Int Aquat Res (2018) 10:275–282
https://doi.org/10.1007/s40071-018-0205-6

ORIGINAL RESEARCH

Assessment of bacterial communities in skin ulceration in cultured sea cucumber *Apostichopus japonicus* (Selenka)

Yi Yang · Yuchun Li · Zhenlin Liang

Received: 13 April 2018 / Accepted: 17 September 2018 / Published online: 5 October 2018

Abstract To determine changes in bacterial composition in skin ulcerations in farmed sea cucumber *Apostichopus japonicus*, full-length *16S rRNA* gene sequencing of different skin samples of *A. japonicus* was performed on a Pacific Biosciences (PacBio) RS platform. The fact that one of them varies in bacterial diversity than the other two may suggest different stage of skin ulceration, but the answer is inconclusive due to inefficiency in size of sampling pool. After comparing them with three skin samples from three healthy sea cucumbers, respectively, the majority of the results were distinguishable at the species level. The bacterial composition, which increased in diversity during the possible early stage of skin ulceration, exhibited a decrease in diversity at possible advanced stages, except for the predominant *Saccharicrinis carchari* (846.85 ± 5.75‰). This work may have the first clue implicating *Ornithinibacillus contaminans* as a potential causative pathogen of skin ulcerations in *A. japonicus.*

Keywords Skin ulceration · Third-generation sequencing · 16S rRNA

Introduction

Apostichopus japonicus is an important species for aquaculture in China. In northern China, *A. japonicus* is cultivated on a commercial scale by sea ranching or in shallow ponds, with the latter being more commonly employed in northern China (Choo 2008). Breeding methods for this species have improved in the past decade, and its transcriptome has been sequenced, although extensive studies on the thermal resistance, disease resistance, and growth rates of *A. japonicas* have been conducted, no effective and practical method to prevent the development of skin ulcerations during its cultivation has been established (Du et al. 2012; Gianasi et al. 2017; Zhao et al. 2017). The skin ulceration usually takes place when water temperature gets low from December to March, and once occurs, it spreads to a whole pond within days and brings a lethal death rate higher than 90%. Besides such potential devastating blow to *A. japonicus* cultivation, skin ulceration can be frequently found on couples of day basis. *A. japonicus* only possesses innate immunity, thereby making it relatively vulnerable to harsh cultivation environments. One preventative measure against skin ulcerations is to administer probiotics or prebiotics (Zhang et al. 2010; Ma et al. 2013; Sun et al. 2012; Yang et al. 2014, 2015). However, these lesions continue to occur despite efforts to elucidate the molecular mechanisms

Y. Yang · Y. Li (✉) · Z. Liang (✉)
Marine College, Shandong University, Weihai 264200, China
e-mail: li_yuchun@sdu.edu.cn

Z. Liang
e-mail: liang@sdu.edu.cn

underlying its immune system (Xue et al. 2015). Therefore, an efficient approach in identifying the pathogens that cause skin ulcerations during the cultivation of *A. japonicus* is warranted.

Several studies on the causative pathogens of skin ulcerations have been conducted; however, none have utilized next-generation or third-generation sequencing (Li et al. 2010; Tiruvayipati et al. 2013). The 16S ribosomal RNA (*16S rRNA*) gene has been employed in profiling bacteria and Archaea since the late 1970s (Woese and Fox 1977). Multiple *16S rRNA* databases have accumulated massive amounts of sequence data, and high-throughput sequencing technologies, predominantly the Roche 454 and Illumina sequencing platforms, have facilitated deep microbial community profiling. However, investigations on bacterial composition do not go beyond the family level (Vishnivetskaya et al. 2011; Besemer et al. 2012; Mosher et al. 2012). Recent studies using the Pacific Biosciences (PacBio) RS platform have revealed genus-level discrimination of *16S rRNA* amplicons from microbial communities (Mosher et al. 2013). Because fewer resources are necessary due to improvements in phylogenetic reconstruction, we performed a comparative assessment of *A. japonicus* from a single local breeding spot using the PacBio RS platform to identify candidates responsible for, or linked to, skin ulcerations. To keep skin microbial consortia samples intact, we did not make any effort to exclude host tissues at the sampling stage. Such limitations may also assess the specificity of the polymerase chain reactions (PCRs) and sequencing depth of the PacBio RS platform, as indicated by the generation of good analyzable mega data without contamination.

Materials and methods

Sampling of *A. japonicus*

We sampled three healthy normal (NM) *A. japonica* and three individuals with skin ulceration deterioration (UD) in a same cultivating pond (122°15′N, 37°01′E) in Weifang, Shandong Province, China, on May 5th, 2016. Those with UD were identified by appearances of positive skin ulceration spots. The healthy group (HG) consists of three NM samples with an average weight of 69.0 ± 21.2 g and the three UD samples had an average weight of 41.8 ± 13.0 g. All samples were transported on ice and dissected later for a tiny piece (2 g) of skin tissue (for UD samples, ulceration dots were collected) within 4 h. We did not exclude host tissues or only tried to brush off some of the microbial consortia on the skin during sampling and instead utilized whole-skin tissue samples in PacBio RS II sequencing to precisely reflect the actual skin microbial composition.

PCR amplification of 16S rRNA genes

Genomic DNA was extracted following the Earth Microbiome Project (EMP) protocol (http://www.earthmicrobiome.org/protocols-and-standards/dna-extraction-protocol/). The purity and concentration of DNA in the final 40-μL eluent that was, respectively, extracted from the samples was tested on both NanoDrop NC2000 (Thermo Scientific, Wilmington, DE, USA) and TBS380 Fluorometer (Turner BioSystems, Sunnyvale, CA, USA). The primers employed to amplify the V1–V9 region of the *16S rRNA* were 27F (3′-AGAGTTTGATCMTGGCTCAG) and 1492R (5′-ACCTTGTTACGACTT). The reaction mixture consisted of 5 μL of a 5× reaction buffer, 5 μL of a 5× GC buffer, 2 μL of each dNTP (2.5 mM), 1 μL of the forward primer (10 μM), 1 μL of the reverse primer (10 μM), 2 μL of the DNA template (4–25 ng μL^{-1}), 8.75 μL of ddH_2O, and 0.25 μL of 5U/μL Q5 DNA polymerase. All the samples were subjected to two-step PCR: (1) 25 cycles of the following: initial denaturation at 98 °C for 3 min, denaturation at 98 °C for 30 s, annealing at 52 °C for 30 s, extension at 72 °C for 180 s, and a final extension at 72 °C for 5 min; (2) 16 cycles of the following: initial denaturation at 98 °C for 3 min, denaturation at 98 °C for 30 s, annealing at 62 °C for 30 s, extension at 72 °C for 180 s, and a final extension at 72 °C for 5 min.

PacBio RS single-molecule real-time (SMRT) sequencing

The PCR products (0.2–0.7 μg) were prepared using Pacific Biosciences Template Prep Kit 1.0 for DNA damage repair and end repair. A total of 50 μL of the damage repair mix was, respectively, incubated at 37 °C for 60 min and cooled at 4 °C. By adding 2 μL of the end-repair eluent, the 52-μL mixture was incubated at 25 °C for 5 min. The reaction mix was cleaned using 0.6× Agencourt AMPure XP beads (Beckman Coulter,

Beverly, MA, USA). Then, the mixtures were subjected to a blunt-ligation reaction preparation step at − 25 °C for 1 h, 65 °C for 10 min, and 4 °C overnight. Ligation failure products were removed by adding exonucleases and incubating at 37 °C for 60 min. The resulting 41-μL mixture containing the library was purified using 0.6× Ampure PB beads twice. Once the primers were annealed and DNA polymerase had bound, the SMRT bell-polymerase complexes were sequenced.

Results

PacBio RS II SMRT chip generated a total of 18,195 reads from the V1–V9 amplicons of all six samples. The circular consensus sequencing data were processed through the SMRT pipeline (https://github.com/PacificBiosciences/smrtflow) to make sure that the error rate was < 10% and full passes were > 3. The length distribution of all reads was as expected of typical V1–V9 amplicons of the *16S rRNA* gene (Fig. 1).

From a total of 16,276 optimized full-length *16S rRNA* reads from all six samples, 97% were clustered to the bacterium *16S rRNA* sequence of the Greengenes database (Release 13.8, http://greengenes.secondgenome.com/) (DeSantis et al. 2006) using QIIME (Quantitative Insights Into Microbial Ecology, v1.8.0, http://qiime.org/) (Caporaso et al. 2010) up to species level (Table 1).

The rarefaction curve depicted well-presentable sequencing depth for each sample (Fig. 2). All samples reached saturation as indicated by the plateau.

The composition of each sample at the species level shows a three-stage trend in Fig. 3.

NM1, 2, and 3 comprised HG *A. japonicus*, UD1 showed the early stages of skin ulceration symptom, and UD2 and 3 exhibited advanced stages of skin ulceration.

To identify the bacterium that potentially causes skin ulceration, we compared the bacterial communities between HG and UD1 and that between UD1 and UD2 and 3. Then, we filtered the data matrix to identify strains that showed at least a twofold increase from the HG group to UD1 and still existed at average densities in UD 2 and 3 (Fig. 4). Our results showed that all the filtered strains decreased in number as *A. japonicus* reached the advanced stages of skin ulceration.

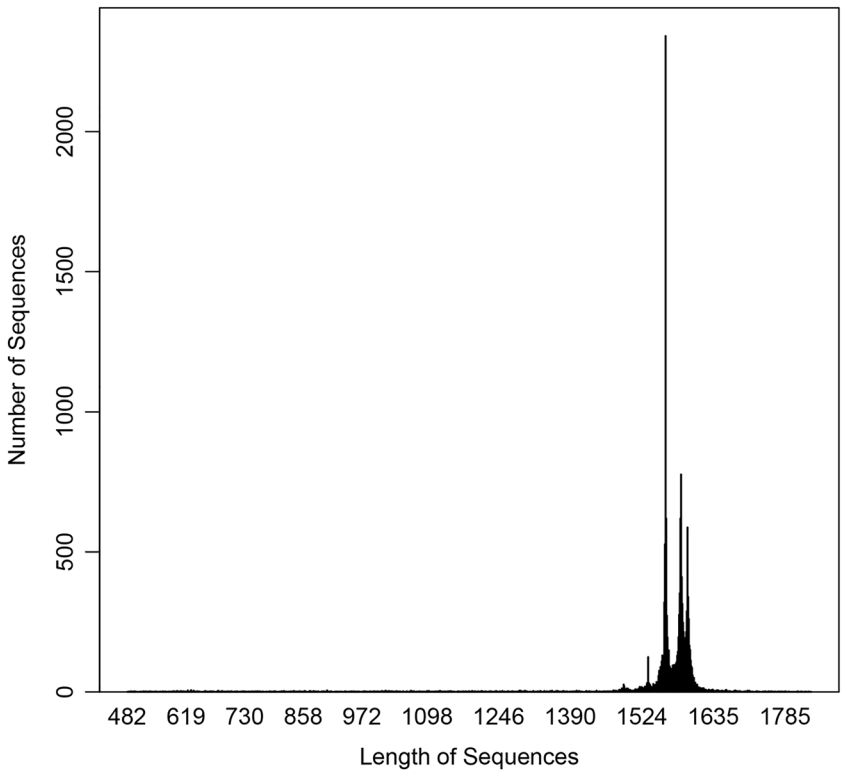

Fig. 1 Length distribution of reads from NM and UD groups using PacBio RS II SMRT chip

Table 1 OTU clustering and classification of outcomes

Sample	Phylum	Class	Order	Family	Genus	Species	Classified vs. unclassified OTU
UD1	8	14	34	64	146	260	824:5
UD2	6	9	18	34	61	94	364:3
UD3	3	5	6	14	27	40	198:2
NM1	8	11	23	42	94	191	646:5
NM2	5	10	17	32	55	123	517:3
NM3	4	8	21	37	59	130	444:6

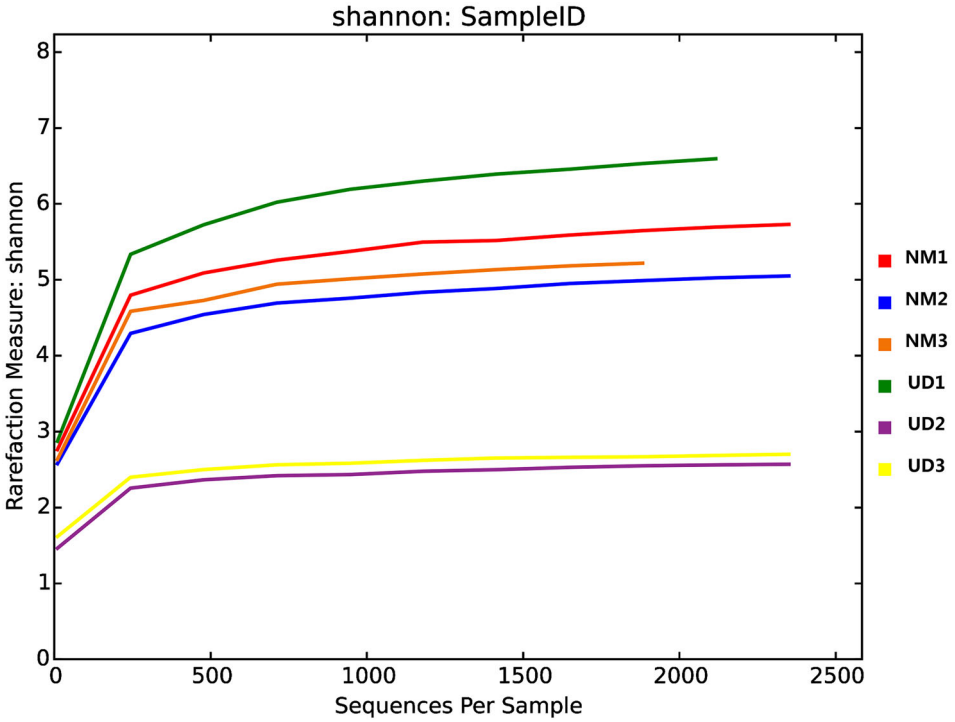

Fig. 2 Rarefaction curves of OTU numbers of the samples

Figure 4A shows a shift in the bacterial community profile from HG to UD1, and that in B depicts similarities in profiles between UD1 and UD 2 and 3. Eight bacterial species exhibited de novo emergence (DE) as reflected in the microbial composition during the early stage of skin ulceration. The specific number of the DEs in UD1 was as follows: *Desulforhopalus singaporensis* (0.44‰), *Lactobacillus fabifermentans* (0.44‰), *Ornithinibacillus contaminans* (0.44‰), *Polaribacter dokdonensis* (0.44‰), *Bizionia argentinensis* (0.87‰), *Rubritalea tangerina* (0.87‰), *Luteolibacter algae* (1.31‰), and *Lutibacter agarilyticus* (4.36‰).

Discussion

D. singaporensis, *L. fabifermentans, P. dokdonensis, B. argentinensis, L. agarilyticus,* and *L. algae* from the DE group are associated with various metabolic pathways related to skin ulcerations but have not been reported as actual pathogens (Lie et al. 1999; Yoon et al. 2006; Bercovich et al. 2008; De Bruyne et al. 2009; Lanzarotti et al. 2011; Park et al. 2013; Aran et al. 2014; Campanaro et al. 2014; Treu et al. 2014; Yoon et al. 2017).

Approximately seven species have been validated in the genus *Ornithinibacillus* (Mayr et al. 2006). Most are free-living bacteria isolated from natural environments, such as marine sediment and saline soil. *O. scapharcae* TW25T is considered to be the only *Ornithinibacillus* that has pathogenic potential. *O.*

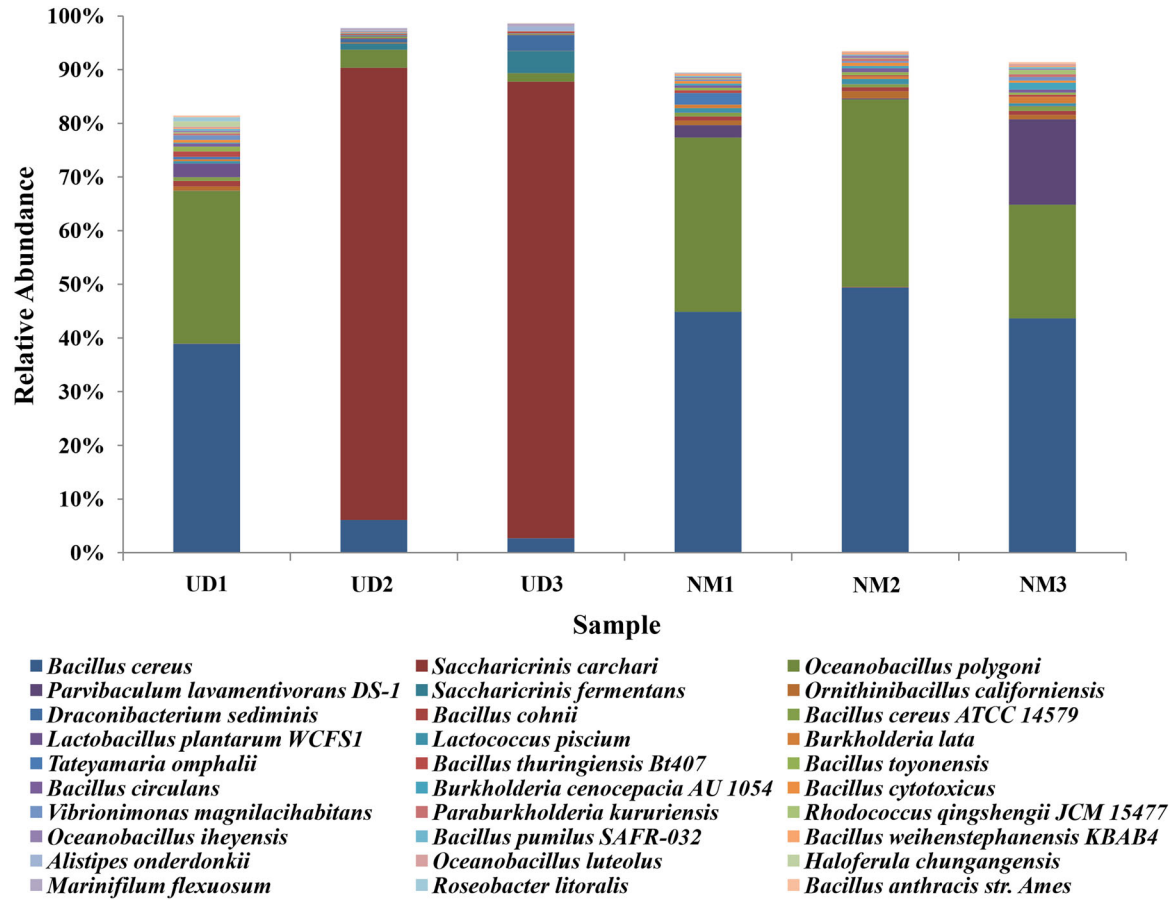

Fig. 3 Main species composition of the samples

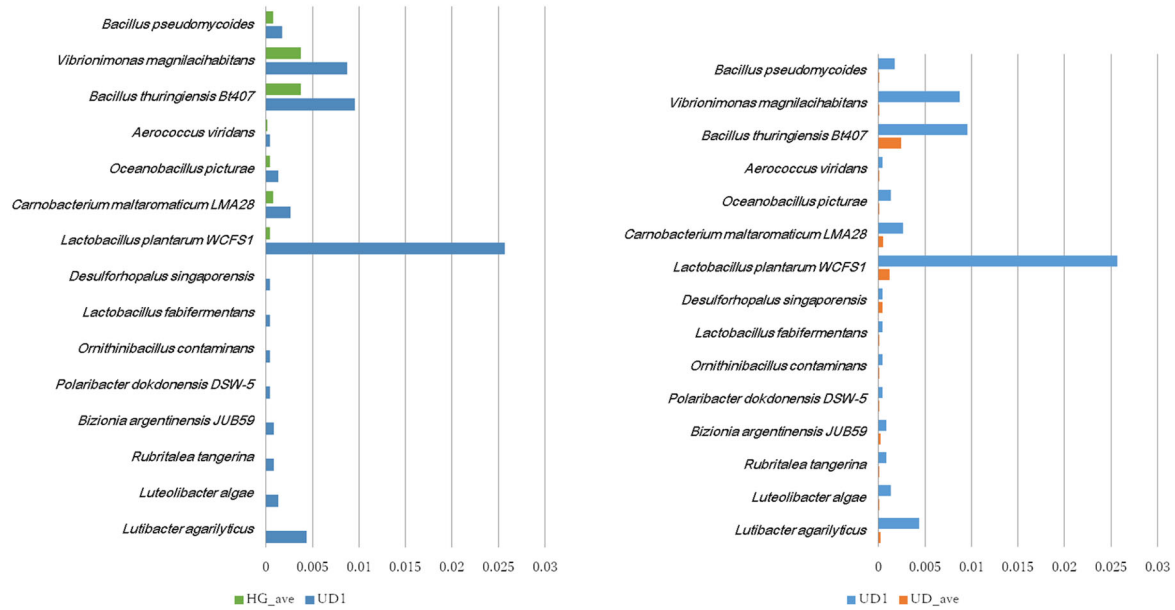

Fig. 4 Strains showing at least a twofold increase in number and a positive average in the UD 2 and 3 groups

contaminans DSM22953T, which emerged in this study, was first isolated from a blood sample of a 75-year-old woman. It is Gram-positive, endospore-forming, and rod-shaped. *O. contaminans* DSM22953T, similar to *O. scapharcae* TW25T, contains a number of virulence-relevant genes, which indicates that it may also be pathogenic. However, no case reports of disease due to *O. contaminans* DSM22953T have been reported to date. The present study thus may suggest a case study of its pathogenicity (Kämpfer et al., 2010; Jiang et al., 2016). Further research is due for conclusions.

R. tangerina is Gram-negative, non-motile, facultatively anaerobic, and coccoid or rod-shaped. *R. tangerina* was isolated from the visceral specimen of an unidentified sea hare collected from Himetsu, Sado, Niigata, Japan, in 2006. Information on this species is limited (Yoon et al. 2007).

Other strains that increased in density during possible different stages of skin ulceration in cultured *A. japonicas* included the following:

1. *Carnobacterium maltaromaticum* LMA28 can be found in the wild and in food products, and can grow anaerobically. This species is pathogenic to fish, whereas cases involving humans have not been reported (Leisner et al. 2007).
2. *Aerococcus viridans* is a member of the bacterial genus *Aerococcus*. It is a causative agent of gaffkaemia, a disease in lobsters (Greenwood et al. 2005).
3. *Bacillus thuringiensis* is a Gram-positive, soil-dwelling bacterium that is commonly used as a biological pesticide. It was first discovered in 1901 by Japanese biologist Shigetane Ishiwata (Roh et al. 2007) and then rediscovered in Germany by Ernst Berliner, who reported that it causes a disease called Schlaffsucht in flour moth caterpillars. *B. thuringiensis* is closely related to *B. cereus*, a soil bacterium, and *B. anthracis*, which causes anthrax; the three organisms differ mainly in their plasmids (Økstad and Kolstø 2011). Besides *B. thuringiensis*, there are a total of 14 members of the genus *Bacillus* that showed an increase in density in UD 1. Among these, *B. thuringiensis* and *B. pseudomycoides* are the only two *Bacillus* species that were observed on UD 2 and 3 at average levels.
4. Seven members of the *Burkholderia* appeared during the early stage of skin ulceration, except for *Burkholderia vietnamiensis* G4, which also emerged in HG. However, none of these species were identified during the advanced stage of skin ulceration. Several members of this genus are considered to be biosecurity threats (Woods and Sokol 2006; Sawana et al. 2014).

The two predominant bacteria of the skin health consortia included *B. cereus* and *Oceanobacillus polygoni*. *B. cereus* averaged 459.93 ± 30.38‰ and *O. polygoni* 295.24 ± 73.53‰ in the HG group. The population of both species dramatically dropped to 43.80 ± 24.20‰ and 24.70 ± 12.17‰, respectively, during the advanced stage of skin ulceration. *B. cereus* is a Gram-positive, rod-shaped, aerobic, motile, β-hemolytic bacterium that is commonly found in soil and food. Some strains of *B. cereus* are harmful, whereas others can be beneficial as probiotics for animals (Charalampopoulos and Rastall 2009). *O. polygoni* is a Gram-positive, peritrichously flagellated, straight, facultatively alkaliphile, rod-shaped bacterium that was first isolated from indigo fermentation fluid in 2013 (Hirota et al. 2013). A strain of *O. polygoni*, MBF-HG6, is salt-tolerant and alkaliphilic and produces bioflocculants (Li et al. 2017). The role of *O. polygoni* in skin ulceration remains unclear.

Saccharicrinis carchari was the predominant bacterium (846.85 ± 5.75‰) during the advanced stage of skin ulceration. However, this bacterium was not observed in all three HG samples and only showed a 1.54-fold increase in percentage from HG to UD1 (0.44‰). *S. carchari* was the only strain that illustrated a shift in the trend wherein it continuously increased. It is a Gram-negative, facultatively anaerobic, gliding, non-endospore-forming, yellow-pigmented, straight or slightly curved, rod bacterium that was initially isolated from shark gill homogenate. This strain is catalase-positive and oxidase-negative (Liu et al. 2014). Its role in skin ulceration remains unclear.

Conclusions

The present study examined changes in the skin microbial consortia of *A. japonicus* with skin ulcerations. PacBio sequencing revealed differences in bacterial communities at the species level. *O. contaminans* and *S. carchari* were the predominant bacterial species in the skin ulcerations. Our approach of keeping skin samples intact and eliminating unrelated sequences by selective PCR for *16S rRNA* sequencing on a PacBio RS

platform proved to be sufficient for performing a full-scale, species-discriminating inspection of the skin consortia community. Our technique may thus be potentially employed in future investigations on skin microbial consortia in aquatic invertebrates.

Acknowledgements The authors thank the Technology Department of Shanghai Personal Biotechnology Co., Ltd., for processing and preparing backups of our raw data. We thank LetPub (www.letpub.com) for its linguistic assistance during the preparation of this manuscript.

Author contributions YY designed all the experiments, prepared all the samples, and wrote the manuscript. YY, YL, and ZL discussed the results and commented on the manuscript. All authors reviewed the manuscript.

Funding This research is supported by Natural Science Foundation of Shandong Province, China.

Compliance with ethical standards

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

Aran M, Smal C, Pellizza L, Gallo M, Otero LH, Klinke S, Goldbaum FA, Ithurralde ER, Bercovich A, Cormack WPM, Turjanski AG, Cicero DO (2014) Solution and crystal structure of BA42, a protein from the Antarctic bacterium *Bizionia argentinensis* comprised of a stand-alone TPM domain. Proteins 82:3062–3078

Bercovich A, Vazquez SC, Yankilevich P, Coria SH, Foti M, Hernandez E, Vidal A, Ruberto L, Melo C, Marenssi S, Criscuolo M, Memoli M, Arguelles M, Cormack WPM (2008) *Bizionia argentinensis* sp. nov., isolated from surface marine water in Antarctica. Int J Syst Evol Microbiol 58:236–2367

Besemer K, Peter H, Logue JB, Langenheder S, Lindström ES, Tranvik LJ, Battin TJ (2012) Unraveling assembly of stream biofilm communities. Microb Popul Community Ecol 6:1459–1468

Campanaro S, Treu L, Vendramin V, Bovo B, Giacomini A, Corich V (2014) Metagenomic analysis of the microbial community in fermented grape marc reveals that *Lactobacillus fabifermentans* is one of the dominant species: insights into its genome structure. Appl Microbiol Biotechnol 98:6015–6037

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R (2010) QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7:335–336

Charalampopoulos D, Rastall RA (2009) Prebiotics and probiotics science and technology. Springer, New York

Choo PS (2008) Population status, fisheries and trade of sea cucumbers in Asia. In: Toral-Granda V, Lovatelli A, Vasconcellos M (eds) Sea cucumbers. A global review of fisheries and trade. FAO Fisheries and Aquaculture Technical Paper, Rome, pp 81–118

De Bruyne K, Camu N, De Vuyst L, Vandamme P (2009) Lactobacillus *fabifermentans sp.* nov. and *Lactobacillus cacaonum* sp. nov., isolated from Ghanaian cocoa fermentations. Int J Syst Evol Microbiol 59:7–12

DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, Huber T, Dalevi D, Hu P, Andersen GL (2006) Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl Environ Microbiol 72:5069–5072

Du H, Bao Z, Hou R, Wang S, Su H, Yan J, Tian M, Li Y, Wei W, Lu W, Hu XL, Wang S, Hu JJ (2012) Transcriptome sequencing and characterization for the sea cucumber *Apostichopus japonicus* (Selenka, 1867). PLoS One 7:e33311

Gianasi BL, Parrish CC, Hamel JF, Mercier A (2017) Influence of diet on growth, reproduction and lipid and fatty acid composition in the sea cucumber *Cucumaria frondosa*. Aquac Res 48:3413–3432

Greenwood SJ, Keith IR, Després BM, Cawthorn RJ (2005) Genetic characterization of the lobster pathogen *Aerococcus viridans* var. *homari* by 16S rRNA gene sequence and RAPD. Dis Aquat Org 63:237–246

Hirota K, Hanaoka Y, Nodasaka Y, Yumoto I (2013) *Oceanobacillus polygoni* sp. nov., a facultatively alkaliphile isolated from indigo fermentation fluid. Int J Syst Evol Microbiol 63:3307–3312

Jiang XW, Cheng H, Zheng BW, Li A, Lv LX, Ling ZX (2016) Comparative genomic study of three species within the genus *Ornithinibacillus*, reflecting the adaption to different habitats. Gene 578:25–31

Kämpfer P, Falsen E, Lodders N, Langer S, Busse HJ, Schumann P (2010) *Ornithinibacillus contaminans* sp. nov., an endospore-forming species. Int J Syst Evol Microbiol 60:2930–2934

Lanzarotti E, Pellizza L, Bercovich A, Foti M, Coria SH, Vazquez SC, Ruberto L, Hernandez EA, Dias RL, Cormack WPM, Cicero DO, Smal C, Nicolas MF, Vasconcelos ATR, Marti MA, Turjanski AG (2011) Draft genome sequence of *Bizionia argentinensis*, isolated from Antarctic surface water. J Bacteriol 193:6797–6798

Leisner JJ, Laursen BG, Prévost H, Drider D, Dalgaard P (2007) Carnobacterium: positive and negative effects in the environment and in foods. FEMS Microbiol Rev 31:592–613

Li H, Qiao G, Li Q, Zhou W, Won KM, Xu DH, Park SI (2010) Biological characteristics and pathogenicity of a highly pathogenic *Shewanella marisflavi* infecting sea cucumber, *Apostichopus japonicus*. J Fish Dis 33:865–877

Li J, Yun Y, Yun L Xing, Song L (2017) Novel bioflocculant produced by salt-tolerant, alkaliphilic strain *Oceanobacillus polygoni* HG6 and its application in tannery wastewater treatment. Biosci Biotechnol Biochem 81:1018–1025

Lie TJ, Clawson ML, Godchaux W, Leadbetter ER (1999) Sulfidogenesis from 2-aminoethanesulfonate (taurine) fermentation by a morphologically unusual sulfate-reducing bacterium, *Desulforhopalus singaporensis* sp. nov. Appl Environ Microbiol 65:3328–3334

Liu QQ, Wang Y, Li J, Du ZJ, Chen GJ (2014) *Saccharicrinis carchari* sp. nov., isolated from a shark, and emended descriptions of the genus *Saccharicrinis* and *Saccharicrinis fermentans*. Int J Syst Evol Microbiol 64:2204–2209

Ma Y, Liu Z, Yang Z, Li M, Liu J, Song J (2013) Effects of dietary live yeast *Hanseniaspora opuntiae* C21 on the immune and disease resistance against *Vibrio splendidus* infection in juvenile sea cucumber *Apostichopus japonicus*. Fish Shellfish Immunol 34:66–73

Mayr R, Busse HJ, Worliczek HL, Ehling-Schulz M, Scherer S (2006) *Ornithinibacillus gen.* nov., with the species *Ornithinibacillus bavariensis* sp. nov. and *Ornithinibacillus californiensis* sp. nov. Int J Syst Evol Microbiol 56:1383–1389

Mosher JJ, Vishnivetskaya TA, Elias DA, Podar M, Brooks SC, Brown SD, Brandt CC, Palumbo AV (2012) Characterization of the Deltaproteobacteria in contaminated and uncontaminated stream sediments and identification of potential mercury methylators. Aquat Microb Ecol 66:271–282

Mosher JJ, Bernberg EL, Shevchenko O, Kan J, Kaplan LA (2013) Efficacy of a 3rd generation high-throughput sequencing platform for analyses of 16S rRNA genes from environmental samples. J Microbiol Methods 95:175–181

Økstad OA, Kolstø AB (2011) Genomics of bacillus species. In: Wiedmann M, Zhang W (eds) Genomics of foodborne bacterial pathogens. Food microbiology and food safety. Springer, New York, pp 29–53

Park SC, Choe HN, Hwang YM, Baik KS, Seong CN (2013) *Lutibacter agarilyticus* sp. nov., a marine bacterium isolated from shallow coastal seawater. Int J Syst Evol Microbiol 63:2678–2683

Roh JY, Choi JY, Li MS, Jin BR, Je YH (2007) *Bacillus thuringiensis* as a specific, safe, and effective tool for insect pest control. J Microbiol Biotechnol 17:547–559

Sawana A, Adeolu M, Gupta RS (2014) Molecular signatures and phylogenomic analysis of the genus Burkholderia: proposal for division of this genus into the emended genus *Burkholderia* containing pathogenic organisms and a new genus *Paraburkholderia gen.* nov. harboring environmental species. Front Genet 5:429

Sun Y, Wen Z, Li X, Meng N, Mi R, Li Y, Li S (2012) Dietary supplement of fructooligosaccharides and *Bacillus subtilis* enhances the growth rate and disease resistance of the sea cucumber *Apostichopus japonicus* (Selenka). Aquac Res 43:1328–1334

Tiruvayipati S, Bhassu S, Kumar N, Baddam R, Shaik S, Gurindapalli AK, Thong KL, Ahmed N (2013) Genome anatomy of the gastrointestinal pathogen, *Vibrio parahaemolyticus* of crustacean origin. Gut Pathog 5:37

Treu L, Vendramin V, Bovo B, Giacomini A, Corich V, Campanaro S (2014) Genome sequence of *Lactobacillus fabifermentans* strain T30PCM01, isolated from fermenting grape marc. Genome Announc 2:e00060-14

Vishnivetskaya TA, Mosher JJ, Palumbo AV, Yang ZK, Podar M, Brown SD, Brooks SC, Gu B, Southworth GR, Drake MM, Brandt CC, Elias DA (2011) Mercury and other heavy metals influence bacterial community structure in contaminated Tennessee streams. Appl Environ Microbiol 77:302–311

Woese CR, Fox GE (1977) Phylogenetic structure of the prokaryotic domain: the primary kingdoms. Proc Natl Acad Sci 74:5088–5090

Woods DE, Sokol PA (2006) The genus Burkholderia. In: Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt E (eds) The prokaryotes. Springer, New York, pp 848–860

Xue Z, Li H, Wang X, Li X, Liu Y, Sun J, Liu C (2015) A review of the immune molecules in the sea cucumber. Fish Shellfish Immunol 44:1–11

Yang ZP, Sun JM, Xu Z, Zhang CC, Zhou Q (2014) Beneficial effects of *Metschnikowia sp.* C14 on growth and intestinal digestive enzymes of juvenile sea cucumber *Apostichopus japonicus*. Anim Feed Sci Technol 197:142–147

Yang Z, Sun JM, Xu Z (2015) Beneficial effects of *Rhodotorula sp.* C11 on growth and disease resistance of juvenile Japanese spiky sea cucumber *Apostichopus japonicus*. J Aquat Anim Health 27:71–76

Yoon JH, Kang SJ, Oh TK (2006) *Polaribacter dokdonensis* sp. nov., isolated from seawater. Int J Syst Evol Microbiol 56:1251–1255

Yoon J, Matsuo Y, Matsuda S, Adachi K, Kasai H, Yokota A (2007) *Rubritalea spongiae* sp. nov. and *Rubritalea tangerina* sp. nov., two carotenoid-and squalene-producing marine bacteria of the family *Verrucomicrobiaceae* within the phylum '*Verrucomicrobia*', isolated from marine animals. Int J Syst Evol Microbiol 57:2337–2343

Yoon K, Song JY, Kwak MJ, Kwon SK, Kim JF (2017) Genome characteristics of the proteorhodopsin-containing marine flavobacterium *Polaribacter dokdonensis* DSW-5. J Microbiol 55:561–567

Zhang Q, Ma H, Mai K, Zhang W, Liufu Z, Xu W (2010) Interaction of dietary *Bacillus subtilis* and fructooligosaccharide on the growth performance, non-specific immunity of sea cucumber, *Apostichopus japonicus*. Fish Shellfish Immunol 29:204–211

Zhao Y, Zhang Q, Yuan L, Liu X (2017) Effects of dietary taurine on the growth, digestive enzymes, and antioxidant capacity in juvenile sea cucumber, *Apostichopus japonicus*. J World Aquacult Soc 48:478–487

Publisher's Note