (10%) after 28 d storage at 2 °C (Linton *et al*., 2003).

Other technologies

Other treatments, such as ozonated water treatment, quick frozen and supercritical fluid CO_2 pasteurization, were also evaluated. The results of quick frozen treatment of oysters at -20 °C showed that the predominant bacteria were *Shewanella* in winter, and *Shewanella*, *Vibrio*, and *Psychrobacter* in summer as well as *Psychrobacter* and *Vibrio* in fall through 16S rDNA identification (Prapaiwong *et al*., 2009a). Cao *et al.* (2010) used ozonated water (5.0×10^{-6} g/L ozone) to treat oysters for 2 min, and the diversity of initial microbiotas were higher than those of treated oyster, which were dominated by *Pseudomonas* and *Vibrionaceae*. As process of cold pasteurization, supercritical fluid CO_2 was also proven to reduce oyster-associated bacteria (Meujo *et al*., 2008; Meujo *et al.*, 2010). MAP was introduced into oyster preservation and was illustrated that appropriate atmosphere composition can inhibit the growth of microbiology and change the bacterial communities in oyster gill (Chen *et al*., 2016). The mechanism on oyster bacterial spoilage should be further investigated focusing not only on the loads and population of total bacteria counts, but also on the characterization of bacterial microbiotas in whole oyster and different tissues.

Prospective

Microbiological analysis in oyster is of vital importance as microbiotas are associated with oyster mortalities, shelf life, spoilage, and human diseases. Most studies on oyster preservation were focused on calculating bacterial counts instead of the spoilage bacterial communities during processing or storage. However, the mechanism of oyster bacterial spoilage should be further revealed by discovering the bacterial microbiotas and re-evaluated the spoilage in different oyster species and tissues, instead of focusing on the loads and population of total bacteria counts. Innovative molecular technologies have been introduced to further characterize microbiotas in oyster. These technologies have been reported as effective way for microbiota investigation, which provide more advantages to study microorganism profile than traditional cultivation. Moreover, those high throughput technologies can be used not only on diversity investigation but also on better understanding of dominant microbiota and illustration of spoilage mechanisms. The microbiota in oyster was well revealed on the basis of the present literatures, while applying state-of-the-art technologies such as metagenome and transcriptome will further clarify the functional roles of bacteria and their co-relationship. Aquaculture location and environmental condition, which determine the initial bacteria and affect the proliferation of dominant bacteria and food borne pathogenic bacteria in oyster, should also be emphasized. Furthermore, although the entire oyster microbiota has been well studied to illustrate the dominant spoilage bacteria at the end of shelf life, the spoilage mechanism needs to be characterized by different tissues. As the part of oyster, the gill and gut with complex microbiological diversity are easily resulted in spoilage before unacceptability of entire oyster, which should be paid more attention at the beginning of spoilage. The novel technologies for multi-target pathogens detection can provide potential application to prevent the outbreak of oyster diseases and human foodborne illness.

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