Food Microbiology 61 (2017) 58–65

Contents lists available at ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

Bacterial microbiota profile in gills of modified atmosphere-packaged oysters stored at 4 °C

Huibin Chen [a, b, c], Meiying Wang [c], Xiangzhi Lin [a, *], Caihua Shi [d], Zhiyu Liu [e, **]

[a] Third Institute of Oceanography, State Oceanic Administration, Xiamen, Fujian, 361005, PR China
[b] School of Life Sciences, Xiamen University, Xiamen, 361102, PR China
[c] Biology Department, Xiamen Ocean Vocational College, Xiamen, 361012, PR China
[d] College of Food Science, University of Guelph, ON, N1G 2W1, Canada
[e] Fujian Collaborative Innovation Center for Exploitation and Utilization of Marine Biological Resources, Fisheries Research Institute of Fujian, Xiamen, Fujian, 361013, PR China

ARTICLE INFO

Article history:
Received 12 March 2016
Received in revised form
30 June 2016
Accepted 19 August 2016
Available online 25 August 2016

Keywords:
Oyster
Gill
Modified atmosphere package
Microbiota
PCR-DGGE

ABSTRACT

As filter-feeding bivalves, oysters can accumulate microorganisms into their gills, causing spoilage and potential safety issues. This study aims to investigate the changes in the gill microbiota of oysters packed under air and modified atmospheres (MAs, 50% CO_2: 50% N_2, 70% CO_2: 30% O_2, and 50% CO_2: 50% O_2) during storage at 4 °C. The diversity of bacterial microbiota in oyster gills was profiled through polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) analysis on the 16S rRNA gene V3 region to describe the variation during the entire storage period. The DGGE profile revealed high bacterial diversity in the air- and MA-packaged oyster gills, and the spoilage bacterial microbiota varied in the MA-packaged oyster gills. Results indicated that CO_2:O_2 (70%:30%) was suitable for oyster MA packaging and that high bacterial loads in oyster gills need to be considered during storage. In addition, *Lactobacillus* and *Lactococcus* species were found to grow dominantly in fresh oyster gills under MA packaging, which supports the potential application of MA packaging for oyster storage.

1. Introduction

Oysters are important marine resources because of their high nutritional value. The annual consumption of oysters was estimated up to 3.89 million tons in China (Chen et al., 2014), half of which was fresh. However, oysters are filter feeders that accumulate microorganisms from the aquatic environment into their gills and digestive glands (Chen et al., 2013; Wang et al., 2014a,b). These microorganisms can induce spoilage during further processing (Chen et al., 2013; La Valley et al., 2009; Zurel et al., 2011). Therefore, understanding the microbial profile of oysters is crucial to improve food safety.

The diversity and community structure of bacteria in fresh oysters depend on seasons (Parveen et al., 2008), seawater temperature (Gonzalez-Acosta et al., 2006; Shen et al., 2009), and living environments (Azandégbé et al., 2012; Chávez et al., 2005; Shen et al., 2009). Different bacterial communities thrive in different oyster tissues, such as gland, gut, stomach, and gill (Chávez et al., 2005; King et al., 2012; La Valley et al., 2009; Wang et al., 2014a,b). Moreover, the diverse and dominant spoilage bacterial community may be altered by subsequent treatments and storage (Chen et al., 2013; Cruz-Romero et al., 2008; Fernandez-Piquer et al., 2012). Previous studies investigated oyster spoilage, particularly the bacterial communities in whole oysters, by using culture-dependent and culture-independent methods (Cao et al., 2009a,b; Cruz-Romero et al., 2008; Fernandez-Piquer et al., 2012; Wood and Arias, 2015). The investigators found the highest diversity and initial aerobic plate counts in gills among other tissues (Chen et al., 2013; Wang et al., 2014a,b). To the best of our knowledge, little has been known about the associations between spoilage bacterial community and oyster gills until recently, when Chen et al. (2013) revealed that the dominant spoilage community in gills changes with storage temperature. Therefore, the microbial community and dynamics within the gills must be analyzed and controlled because of their high ability to filter and accumulate microorganisms.

Among various preservative methods, modified atmosphere

* Corresponding author.
** Corresponding author.
E-mail addresses: vipin_chen@163.com (H. Chen), xzlin@tiosoa.org (X. Lin), negroliu@163.com (Z. Liu).

http://dx.doi.org/10.1016/j.fm.2016.08.006
0740-0020/

(MA) packaging effectively prolongs the shelf life of seafood by inhibiting microorganism growth and thus reduces the quality loss for most fish and shellfish products (Caglak et al., 2008; Parlapani et al., 2015; Simoes et al., 2015; Soccol and Oetterer, 2003). It was also find that high CO_2 levels inhibit microorganism growth and oxidation (Sivertsvik et al., 2002). Compared with regular packaging, MA packaging can increase the shelf life of shellfish by approximately 40% (Caglak et al., 2008; Cann, 1988; Goulas et al., 2005), hence shows a considerable potential in controlling oyster microbiota.

Various methods have been applied to investigate the microbial profile of oysters. PCR-DGGE is an advanced and widely recognized molecular technique that allows microbial profile monitoring through its capacity to separate DNA fragments on the basis of sequence composition and size (Nicolaisen and Ramsing, 2002). This study utilized PCR-DGGE to monitor the efficacy of MA packaging in controlling oyster microbial communities and dynamics during storage. To illustrate the quality changes, the microbiological enumeration was used together with the sensory evaluation and pH analysis. These profiles provide detailed insight into the spoilage in oyster gills during storage under MA packaging.

2. Material and methods

2.1. Oyster sample preparation

Commercial oyster (*Crassostrea plicatula*) samples with similar sizes were obtained from a farmed field in Lianjiang, Fujian Province. Oysters were harvested on 24th September 2012 and stored in foam boxes with ice and transported to our laboratory immediately after harvest. After washing and cleaning, all oysters were manually shucked with a sterile knife and randomly divided into five groups.

2.2. Modified atmosphere package and storage

All oyster samples including the control group were individually arranged in a monolayer. Thirty oyster samples were packed in a 500 mL high-density polyethylene semi-rigid tray (O_2 transmission rate of 0.056 mL/(m^2·d·atm), CO_2 transmission rate of 0.23 mL/(m^2·d·atm), at 0 °C, 0% RH) that was previously sterilized under UV light for 20 min. Afterward, the tray was transferred to the automatic Modified Atmosphere Packaging machine (MAP380, Deshun, Zhangjiagang, China). After air evacuation, the tray was refilled with pre-mixed gases (50% CO_2: 50% N_2, 70% CO_2: 30% O_2, and 50% CO_2: 50% O_2, respectively). Subsequently, the Modified Atmosphere Packaging machine automatically heat-sealed the tray, which was covered with a 35 μm polypropylene film (O_2 transmission rate of 0.025 mL/(m^2·d·atm), CO_2 transmission rate of 0.102 mL/(m^2·d·atm) sterilized by UV light, at 0 °C, 0% RH). The film cover maintained a positive pressure in the inner tray. The controls were packaged and refilled with sterile filtered air. All packages were refrigerated at 4 ± 1 °C and taken for DGGE analyses on days 0, 4, 8, 12, and 16.

2.3. Microbiological activity and pH value test

Microbiological activity and pH value tests were conducted every 2 days. On each sampling day, oyster samples were collected for replicate testing. Within each replicate, 5 g of oyster gills was weighed and mixed in 45 mL of distilled water (pH 7.0). The solution was homogenized in a sterile stomacher bag for 1 min. The pH value was tested using a pH meter (PHS-3C; Rex Instrument Factory, Shanghai, China).

Oyster gills (10 g) were aseptically removed from the oyster samples. The oyster gill matrix and 90 mL of sterile peptone saline water (8.5 g/L NaCl and 1 g/L peptone solution at pH 7) were homogenized in a sterile stomacher bag for 1 min, resulting in a 1:10 dilution. The serial dilutions were prepared and a 0.1 mL aliquot of the serial dilution was spread on the surface of Plate count agar (Oxoid, CM0325B) supplement with 10 g/L NaCl and then incubated at 30 °C for 48 h. Aerobic plate counts were enumerated by counting colonies and described by logarithmic conversion to colony forming units (CFU) per gram of the sample.

2.4. Sensory

Sensory analysis was measured every 2 days. On sampling day, six trained panellists evaluated oyster according to the freshness grade guide described by Cao et al. (2010) based on odor, body color, fluid, and texture. Four parameters were awarded the score on a range from 0 (extremely undesirable) to 3 (extremely desirable). The sum of the four parameter scores were counted as “freshness score” (from 0 to 12), which score of under 6 was regarded as unacceptability.

2.5. Total bacterial genomic DNA extraction

Bacterial genomic DNA was extracted in accordance with the method as demonstrated (Chen et al., 2013). In brief, 3 g of the oyster gill was aseptically removed from fresh, air-packaged, and MA-packaged oyster samples. After homogenizing in a stomacher bag with 10 mL of saline water for 3 min, the homogenizing suspension was centrifuged at 100 × g for 5 min (Biofuge Fresco; Kendro Laboratory Products, Langenselbold, Germany), and the precipitation was dissolved in 30 mL of saline water and then centrifuged again. The two centrifuged supernatants were combined and centrifuged at 10000 × g for 5 min. After removing the supernatants, the pellets were used for total bacterial genomic DNA extraction. The total bacterial genomic DNA was extracted using the DNA Tissue Kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's protocol. Finally, DNA was eluted with TE buffer and stored at −20 °C.

2.6. PCR protocol

Nested PCR was performed in a programmable heating incubator (Eppendorf, Hamburg, Germany). The 16S rRNA gene was amplified using the forward primer 8-27f (5′-AGA GTT TGA TCC TGG CTC AG-3′) and the reverse primer 1492r (5′-GGT TAC CTT GTT ACG ACT T-3′) (Baer et al., 2004). Each PCR mixture (final volume, 25 μL) consisted of 0.8 μL of genomic DNA template, 12.5 μL of High-Fidelity PCR SuperMix (Invitrogen, Carlsbad, CA, USA), 2 μL of 0.2 μmol/L primers (8-27f and 1492r), and 9.7 μL of ddH_2O. The PCR amplification program was designed as follows: initial denaturation at 94 °C for 5 min; 30 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, extension at 72 °C for 1.5 min; and a final extension at 72 °C for 10 min. A second PCR experiment was carried out by utilizing the 16S rDNA amplicons as the template DNA for the V3 region.

Amplicons of approximately 230 bp were amplified from the hypervariable V3 region of the 16S rRNA gene using the forward primer 338f (5′-CC TAC GGG AGG CAG CAG-3′) with a 40 base GC clamp (5′-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG G-3′) and the reverse primer 518r (5′-ATT ACC GCG GCT GCT GG-3′) (Muyzer et al., 1993). The PCR mix contained 2 μL of template DNA, 3.2 μL of 10 μmol/L primers (gc338f and 518r), 5 μL of 10 × PCR buffer, 3 μL of Mg^{2+}, 4 μL of 2.5 mmol/L dNTP, and 0.4 μL of 5 U/μL TaKaRa Taq polymerase (Takara Biotechnology Co., Ltd, Dalian, China). Then, 32.4 μL of ddH_2O was added to prepare the final total reaction volume of 50 μL. The amplification conditions