Symbiotic bacteria associated with puffer fish *Gastrophysus spadiceus* and evaluation of their antimicrobial activities

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Abstract The present study reports the diversity of culturable bacteria associated with the puffer fish *Gastrophysus spadiceus*. During the study, a total of 31 strains affiliated to the genera *Pseudomonas*, *Janthinobacterium*, *Rahnella*, and *Psychrobacter* were isolated from liver, intestines, and flesh of *G. spadiceus*. These strains exhibited a diverse range of metabolites as indicated by the HPLC and TLC profiles of the chemical extracts of their fermentation products. Some of these crude extracts showed strong antimicrobial activities against pathogenic bacterial strains. In addition, few crude extracts exhibit insecticidal activity against *Artemia salina*.

Keywords *Gastrophysus spadiceus* · Symbiotic bacteria · Antimicrobial activity · Insecticidal activity · South China Sea

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Introduction

Antibacterial drugs (antibiotics) were considered to be wonder drugs for treatment of deadly diseases (Brown 2001). However, with the rise of antibiotic resistance in bacteria, many antibiotics have become less effective (Ventola 2015). Despite the increasing demand for new antibiotics to meet urgent clinical needs, discovery of new groups of antibacterial drugs have been on decline (Conly and Johnston 2005). Since the terrestrial bacteria especially the genus *Streptomyces* have long been investigated as a source of bioactive compounds leading to successful applications in pharmaceutical and biotechnological industries, it is time for us to shift our focus to niches which has not been exploited to the same extent, but has a great potential (Monciardini et al. 2014).

Among these different niches, one important source of new antimicrobial compounds is marine environment and its associated marine organisms (Liu et al. 2010); and among all the marine organisms, the puffer fish is no doubt a hot spot. Puffer fish are considered a celebrated delicacy in many oriental countries especially Japan (Yong et al. 2013). Several species of puffer fish have, however, been found to be bioaccumulated with tetrodotoxin (TTX), an extremely potent marine neurotoxin (Noguchi and Arakawa 2008; Bane et al. 2016). In a toxicity assessment of seven species of common puffer fish caught from South China Sea, only the species *Gastrophysus spadiceus* have been determined to contain low TTX content (below 10 MU g⁻¹) and is considered safe for human consumption (Liu 2011). Since no systematic study has been conducted to assess the symbiotic bacterial diversity in puffer fish, we tried to analyze the diversity of culturable bacterial diversity in different tissues of *G. spadiceus*.

Association with symbiotic bacteria has provided ecological advantages to the host. For example, *Bacillus* species found in the gut of termites enable them to degrade lignocelluloses (Brune 2014). Corals have also been found to form characteristic associations with symbiotic nitrogen-fixing bacteria (Lema et al. 2012). Symbiotic, chemosynthetic bacteria that have been discovered associated with mussels (*Bathymodiolus*) located near hydrothermal vents have a gene that enables them to utilize hydrogen as a source of energy, in preference to sulphur or methane (Duperron et al. 2009). In the present study, we screened the bacteria isolated from the puffer fish *G. spadiceus* for their antimicrobial activities. Crude metabolite extracts from these bacteria were also subjected to insecticidal activity.

Materials and methods

Sampling and isolation of bacteria

Three specimens of puffer fish *G. spadiceus* were collected from the South China Sea during summer and transported to laboratory. Each puffer fish was immediately dissected under aseptic condition, and liver, intestines, and flesh samples were collected. Each tissue was homogenized in a sterile blender with 10 mL of sterile seawater. The homogenates were serially diluted to 10^{-2} and 10^{-3} , and plated on Potato Dextrose Agar (PDA), Gause No. 1, Trypticase Soy Agar (TSA) and a synthetic medium (with the composition: glucose 10 g, sucrose 10 g, beef extract 3 g, yeast extract 3 g, peptone 3 g, agar 20 g, distilled water 1000 mL, pH 7.2). The isolation plates were incubated at 28 °C for 21 days. Single colonies were cultured and purified using the same medium. Purified strains were regularly maintained in TSA/PDA and preserved as glycerol suspension (20% w/v) at -80 °C.

Genomic DNA extraction, amplification, and sequencing

All the strains were identified at the genus level based on the 16S rRNA gene sequence. Genomic DNAs of the strains were extracted using Chelex-100 (de Lamballerie et al. 1992). About 10 mg of the freshly grown culture was taken in an autoclaved 200 μ L PCR tube. To the culture, 50 μ L Chelex buffer (5% Chelex-100) were added, thoroughly mixed and incubated at 100 °C for 40 min. The mixture was centrifuged (12,000 rpm, 10 min) and the supernatant transferred to another sterile PCR tube. The collected samples were stored at -20 °C as the crude DNA extracts for further use.

PCR amplification of the 16S rRNA gene was performed using the primer pair PA-PB (PA: 5'-CAGA GTTGATCCTGGCT-3'; PB: 5'-AGGAGGTGATCCA GCCGCA-3'). The amplicons were purified and sequenced by Sangon Biotech (Shanghai, China).

Phylogenetic diversity

The sequences obtained were quality-checked, assembled, and identified using the EzBioCloud server database (Yoon et al. 2017). These sequences were aligned, and phylogenetic dendrogram based on neighbour-joining algorithm generated using MEGA 5.1 software package (Tamura et al. 2011). Evolutionary distances in the tree were calculated according to Kimura's two-parameter model (Kimura 1983).

Fermentation, metabolite extraction, and screening for chemical diversity

Three different liquid media, AM3# (composition, g L⁻¹: soluble starch 10; soya peptone 5; glycerol 10; peptone 15; CaCO₃ 2), ISP2 broth (yeast extract 4; glucose 4; malt extract 10; pH 7.2) and YIM61# (soluble starch 2; glucose 20; yeast extract 2; peptone 2; K₂HPO₄ 10.5; MgSO₄·7H₂O 0.5; CaCO₃ 2; NaCl 4; pH 7.2) were used as antibiotic production media for evaluation of chemical diversity and further screening of bioactivities. The fermentation flasks containing 50 mL each of these media (in duplicate) were inoculated with one loopful each of the different cultures from fully grown plates. The flasks were, then, cultured under shaking conditions (180 rpm, 28 °C, 7 days). The supernatant obtained from the culture filtrate was extracted with an equal volume of butanone and kept under static condition for 24 h. The organic fraction was then concentrated in vacuo (120 Pa, 45 °C, 10 min). One set of the chemical extracts was dissolved in minimal volume of methanol for screening of chemical diversity, while the other set dissolved in DMSO for antimicrobial screening.

Chemical diversity of the organic extracts was determined by the number of peaks in high-performance liquid chromatography (HPLC) and/or spots in Thin-Layer Chromatography (TLC). HPLC was performed using Waters' reverse phase column (SunFire® C18 5 μ m, 4.6 $\times$ 250 mm column). Elution was done at column flow rate of 1.0 mL/min with the following solvent systems: A (15% acetonitrile, 85% ddH₂O, 1% glacial acetic acid) and B (85% acetonitrile, 15% ddH₂O, 1% glacial acetic acid), using the gradient program: 0–20 min, B 0–80%; 20–22 min, B 80–100%; 22–25 min, B 100%; 25–25.1 min, B 100–0%;

25.1–30 min, B 0%. The extracts were also developed on a silica gel TLC sheet (Si60 F₂₅₄; HAIYANG) using two previously optimized mobile phases, chloroform–methanol (9:1, v/v) and petroleum ether–ethyl acetate (6:4, v/v). The TLC sheets were dried at ambient temperature for 2 h and analyzed under UV light (254 nm).

Antibacterial screening using chemical extracts

The indicator bacterial pathogens used for antibacterial screening were: *Bacillus thuringiensis* ATCC 10792, *Enterococcus faecalis* ATCC 29212, *Micrococcus luteus* ATCC 4698, *Staphylococcus aureus* ATCC 29213, and *Acinetobacter baumannii* ATCC 19606. All the test pathogens were procured from American Type Culture Collection (ATCC). Antibacterial activity was assessed against these test pathogens using the disc-diffusion method (Hugo and Russell 1983). Prior to screening, each of the pathogenic strains was inoculated in 5 mL LB broth and kept incubated under shaking conditions (180 rpm, 28 °C, 24 h). 100 µL of the overnight grown culture were spread on the LB agar plates and kept it for 1 h. Sterile discs (6 mm) impregnated with 10 µL of each chemical extract dissolved in DMSO were then placed on the LB plates previously spread with pathogenic strains. For the control, sterile discs were impregnated with the media extract containing no culture. The plates were kept in upright position to allow the organic extracts to diffuse in the agar plates and then kept incubated for 15 h. Development of inhibitory zones around the discs was considered as positive results.

Screening for insecticidal activity

Insecticidal activities of the chemical extracts were assessed following the inhibition of growth of larva of brine shrimp *Artemia salina* on a 96-well microtitre plates. Prior to the experiment, the cyst (eggs) of the shrimp was hatched into nauplii (larva) by placing 2 g of *A. salina* cyst into 2.4 L of nutrient solution (composition in g L⁻¹ distilled water: NaHCO₃ 1; sea salt 33, pH 8–8.5) and keeping it aerated at 28 °C for 24 h. Active larvae of *A. salina* were selected based on the reaction of the larva to the light. Approximately 20 larva were then placed in each well of the microtitre plates containing 195 µL nutrient solution. To each well, 5 µL of the chemical extract were added. Potassium permanganate (8 mg mL⁻¹) was used as positive control and nutrient solution without any chemical extract as the blank. The microtitre plates were kept incubated at 28 °C for 24 h and active larvae in each well were then counted manually.

Results

Diversity of bacteria associated with *Gastrophysus spadiceus*

A total of 31 bacterial strains representing nine genera from three phyla were isolated from the puffer fish *G. spadiceus* collected from South China Sea. Maximum isolates were obtained from flesh (16 strains), followed by intestine (9) and liver (6) (Table 1). The phylum *Proteobacteria* dominated the bacteria associated with *G. spadiceus*, representing 83.9% of the total isolates. The remaining isolates were represented by the phyla *Actinobacteria* and *Firmicutes* which constitute 12.9 and 3.2%, respectively. The most predominantly isolated genera among these isolates were *Pseudomonas* (13 isolates), *Janthinobacterium* (6) and *Rahnella* (4). While the genera *Arthrobacter*, *Psychrobacter*, and *Rhodococcus* comprised of two isolates each, the genera *Brochothrix* and *Methylobacterium* contained one isolate each. The 16S rRNA gene sequence profiles for these isolates is given in Table 2 and the phylogenetic relationship between these strains is represented in a neighbour-joining dendrogram (Fig. 1) based on the 16S rRNA gene sequences.

Chemical diversity

Almost all the strains had high chemical diversity as implicated in the TLC and HPLC profiles (Table 3). However, this diversity is highly influenced by the antibiotic-producing media (Table 3).

Antibacterial activities

Antibacterial activity was assessed against a set of indicator organisms. The antibacterial profiles of the chemical extracts of the bacteria associated with *G. spadiceus* are shown in Table 3. Of the 31 isolates, 13 exhibited antibacterial activities against at least one of the test pathogens. In case of *B. thuringiensis*, three strains showed inhibition, of which strain SYSU P06 showed higher zone of inhibition. Against *E. faecalis*, nine strains exhibited antagonistic activities of which only strain SYSU P06 produced antibacterial compounds in all the three media. The frequencies of isolates exhibiting antagonistic properties against *M. luteus*, *S. aureus*, and *A. baumannii* are two, four, and eight isolates, respectively. Among all the isolates, the strains SYSU P06, SYSU P62, and SYSU P63 were broad spectrum antibiotic producer, and exhibited antagonistic activities against four of the five pathogenic strains. The strains SYSU P04 and SYSU P13 exhibited antibacterial activities against two pathogenic strains,

Table 1 Generic distribution of bacteria isolated from the different tissues of the puffer fish *Gastrophysus spadiceus*

Genus	Liver	Intestine	Flesh	Total
<i>Arthrobacter</i>	0	1	1	2
<i>Brochothrix</i>	0	0	1	1
<i>Janthinobacterium</i>	2	3	1	6
<i>Methylobacterium</i>	0	0	1	1
<i>Psychrobacter</i>	0	1	1	2
<i>Pseudomonas</i>	4	2	7	13
<i>Rahnella</i>	0	1	3	4
<i>Rhodococcus</i>	0	1	1	2
Total	6	9	16	31

while strains SYSU P02, SYSU P07, SYSU P15, SYSU P17, SYSU P24, and SYSU P38 produced antagonistic activities against one of the test pathogens.

Insecticidal activity

Among all the chemical extracts, 10 were found to exhibit strong insecticidal activity against *A. salina*. The most significant among these extracts was of strain SYSU P17. The strain produced insecticidal compounds in all the three antibiotic-producing media. The results were, however, not consistent with the other strains. Strains SYSU P06 and SYSU P24 produced insecticidal compounds in two of the

Table 2 16S rRNA gene sequence analysis profile of the symbiotic bacteria

Strain	Accession number	Closest homolog	Pairwise similarity
SYSU P02	KY817997	<i>Janthinobacterium lividum</i> DSM 1522 ^T	99.8
SYSU P03	KY817998	<i>Psychrobacter cibarius</i> JG-219 ^T	100
SYSU P04	KY817999	<i>Pseudomonas helmanticensis</i> OHA11 ^t	99.7
SYSU P05	KY818000	<i>Pseudomonas mohnii</i> Ipa-2 ^T	99.4
SYSU P06	KY818001	<i>Janthinobacterium svalbardensis</i> JA-1 ^T	100
SYSU P07	KY818002	<i>Methylobacterium tarhaniae</i> N4211 ^T	99.8
SYSU P09	KY818003	<i>Pseudomonas migulae</i> CIP 105470 ^T	99.2
SYSU P11	KY818004	<i>Rahnella aquatilis</i> CIP 78.65 ^T	100
SYSU P12	KY818005	<i>Rhodococcus cercidiphylli</i> YIM 65003 ^T	100
SYSU P13	KY818006	<i>Brochothrix thermosphacta</i> DSM 20171 ^T	100
SYSU P15	KY818007	<i>Psychrobacter faecalis</i> Iso-46 ^T	99.7
SYSU P16	KY818008	<i>Janthinobacterium lividum</i> DSM 1522 ^T	99.8
SYSU P17	KY818009	<i>Pseudomonas mohnii</i> Ipa-2 ^T	99.5
SYSU P18	KY818010	<i>Pseudomonas lundensis</i> DSM 6252 ^T	99.7
SYSU P19	KY818011	<i>Arthrobacter alpinus</i> DSM 22274 ^T	98.7
SYSU P22	KY818012	<i>Arthrobacter alpinus</i> DSM 22274 ^T	98.7
SYSU P23	KY818013	<i>Pseudomonas weihenstephanensis</i> DSM 29166 ^T	99.8
SYSU P24	KY818014	<i>Pseudomonas panacis</i> CG20106 ^T	99.3
SYSU P28	KY818015	<i>Pseudomonas veronii</i> DSM 11331 ^T	99.8
SYSU P34	KY818016	<i>Pseudomonas veronii</i> DSM 11331 ^T	99.8
SYSU P35	KY818017	<i>Rahnella bruchi</i> FRB 226 ^T	99.8
SYSU P38	KY818018	<i>Rahnella bruchi</i> FRB 226 ^T	99.8
SYSU P40	KY818019	<i>Rahnella aquatilis</i> CIP 78.65 ^T	100
SYSU P42	KY818020	<i>Pseudomonas gessardii</i> CIP 105469 ^T	99.8
SYSU P44	KY818021	<i>Pseudomonas gessardii</i> CIP 105469 ^T	100
SYSU P47	KY818022	<i>Janthinobacterium svalbardensis</i> JA-1 ^T	100
SYSU P48	KY818023	<i>Rhodococcus yunnanensis</i> NBRC 103083 ^T	100
SYSU P50	KY818024	<i>Janthinobacterium svalbardensis</i> JA-1 ^T	100
SYSU P51	KY818025	<i>Janthinobacterium lividum</i> DSM 1522 ^T	100
SYSU P62	KY818026	<i>Pseudomonas veronii</i> DSM 11331 ^T	99.8
SYSU P63	KY818027	<i>Pseudomonas veronii</i> DSM 11331 ^T	99.7

Fig. 1 Neighbour joining phylogenetic dendrogram based on the 16S rRNA gene sequences of the bacterial isolates associated with *Gastrophysus spadiceus*. Fonts in different colours indicate different phyla: red *Proteobacteria*, green *Firmicutes*, blue *Actinobacteria*

media, while SYSU P04, SYSU P05, SYSU P07, SYSU P11, SYSU P28, SYSU P38, and SYSU P63 produced in only one of the three media.

Discussion

Fishes are used both as ornamental pets (Smith et al. 2012) and as food items. Market for the earlier case is ever increasing, and Southeast Asia is a major shareholder of the exports of ornamental fishes (Smith et al. 2009). Under any unintended outbreak of potential pathogens, this market

can suffer big losses (Trust and Barlett 1974; Smith et al. 2008). For the latter prospect, there are many instances, where fish was once considered a poor's man food and has later been made into gourmet dinner. Among such delicacies are species belonging to puffer fishes. The consumption of these fishes is, however, consider risky due to high accumulation of neurotoxin TTX produced by certain symbiotic bacteria (Mosher et al. 1964; Narahashi 2001). Therefore, considering the importance of fish in both ornamental and food industries, it is imperative that we understand the microbial diversity associated with them for future planning in its management.

Table 3 Chemical diversity, antibacterial and insecticidal activities of the chemical extract

Strain designation	Chemical diversity						Test pathogen					
	AM3#		ISP2		YIM61#		<i>Bacillus thuringiensis</i>			<i>Enterococcus faecalis</i>		
	HPLC	TLC	HPLC	TLC	HPLC	TLC	AM3#	ISP2	YIM61#	AM3#	ISP2	YIM61#
SYSU P02	++	++	+	—	—	—	—	—	—	—	—	—
SYSU P03	++	+	++	+	++	+	—	—	—	—	—	—
SYSU P04	+++	++	+++	+	+++	+	—	—	—	+	—	—
SYSU P05	++	+	+	++	—	—	+	—	—	—	—	—
SYSU P06	+++	+	++	++	++	++	—	++	++	+	+	++
SYSU P07	++	++	++	+	++	+	—	—	—	—	—	—
SYSU P09	++	++	+	++	+	+	—	—	—	—	—	—
SYSU P11	++	+++	+	+	+	+	—	—	—	+	—	+
SYSU P12	++	+	++	—	+	—	—	—	—	—	—	—
SYSU P13	+++	+	+	+	+	—	—	—	—	—	—	—
SYSU P15	+	—	—	—	+	—	—	—	—	—	—	—
SYSU P16	+	++	+	—	+	—	—	—	—	—	—	—
SYSU P17	++	+	—	—	+	—	—	—	—	+	—	+
SYSU P18	++	+	++	+	+	+	—	—	—	—	—	—
SYSU P19	++	++	++	+	+	+	—	—	—	—	—	—
SYSU P22	++	++	++	+	+	+	—	—	—	—	—	—
SYSU P23	—	—	—	—	—	—	—	—	—	—	—	—
SYSU P24	+++	+++	++	+	+	—	—	—	—	—	—	+
SYSU P28	+	—	—	—	—	—	—	—	—	—	—	—
SYSU P34	+++	+	+	—	+	—	—	—	—	—	—	—
SYSU P35	+++	+++	+++	++	++	+	—	—	—	—	—	—
SYSU P38	+++	+++	+++	++	+++	+	—	—	—	+	—	—
SYSU P40	+++	+++	++	++	+++	++	—	—	—	—	—	—
SYSU P42	+	—	+	—	—	—	—	—	—	—	—	—
SYSU P44	+++	—	+	—	+	—	—	—	—	—	—	—
SYSU P47	+++	++	++	—	+	—	—	—	—	—	—	—
SYSU P48	+++	+	+	—	++	+	—	—	—	—	—	—
SYSU P50	+++	+	+	+	++	—	—	—	—	—	—	—
SYSU P51	+++	+++	++	—	+	—	—	—	—	—	—	—
SYSU P62	+++	—	+	—	++	—	—	—	—	++	—	—
SYSU P63	+++	—	++	—	++	—	—	—	+	—	—	+
Total	30	24	27	16	26	13	1	1	2	6	1	5

Strain designation	Test pathogen									Insecticidal activity		
	<i>Micrococcus luteus</i>			<i>Staphylococcus aureus</i>			<i>Acinetobacter baumannii</i>					
	AM3#	ISP2	YIM61#	AM3#	ISP2	YIM61#	AM3#	ISP2	YIM61#	AM3#	ISP2	YIM61#
SYSU P02	—	—	—	—	—	—	+	—	—	—	—	—
SYSU P03	—	—	—	—	—	—	—	—	—	—	—	—
SYSU P04	—	—	—	—	—	—	—	—	++	—	—	+
SYSU P05	—	—	—	—	—	—	—	+	++	—	+	—
SYSU P06	—	—	++	—	++	++	—	—	—	+	—	+
SYSU P07	—	—	—	—	—	—	+	—	—	+	—	—
SYSU P09	—	—	—	—	—	—	—	—	—	—	—	—
SYSU P11	—	—	—	—	—	—	—	—	—	+	—	—
SYSU P12	—	—	—	—	—	—	—	—	—	—	—	—

Table 3 continued

Strain designation	Test pathogen									Insecticidal activity		
	<i>Micrococcus luteus</i>			<i>Staphylococcus aureus</i>			<i>Acinetobacter baumannii</i>			AM3#	ISP2	YIM61#
	AM3#	ISP2	YIM61#	AM3#	ISP2	YIM61#	AM3#	ISP2	YIM61#			
SYSU P13	—	—	—	—	—	+	++	—	—	—	—	—
SYSU P15	—	—	—	—	—	—	+	—	—	—	—	—
SYSU P16	—	—	—	—	—	—	—	—	—	—	—	—
SYSU P17	—	—	—	—	—	—	—	—	—	+	+	+
SYSU P18	—	—	—	—	—	—	—	—	—	—	—	—
SYSU P19	—	—	—	—	—	—	—	—	—	—	—	—
SYSU P22	—	—	—	—	—	—	—	—	—	—	—	—
SYSU P23	—	—	—	—	—	—	—	—	—	—	—	—
SYSU P24	—	—	—	—	—	—	—	—	—	—	+	+
SYSU P28	—	—	—	—	—	—	—	—	—	—	+	—
SYSU P34	—	—	—	—	—	—	—	—	—	—	—	—
SYSU P35	—	—	—	—	—	—	—	—	—	—	—	—
SYSU P38	—	—	—	—	—	—	—	—	—	—	+	—
SYSU P40	—	—	—	—	—	—	—	—	—	—	—	—
SYSU P42	—	—	—	—	—	—	—	—	—	—	—	—
SYSU P44	—	—	—	—	—	—	—	—	—	—	—	—
SYSU P47	—	—	—	—	—	—	—	—	—	—	—	—
SYSU P48	—	—	—	—	—	—	—	—	—	—	—	—
SYSU P50	—	—	—	—	—	—	—	—	—	—	—	—
SYSU P51	—	—	—	—	—	—	—	—	—	—	—	—
SYSU P62	++	—	—	++	—	—	++	—	—	—	—	—
SYSU P63	—	—	—	—	—	+	—	—	++	—	—	+
Total	1	0	1	1	1	3	5	1	3	4	5	5

Chemical profile/bioactivity: +++, high; ++, moderate; +, low; —, negative

Several studies on bacterial diversity have been conducted in fishes including ornamental fishes using both culture dependent and independent methods (Sugita et al. 2005; Raja et al. 2006; Sogin et al. 2006; Huber et al. 2007; Han et al. 2010; Smith et al. 2012; Wong and Rawls 2012; He et al. 2016; Hennersdorf et al. 2016; Zheng et al. 2017). However, except for some toxicity tests, no such studies have been conducted on puffer fishes (Kan et al. 1987; Mahmud et al. 1999, 2003; Ahasan et al. 2004; Ahmed 2006; Noguchi et al. 2006; Jang and Yotsu-Yamashita 2006; Liu 2011). The present report is a comprehensive study on the distribution of symbiotic bacteria in three different tissues of puffer fish *Gastrophysus spadiceus*. A cultivation-based technique for the isolation of bacteria is employed in this study, and it resulted in the isolation of 31 bacterial strains. Isolation of bacteria representing nine genera from three phyla indicated that puffer fish is a rich reservoir for bacteria.

In the present study, the genus *Pseudomonas* (phylum *Proteobacteria*) represented the dominant species with 13 strains being isolated from three different tissues of the

fish. The phylum *Proteobacteria* which is the dominant genera in the study is reported as the dominant bacterial phylum with other fishes as well (Han et al. 2010; Smith et al. 2012; Hennersdorf et al. 2016). Another finding is that strains of the genus *Janthinobacterium* (the second most abundant species in this study) have not been reported from puffer fishes in any of the earlier studies. This genus has been reported to manifest a variety of diverse energy metabolism and tolerate environmental stressors (Pantarella et al. 2006; Yin et al. 2015). This report further suggests the importance of the present study.

Symbiotic bacteria of marine origin are often associated of antimicrobial activities (Anand et al. 2006; Margassery et al. 2012; Ruiz-Rodríguez et al. 2012; Mohan et al. 2016). In the present study, the frequencies of bioactive strains were low (in the range of 0–19.4%). The most significant antimicrobial activity was found against *E. faecalis* and *A. baumannii*. Unlike the other strains, the strain SYSU P06 which is phylogenetically related to *Janthinobacterium svalbardensis* JA-1^T showed antimicrobial profile against *E. faecalis* in three antibiotic-producing media. The

bioactivity of this *Janthinobacterium* strain might be related to the high metabolic potential of the genus (O'Sullivan et al. 1990; Pantanella et al. 2006; Schloss et al. 2010; Hornung et al. 2013; Asencio et al. 2014). Furthermore, strain SYSU P06 (*Janthinobacterium* sp.), SYSU P62 (*Pseudomonas* sp.), and SYSU P63 (*Pseudomonas* sp.) are found to exhibit moderate antimicrobial activities against more than one pathogenic strain.

Symbiotic bacteria associated with nematodes were found to display insecticidal activity (Sergeant et al. 2006; de Marco Salvadori et al. 2012). However, it is not specifically restricted to nematodes only. Several other symbionts related to integrated pest management or to pest insect have also exhibited insecticidal activity (Kikuchi et al. 2012; Ruii 2015). We, therefore, considered this particular activity to be of good value for industrial application of our symbiotic strains isolated from *G. spadiceus*. Even though the strains showed low level of insecticidal activity, the number of strains exhibiting this activity is proportional high (32.3% of the total isolates). The most interesting among these strains is SYSU P17 (*Pseudomonas* sp.). Chemical extracts of SYSU P17 from all three antibiotic-producing media showed inhibitory action against *A. salina*. Similar reports have also been published, where pseudomonads are found to be promising plant pest control organisms because of their insecticidal activities (Kim et al. 2011; Kupferschmied et al. 2013).

In the current study, AM3# and YIM61# were found to be better antibiotic-producing media as compared to ISP2 broth. If one compare the chemical composition of these media, the constituent of AM3# is more complex followed by YIM61# and ISP2, respectively. This might have induced the production of bioactive metabolites. Similar findings have also been reported in many studies, whereby media composition play a major role in the production of the antibiotics and/or are found to enhance the production of antibiotics (El-Marsafy et al. 1977; Gesheva et al. 2005; Antoniao et al. 2012). However, if we compare the level of metabolites produced in these media, the level produced in ISP2 is nevertheless higher than YIM61# (Table 3). This indicates that the quantity of metabolites produced cannot be necessarily correlated with its bioactivity.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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