


RESEARCH ARTICLE

Open Access



# Potential of fascaplysin and palauolide from *Fascaplysinopsis cf reticulata* to reduce the risk of bacterial infection in fish farming

Tepoerau Mai<sup>1\*</sup> , Jordan Toullec<sup>1,2</sup>, Simon Van Wynsberge<sup>1,3,4</sup>, Marc Besson<sup>5,6</sup>, Stephanie Soulet<sup>1</sup>, Sylvain Petek<sup>1,2</sup>, Emmanuelle Aliotti<sup>1</sup>, Merrick Ekins<sup>7,8</sup>, Kathryn Hall<sup>7</sup>, Dirk Erpenbeck<sup>9</sup>, David Lecchini<sup>5</sup>, Mehdi A. Beniddir<sup>10</sup>, Denis Saulnier<sup>1</sup> and Cécile Debitus<sup>1,2</sup>

## Abstract

Marine natural products isolated from the sponge *Fascaplysinopsis cf reticulata*, in French Polynesia, were investigated as an alternative to antibiotics to control pathogens in aquaculture. The overuse of antibiotics in aquaculture is largely considered to be an environmental pollution, because it supports the transfer of antibiotic resistance genes within the aquatic environment. One environmentally friendly alternative to antibiotics is the use of quorum sensing inhibitors (QSIs). Quorum sensing (QS) is a regulatory mechanism in bacteria which control virulence factors through the secretion of autoinducers (AIs), such as acyl-homoserine lactone (AHL) in gram-negative bacteria. *Vibrio harveyi* QS is controlled through three parallel pathways: HAI-1, AI-2, and CAI-1. Bioassay-guided purification of *F. cf reticulata* extract was conducted on two bacterial species, i.e., *Tenacibaculum maritimum* and *V. harveyi* for antibiotic and QS inhibition bioactivities. Toxicity bioassay of fractions was also evaluated on the freshwater fish *Poecilia reticulata* and the marine fish *Acanthurus triostegus*. Cyclohexanic and dichloromethane fractions of *F. cf reticulata* exhibited QS inhibition on *V. harveyi* and antibiotic bioactivities on *V. harveyi* and *T. maritimum*, respectively. Palauolide (1) and fascaplysin (2) were purified as major molecules from the cyclohexanic and dichloromethane fractions, respectively. Palauolide inhibited QS of *V. harveyi* through HAI-1 QS pathway at 50  $\mu\text{g ml}^{-1}$  (26  $\mu\text{M}$ ), while fascaplysin affected the bacterial growth of *V. harveyi* (50  $\mu\text{g ml}^{-1}$ ) and *T. maritimum* (0.25  $\mu\text{g}$ ). The toxicity of fascaplysin-enriched fraction (FEF) was evaluated and exhibited a toxic effect against fish at 50  $\mu\text{g ml}^{-1}$ . This study demonstrated for the first time the QSI potential of palauolide (1). Future research may assess the toxicity of both the cyclohexanic fraction of the sponge and palauolide (1) on fish, to confirm their potential as alternative to antibiotics in fish farming.

**Keywords:** Porifera, Marine natural products, Quorum sensing inhibitors, Antibiotic, *Fascaplysinopsis cf reticulata*

## Background

The overuse of antibiotics in the environment may have important economic and sanitary outcomes (Martinez 2009; Hatosy and Martiny 2015). Indeed, the release of antibiotics in natural environments exerts a strong pressure on bacteria strains and supports the selection of resistant bacteria. The recurrent use of antibiotics decreases their effectiveness over time (Blair et al. 2015). To reduce the overuse of antibiotics and minimize the impacts to the environment and human society, there is

an urgent need for alternatives to antibiotics (Editorials 2013; Spellberg and Gilbert 2014).

In aquaculture antibiotic resistance causes mass mortality of cultured species (Karunasagar et al. 1994) which result in economic loss for farmers (Shrestha et al. 2018). Aquaculture itself largely contributes to the dissemination of antibiotics resistance genes in the aquatic environment (WHO, 2006; Shah et al. 2014), which increases the risks on human health (Aly and Albutti 2014). Policy on antibiotics in aquaculture is becoming more strict, and antibiotics are forbidden in some countries (Lulijwa et al. 2019). Finding antibiotic alternatives in this field is the focus of the current research (Pérez-Sánchez et al. 2018) due to

\* Correspondence: [maitepoerau@live.fr](mailto:maitepoerau@live.fr)

<sup>1</sup>EIO, ILM, IFREMER, IRD, UPF, BP 6570, 98702 FAA'A, Tahiti, French Polynesia  
Full list of author information is available at the end of the article



the promising market they represent. To reduce the selective pressure exerted on bacteria strains, novel strategies target natural products that inhibit the expression of virulence genes without exerting a strong bactericide activity (Moloney 2016; Spellberg and Gilbert 2014). Such promising products include inhibitors of quorum sensing (Chen et al. 2018; Pérez-Sánchez et al. 2018) which exhibited in vitro and in vivo effectiveness in aquaculture (Manefield et al. 2000; Brackman et al. 2008; Pande et al. 2013).

Quorum sensing (QS) is a cell-to-cell communication process in bacteria based on the secretion and detection of signal molecules (i.e., autoinducers) by bacteria. Specifically for gram-negative bacteria, autoinducers (AIs) consist of small molecules, mainly acyl-homoserine lactone (AHL) derivatives (Waters and Bassler 2005). Quorum sensing allows the expression of target genes involved in biofilm formation, toxin secretion, and bioluminescence (Henke and Bassler 2004a). It is influenced by the concentration in AIs related to the bacterial density and the genetic similarity of bacteria neighbors (Schluter et al. 2016).

A model species for testing the relevance of antibiotic alternatives in aquaculture is *V. harveyi*. *Vibrio harveyi* is a luminescent bacteria inhabiting the marine environment and pathogens in aquaculture, specifically when it is associated with *Tenacibaculum maritimum* (Reverter et al. 2016). The QS of *V. harveyi* is well documented, with three parallel QS systems that are regulated by three couples of signal molecules and cognate sensors: *V. harveyi* autoinducer-1 (HAI-1) and LuxN sensor; autoinducer-2 (AI-2) and LuxPQ sensor; *Cholerae* autoinducer-1 (CAI-1); and CqsS sensor (Henke and Bassler 2004a). Together these three systems encode bioluminescence and virulence factors as biofilm formation, type III secretion, and a secreted metalloprotease genes (Henke and Bassler 2004a; Henke and Bassler 2004b).

Quorum sensing inhibitors (QSIs) of *V. harveyi* have already been identified from a variety of marine organisms, including bacteria, algae, and sponges (Givskov et al. 1996; Peters et al. 2003; Rasch et al. 2004; Teasdale et al. 2009; Dobretsov et al. 2011; Natrah et al. 2011; Kalia 2013; Tello et al. 2013; Saurav et al. 2017). Marine sponges are promising sources of antibiotic alternatives because (i) they are known to be a reservoir of diverse microbial communities (Thomas et al. 2016) and (ii) as primitive sessile organisms featured with a simple multicellular structure, their main defense against pathogen rely on the production of secondary metabolites with antibiotic and antibiofilm (Feng et al. 2013), and QS inhibition activities against pathogens (Blunt et al. 2005; Müller et al. 2013; Quévrain et al. 2014). In this study, *Vibrio harveyi* and *T. maritimum* were used as model species to test antibiotic and QS inhibition bioactivities of two compounds isolated from the sponge *Fascaplysinopsis cf reticulata* collected in French Polynesia. Because

of the cytotoxicity of fascaplysin (2) reported in the literature (Hamilton 2014), we also evaluated the toxicity of *F. cf reticulata* extract on two fish species (*Poecilia reticulata* and *Acanthurus triostegus*) to check the safety of using this sponge in fish farming.

## Methods

### Sponge sampling

Sponge samples were collected manually using SCUBA, between 45 and 65 m depth in the Tuamotu Archipelago (French Polynesia) during the 2011 Tuam expedition aboard the Alis vessel (Debitus 2011), on the outer reef of Anuanuaro Atoll (20°25.394'S, 143°32.930'W). Samples were frozen immediately at -20 °C on board until being processed.

### Purification and characterization of secondary metabolites.

The sponge collected was freeze dried and grounded to obtain 95 g of dry sponge powder. It was extracted using 100 ml of 80% ethanol and then rinsed twice in 100% ethanol. The solvent was evaporated under reduced pressure, and the remaining ethanolic extract was dissolved in water and successively partitioned three times with cyclohexane and three times with dichloromethane. The cyclohexanic fraction was subjected to silica gel chromatography (40–60- $\mu$ m mesh) and then eluted with cyclohexane and ethyl acetate mixtures of increasing polarity. Further semi-preparative HPLC on normal phase column eluted with cyclohexane/ethyl acetate 55/45 vol/vol allowed the isolation of palauolide (1) (5 mg). The purification of the dichloromethane fraction (called fascaplysin-enriched fraction (FEF)) using reverse phase HPLC (column: Interchrom Uptisphere strategy, 5  $\mu$ m; solvent: (water/acetonitrile 70:30), TFA 0.1%) led to the isolation of fascaplysin (2) (17% of FEF, 0.02% dry sponge weight, 19 mg). High-performance liquid chromatography analysis was performed on HPLC (Agilent Technologies 1260 Infinity) with diode array (Agilent G1315C) and evaporative light-scattering (Agilent G4260C) detection. Yields were calculated using the ratio compound weight/freeze-dried sponge weight. Structure elucidation of the two known compounds was performed on the basis of  $^1\text{H}$  and  $^{13}\text{C}$  NMR and mass spectra.

### Fish toxicity bioassay

The toxicity effect of *F. cf reticulata*'s FEF on fish was evaluated on two fish species that can be easily found in French Polynesia and reared in the laboratory: *P. reticulata* (the guppy or mosquito fish) and *A. triostegus* (the convict tang fish). *Poecilia reticulata* specimens (5–8 cm length) were collected from a freshwater pool at Tahiti at night. *Poecilia reticulata* specimens were appealed with a flashlight and then caught with a landing net (5-mm mesh size) and kept in 3 L plastic jar containing

freshwater. Young settlers (or recruits, 1.5, 2 cm length) and juveniles (3–7 cm length) of *A. triostegus* (at the two distinct developmental stages) were caught during full moon nights on the foreshore puddles and on the reef crest using a net of the northeast coast of Moorea Island (17°29'52.19"S, 149°45'13.55"W). *Acanthurus triostegus* recruits (fish larvae undergoing metamorphosis) were transparent at the time of capture, demonstrating that they had just entered the reef following their pelagic larval stage, while the juveniles (old settlers, already metamorphosed and settled when captured) were already fully pigmented when caught, demonstrating that they had already settled in this reef area for at least a week (Lecchini et al. 2004).

A preliminary assay was performed on *P. reticulata* by balneation, as described previously for environmental toxicity studies of acetylcholinesterase (AChE) inhibitor pesticides (Wester and Vos 1994; Bocquené and Galgani 2004; El-Demerdash et al. 2018). Fascaplysin-enriched fraction ethanolic solution was further tested in duplicate at 1 and 5  $\mu\text{g ml}^{-1}$  during 72 h (chronic toxicity) and at 50  $\mu\text{g ml}^{-1}$  during 1 h (acute toxicity) in 2-L tanks, each containing five fishes. Solvent controls were run for each experiment. For the 72-h experiment, water, FEF, and EtOH were renewed, and fishes were fed once a day with commercial flakes. Abnormal behavior of fishes after exposure to FEF was evaluated qualitatively, such as swimming difficulties (i.e., irregularity of swim velocity, asymmetric pectorals fins movements, upside down swimming, and quick jumps) and loss of appetite.

Since the preliminary assay highlighted a modification of *P. reticulata* behavior by FEF (see results section), a second toxicity assay was performed on *A. triostegus* focusing on feeding behavior by using a quantitative method. The effect on FEF exposition on *A. triostegus* feeding behavior was assessed on two distinct developmental stages in order to compare the activity of FEF at both stages of development. The bioassays on *A. triostegus* were performed in 3-L tanks. Fishes (young settlers or juveniles) were exposed to FEF at 1  $\mu\text{g ml}^{-1}$  in groups of four or five individuals during 24, 48, and 72 h. Rubble with encrusting turf algae were placed in the tank for fish to feed on 1 h per day during 3 days. The feeding behavior was assessed by counting the number of bites on the algae encrusted rubble in each aquarium. Six video sequences of 5 or 10 min per aquarium per day were analyzed. Results are expressed in number of bites per fish per hour.

#### Antibacterial and quorum sensing inhibition bioassays on *Vibrio harveyi*

Every purified compound was tested in triplicate at four concentrations, 1, 5, 10, and 50  $\mu\text{g ml}^{-1}$  against the wild strain *V. harveyi* BB120 (Johnson and Shunk 1936; Bassler

et al. 1997), and three derived mutants, JAF 375 (Freeman and Bassler 1999), JMH 597, and JMH 612 (Henke and Bassler 2004a). All strains were obtained from Bassler laboratory (Bassler et al. 1997; Freeman and Bassler 1999; Henke and Bassler 2004a). Each mutant only expressed one of the three QS systems of *V. harveyi*: JAF 375 (CAI-1 activated), JMH 597 (AI-2 activated), or JMH 612 (HAI-1 activated) (Freeman and Bassler 1999; Henke and Bassler 2004a). Quorum sensing inhibition bioassay was performed by combining simultaneously luminescence kinetics (in relative luminescence units, RLU) and absorbance kinetics (at  $\lambda = 600\text{ nm}$ ) (Givskov et al. 1996; Brackman et al. 2008; Steenackers et al. 2010). Absorbance kinetics was used to measure the growth of *V. harveyi* with any tested compound or controls. Data was obtained using a Fluostar Omega spectrophoto-luminometer (BMG Labtech Fluostar OPTIMA, Ortenberg, Germany).

The quorum sensing inhibition bioassay was modified from Mai et al. (2015). A *V. harveyi* colony was grown on Zobell agar plates (BD Bacto™ peptone, 5 g; BD BBL™ yeast extract, 1 g; BD Bacto™ agar, 17 g; sterilized sea water, 1 L) for 24 h. The plates were then suspended in liquid Lennox L broth base medium (Invitrogen, Carlsbad, CA, USA) which was supplemented with artificial sea salts (Sigma Aldrich Co., St Louis, MO, USA) at 40  $\text{g l}^{-1}$  and was then incubated for 16 h under constant orbital stirring at 27 °C. This suspension (50  $\mu\text{l}$ ) was then diluted in Marine Broth (CONDA®, Madrid, Spain) (10 ml) and was incubated for 30 min while stirring at 27 °C. Compounds were dissolved in absolute ethanol, deposited in sterile 96- $\mu\text{Clear}^{\circ}$  bottom wells microplates (Greiner Bio-One, Germany) that were dried at room temperature under a laminar flow hood. Each sample was tested in triplicate for each concentration of purified compound tested (1, 5, 10, and 50  $\mu\text{g ml}^{-1}$ ). Compounds were then dissolved in Marine Broth (100  $\mu\text{l}$ ) by sonication at 50/60 Hz for 30 min, and a bacterial suspension (100  $\mu\text{l}$ ) was added in the appropriate wells. The 96 wells plates were incubated at 27 °C for 12 h in a microplate incubator reader, with luminescence and absorbance reading conducted every 10 min, after 1 min of double orbital stirring. The sterility of the culture medium was checked throughout the experiment, as well as the absorbance of each tested compound. Luminescence and absorbance data at the *N*-cycle reading ( $L_{N\text{-cycle}}$  and  $A_{N\text{-cycle}}$ ) were respectively obtained after subtracting the mean of the first ten cycles of the luminescence and absorbance ( $L_{\text{mean first 10 cycles}}$  and  $A_{\text{mean first 10 cycles}}$ ) from the raw data ( $L_{N\text{-cycle raw data}}$  and  $A_{N\text{-cycle raw data}}$ ) (Eqs. 1 and 2).

$$L_{N\text{-cycle}} = L_{N\text{-cycle raw data}} - L_{\text{mean first 10 cycles}} \quad (1)$$

$$A_{N\text{-cycle}} = A_{N\text{-cycle raw data}} - A_{\text{mean first 10 cycles}} \quad (2)$$

The kinetic curves obtained were sigmoidal. Any delay or inhibition of both growth and luminescence curves

compared to the control curves (which mean an inhibition of growth rate) is translated to an antibiotic effect of the compound. By contrast, no change in bacterial growth between tested and control curves associated with a delay of luminescence between tested and control curves translated to a QSI effect of the compound.

#### Antibacterial activity on *Tenacibaculum maritimum*

Antibiotic activity on *T. maritimum* could not be performed through the absorbance kinetics method as previously described for *V. harveyi* strains, because *T. maritimum* precipitated at the beginning of the experiment which prevented measuring absorbance. Antibiotic activity on *T. maritimum* was tested using the disk diffusion method on solid agar medium (Bauer et al. 1966). This bioassay was performed on a strain of the marine bacteria named TFA4 (Reverter et al. 2016). Pure compounds were dissolved in 100% ethanol to obtain impregnated disks (cellulose disks, 6 mm diameter) with 0.5, 0.25, 0.125, and 0.0625  $\mu\text{g}$  of compound. Disks were air-dried in a laminar flow cabinet and then deposited on Zobell agar plates, previously seeded with TFA4 strain. Petri dishes were incubated at 27 °C for 2 days.

#### Statistical analyses

Absorbance was modeled as a logistic function of time ( $t$ ) (Kingsland 1982) according to Equation 3, where  $A_{max}$  is the maximum or asymptotic value of absorbance,  $k$  is the steepness of the curve, and  $t_0$  is the  $x$  value of the sigmoid's midpoint.

$$Y = \frac{A_{max}}{1 + \exp(-k(t-t_0))} \quad (3)$$

$$Y = \frac{L_{max}}{1 + a \exp(-k(t-t_0))} \quad (4)$$

Luminescence was also modeled as a logistic function, following Equation 4, where  $L_{max}$  is the maximum or asymptotic value of luminescence. Equation 4 includes an  $a$  parameter to adequately model the high steepness found for luminescence curves. For each compound and concentration tested, the parameters of the logistic curve were fitted using the function "nls" of the package "stat" in R.3.1.0. The effect of compounds on the growth and the bioluminescence of *V. harveyi* populations were evaluated by comparison of the growth rate (assimilated to the parameter  $k$ ) and the curve inflection points. For the absorbance kinetics, the inflection point was equal to  $t_0$ . For the luminescence kinetics, the derivative ( $Y'$ ) of the sigmoid function was calculated, and the inflection point was identified as the time for which  $Y'$  was maximal. Furthermore, to provide comparable values of bioluminescence, luminescence values were compared at a fixed

bacterial concentration (i.e., fixed absorbance  $A = 0.055$ , which corresponded to half the maximum absorbance  $A_{max}$  of control).

For all parameters involved in QS activity ( $k$  and inflection points) as well as in toxicity (number of bites per unit of time per fish), differences between concentration were tested using the non-parametric *Kruskal-Wallis* test (function `kruskal.test` of `pgirmess` package in R.3.1.0) and a multiple comparison test after *Kruskal-Wallis* (function `kruskalmc`), suitable for small samples. A QSI activity was evidenced when (1) *V. harveyi* population growth rate ( $k$ , see Eq. 3) was not significantly lower with compound (or extract) compared to control (*Kruskal-Wallis* test and multiple comparison test after *Kruskal-Wallis*,  $\alpha = 0.05$ ) and (2) the inflection point of luminescence is significantly higher with compound (or extract) compared to control (*Kruskal-Wallis* test and multiple comparison test after *Kruskal-Wallis*,  $\alpha = 0.05$ ).

## Results

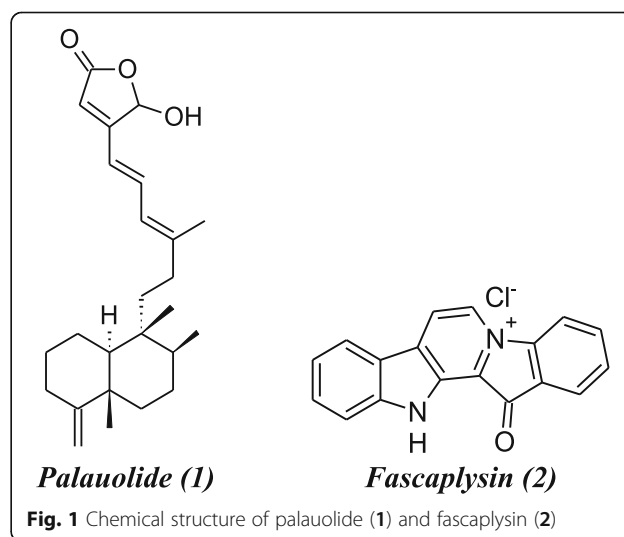
#### Purification of the *Fascaplysinopsis cf reticulata* extract

The hydro-alcoholic extraction of sponge powder (95 g) provided 2.8 g of extract. The partitioning of this extract led to cyclohexanic (1.46 g, yield 1.54% w/w) and dichloromethane (0.112 g, yield 0.11% w/w) fractions. The purification of the cyclohexanic fraction conducted to the known palauolide (1) (0.005 g, yield 0.005% w/w) and the dichloromethenic fraction to the alkaloid fascaplysin (2) (0.019 g, yield 0.02% w/w) (Fig. 1).

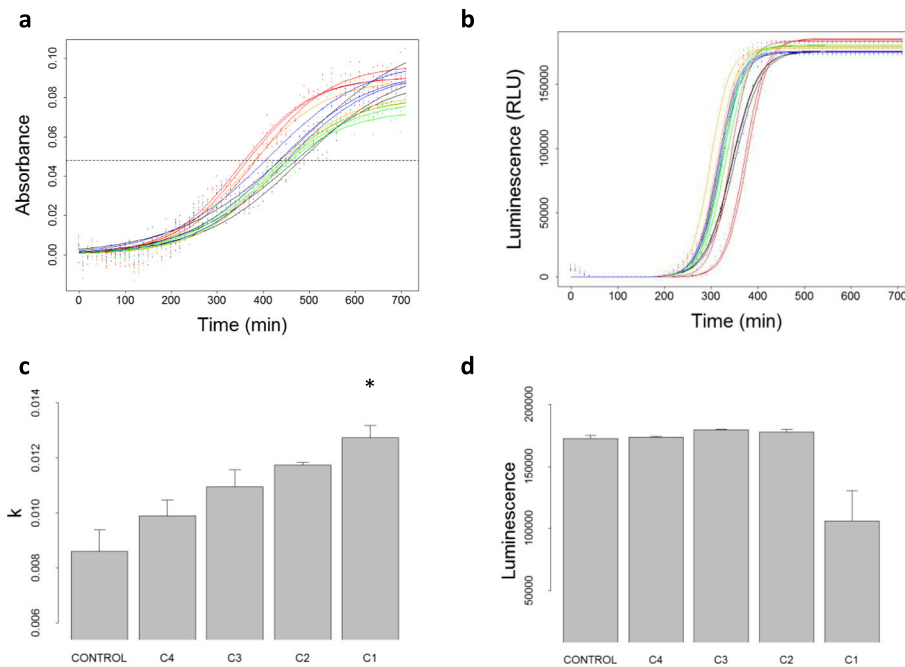
#### Quorum sensing inhibition

##### Effect of palauolide

Absorbance and luminescence kinetics of the *V. harveyi* wild strain (Fig. 2 a and b) highlighted a dose-dependent effect of palauolide (1) on BB120 bacterial growth. During the growth of *V. harveyi* bacterial strains, the growth







**Fig. 2** Effect of palauolide (1) on BB120 strain. (a) Absorbance kinetics, (b) luminescence kinetics (RLU), (c) steepness data ( $k$ ) of absorbance kinetic, (d) luminescence value (RLU) measured for absorbance at 0.055 (represented on (A) by a dashed line, corresponding to the absorbance value at the inflection point of control) without palauolide (black, control), with palauolide  $1 \mu\text{g ml}^{-1}$  (blue, C4),  $5 \mu\text{g ml}^{-1}$  (green, C3),  $10 \mu\text{g ml}^{-1}$  (orange, C2), and  $50 \mu\text{g ml}^{-1}$  (red, C1). Data are reported as means  $\pm$  SD from three technical replicates (\*significant *Kruskal-Wallis*  $p$  value  $< 0.05$  by comparing with control)

rate ( $k$  parameter) of absorbance increased as the concentration of palauolide (1) increased (Table 1, Fig. 2c). As a consequence, the sigmoid midpoint ( $t_0$ ) decreased as concentration of palauolide (1) increased (data not shown). At  $50 \mu\text{g ml}^{-1}$  of palauolide (1), the growth rate of absorbance ( $k = 0.0127 \pm 0.0005$ ) reached values significantly higher than for controls ( $k = 0.0086 \pm 0.0008$ ; multiple comparison test after *Kruskal-Wallis*;  $p < 0.05$ ). Also not significant due to the lack of statistical power, similar trends were obtained for the three derived QS mutants (Table 1). Despite the stimulating effect of palauolide (1) on *V. harveyi* growth, a delay in luminescence activation of approximately 17 min was observed for the highest concentrations tested  $50 \mu\text{g ml}^{-1}$ , compared to the luminescence curve of the control (Fig. 2b, red and black curves, respectively). At the same growth stage ( $A = 0.055$ ), a decrease in RLU was observed for the highest concentration of palauolide (1) compared to control. Such decrease was found for the BB120 wild strain (RLU respectively at  $106\,210 \pm 24\,385$  at  $50 \mu\text{g ml}^{-1}$  ( $26 \mu\text{M}$ ) of palauolide (1) compared to  $172\,416 (\pm 2\,489)$  for control; Table 1; Fig. 2d) and the JMH 612 mutant only (RLU respectively at  $99\,806 \pm 18\,002$  at  $50 \mu\text{g ml}^{-1}$  ( $26 \mu\text{M}$ ) of palauolide (1) compared to  $189\,392 \pm 2\,609$  for control; Table 1; Fig. 2d). For the JMH 612 mutant, the delay between the luminescence kinetics at  $50 \mu\text{g ml}^{-1}$  and the luminescence kinetics of control was

50 min in average. These results indicate that palauolide (1) boosted bacterial growth and inhibited *V. harveyi* QS through HAI-1 QS pathway.

#### Effect of faspaplysin

*Vibrio harveyi* BB120 population growth rate ( $k$ , see Eq. 3) was significantly lower with faspaplysin (2) at  $50 \mu\text{g ml}^{-1}$  ( $k = 0.0021$ ) compared to control ( $k = 0.0121$ ;  $p$  value  $< 0.05$ ). Similar results were obtained for mutant JAF 375, with lower growth rate ( $k = 0.0036$ ) and with faspaplysin (2) at  $50 \mu\text{g ml}^{-1}$  compared to control ( $k = 0.0119$ ). Strong decreases of population growth rate were also obtained for mutants JMH 597 and JMH 612 with faspaplysin (2) at  $50 \mu\text{g ml}^{-1}$  compared to control. For several replicates involving the two last mutants, population growth was null or negative with faspaplysin (2) at  $50 \mu\text{g ml}^{-1}$ , which prevented the growth model to be fitted and  $k$  estimates to be provided (Table 2; Additional file 1). This suggests an antibiotic effect of faspaplysin (2) on *V. harveyi* and prevents concluding on a QS inhibition effect.

#### Antibiotic bioassay

Palauolide did not display any antibiotic activity against the marine pathogen *T. maritimum*. By contrast faspaplysin (2) displayed antibiotic activity at  $0.25 \mu\text{g}$  per disk (11 mm) and  $0.5 \mu\text{g}$  per disk (18 mm) against *T. maritimum* (TFA4) (disk diffusion bioassay).

**Table 1** Steepness of absorbance kinetic ( $k$ ) and luminescence value measured for absorbance at 0.055 (RLU) estimated for various concentration of palauolide (1) and *Vibrio harveyi* strains

| Strain  | Concentration of palauolide | Replicate               | $k$                    | RLU                  |
|---------|-----------------------------|-------------------------|------------------------|----------------------|
| BB120   | Control                     | 1                       | 0.0093                 | 173985               |
|         |                             | 2                       | 0.0088                 | 173717               |
|         |                             | 3                       | 0.0077                 | 169546               |
|         |                             | All                     | 0.0086 ( $\pm$ 0.0008) | 172416 ( $\pm$ 2489) |
|         | C4                          | 1                       | 0.0100                 | 173727               |
|         |                             | 2                       | 0.0104                 | 174212               |
|         |                             | 3                       | 0.0093                 | 172441               |
|         |                             | All                     | 0.0099 ( $\pm$ 0.0006) | 173460 ( $\pm$ 915)  |
|         | C3                          | 1                       | 0.0105                 | 178786               |
|         |                             | 2                       | 0.0116                 | 179795               |
|         |                             | 3                       | 0.0107                 | 179754               |
|         |                             | All                     | 0.0110 ( $\pm$ 0.0006) | 179445 ( $\pm$ 571)  |
|         | C2                          | 1                       | 0.0119                 | 174835               |
|         |                             | 2                       | 0.0116                 | 178950               |
|         |                             | 3                       | 0.0117                 | 178843               |
|         |                             | All                     | 0.0117 ( $\pm$ 0.0001) | 177542 ( $\pm$ 2345) |
| C1      | 1                           | 0.0122                  | 107211                 |                      |
|         | 2                           | 0.0131                  | 81339                  |                      |
|         | 3                           | 0.0129                  | 130080                 |                      |
|         | All                         | 0.0127 ( $\pm$ 0.0005)* | 106210 ( $\pm$ 24385)  |                      |
| JAF 375 | Control                     | 1                       | 0.0144                 | 131953               |
|         |                             | 2                       | 0.0140                 | 134519               |
|         |                             | 3                       | 0.0135                 | 131708               |
|         |                             | All                     | 0.0139 ( $\pm$ 0.0005) | 132727 ( $\pm$ 1557) |
|         | C4                          | 1                       | 0.0160                 | 129253               |
|         |                             | 2                       | 0.0168                 | 131105               |
|         |                             | 3                       | 0.0183                 | 130501               |
|         |                             | All                     | 0.0170 ( $\pm$ 0.0012) | 130286 ( $\pm$ 944)  |
|         | C3                          | 1                       | 0.0151                 | 146894               |
|         |                             | 2                       | 0.0154                 | 146197               |
|         |                             | 3                       | 0.0171                 | 145802               |
|         |                             | All                     | 0.0159 ( $\pm$ 0.0011) | 146298 ( $\pm$ 553)  |
|         | C2                          | 1                       | 0.0172                 | 140852               |
|         |                             | 2                       | 0.0163                 | 141282               |
|         |                             | 3                       | 0.0160                 | 140905               |
|         |                             | All                     | 0.0165 ( $\pm$ 0.0006) | 141014 ( $\pm$ 235)  |
| C1      | 1                           | 0.0170                  | 159632                 |                      |
|         | 2                           | 0.0179                  | 158643                 |                      |
|         | 3                           | 0.0165                  | 158767                 |                      |
|         | All                         | 0.0171 ( $\pm$ 0.0007)  | 159014 ( $\pm$ 539)    |                      |
| JMH 597 | Control                     | 1                       | 0.0094                 | 147880               |
|         |                             | 2                       | 0.0081                 | 146686               |
|         |                             | 3                       | 0.0100                 | 147955               |

**Table 1** Steepness of absorbance kinetic (*k*) and luminescence value measured for absorbance at 0.055 (RLU) estimated for various concentration of palauolide (1) and *Vibrio harveyi* strains (Continued)

| Strain  | Concentration of palauolide | Replicate         | <i>k</i>          | RLU             |
|---------|-----------------------------|-------------------|-------------------|-----------------|
| JMH 612 | C4                          | All               | 0.0091 (± 0.0010) | 147507 (± 712)  |
|         |                             | 1                 | 0.0120            | 150354          |
|         |                             | 2                 | 0.0118            | 147321          |
|         |                             | 3                 | 0.0121            | 147103          |
|         | C3                          | All               | 0.0119 (± 0.0001) | 148259 (± 1818) |
|         |                             | 1                 | 0.0132            | 154515          |
|         |                             | 2                 | 0.0138            | 152906          |
|         |                             | 3                 | 0.0140            | 153875          |
|         | C2                          | All               | 0.0137 (± 0.0004) | 153765 (± 810)  |
|         |                             | 1                 | 0.0129            | 152211          |
|         |                             | 2                 | 0.0123            | 151867          |
|         |                             | 3                 | 0.0122            | 152261          |
|         | C1                          | All               | 0.0125 (± 0.0003) | 152113 (± 214)  |
|         |                             | 1                 | 0.0159            | 150802          |
|         |                             | 2                 | 0.0183            | 161802          |
| 3       |                             | 0.0145            | 157737            |                 |
| Control | All                         | All               | 0.0162 (± 0.0019) | 156780 (± 5562) |
|         |                             | 1                 | 0.0057            | 186864          |
|         |                             | 2                 | 0.0083            | 192076          |
|         |                             | 3                 | 0.0087            | 189235          |
|         | C4                          | All               | 0.0076 (± 0.0017) | 189392 (± 2609) |
|         |                             | 1                 | 0.0074            | 186349          |
|         |                             | 2                 | 0.0076            | 189886          |
|         |                             | 3                 | 0.0087            | 189302          |
|         | C3                          | All               | 0.0079 (± 0.0007) | 188512 (± 1896) |
|         |                             | 1                 | 0.0088            | 187819          |
|         |                             | 2                 | 0.0076            | 174498          |
|         |                             | 3                 | 0.0078            | 184672          |
|         | C2                          | All               | 0.0081 (± 0.0007) | 182330 (± 6963) |
|         |                             | 1                 | 0.0089            | 190911          |
|         |                             | 2                 | 0.0086            | 188991          |
|         |                             | 3                 | 0.0089            | 188746          |
| C1      | All                         | 0.0088 (± 0.0002) | 189549 (± 1186)   |                 |
|         | 1                           | 0.0106            | 120398            |                 |
|         | 2                           | 0.0097            | 91967             |                 |
|         | 3                           | 0.0091            | 87053             |                 |
| All     | 0.0098 (± 0.0008)           | 99806 (± 18002)   |                   |                 |

Control, C4, C3, C2, and C1, respectively, refer to palauolide (1) concentration at 0, 1, 5, 10, and 50 µg ml<sup>-1</sup>. Estimated values of *k* and RLU are provided for each replicate (*n* = 3) and for all replicates (mean ± standard deviation over the three replicated). Values significantly different from controls ( $\alpha$  = 0.05) are marked with a star (\*)

**Fish toxicity assay**

At 50 µg ml<sup>-1</sup> of FEF, *P. reticulata* exhibited signs of hyperventilation as well as motility disarrangement (i.e., jerky movements with sudden accelerations or motionless

periods) within the first hour of treatment. None motility disarrangement was observed at 1 µg ml<sup>-1</sup> FEF solutions, but changes to the feeding behavior were noticed for *P. reticulata*, i.e., *P. reticulata* tasted the food flakes but did

**Table 2** Steepness of absorbance kinetic ( $k$ ) estimated with faspaplysin (2) at  $50 \mu\text{g ml}^{-1}$  (C1), and without faspaplysin (control), for the various *Vibrio harveyi* strains

| Strain  | Dose    | Replicate | $k$    |
|---------|---------|-----------|--------|
| BB120   | Control | 1         | 0.0118 |
|         |         | 2         | 0.0123 |
|         |         | All       | 0.0121 |
|         | C1      | 1         | 0.0024 |
|         |         | 2         | 0.0017 |
|         |         | All       | 0.0021 |
| JAF 375 | Control | 1         | 0.0117 |
|         |         | 2         | 0.0120 |
|         |         | All       | 0.0119 |
|         | C1      | 1         | 0.0051 |
|         |         | 2         | 0.0021 |
|         |         | All       | 0.0036 |
| JMH 597 | Control | 1         | 0.0108 |
|         |         | 2         | 0.0118 |
|         |         | All       | 0.0113 |
|         | C1      | 1         | 0.0019 |
|         |         | 2         | < 0    |
|         |         | All       | -      |
| JMH 612 | Control | 1         | 0.0137 |
|         |         | 2         | 0.0119 |
|         |         | All       | 0.0128 |
|         | C1      | 1         | < 0    |
|         |         | 2         | < 0    |
|         |         | All       | -      |

not ingest them. At  $5 \mu\text{g ml}^{-1}$  of FEF, all of the *P. reticulata* died within 12 h.

The experiment on *A. triostegus* was only performed at  $1 \mu\text{g ml}^{-1}$  of FEF. For each time of incubation (24, 48, and 72 h), the number of bites of *A. triostegus* (both recruits and juveniles) decreased significantly compared to control *A. triostegus* (Fig 3). After 24 h of incubation with  $1 \mu\text{g ml}^{-1}$  FEF solution, the number of bites decreased by 91.3% ( $\pm 1.6\%$ ,  $p$  value < 0.01) for recruits and by 95.9% ( $\pm 0.8\%$ ,  $p$  value < 0.001) for juveniles compared to the control *A. triostegus* (Fig 3). This trend was confirmed for others times of exposition.

## Discussion

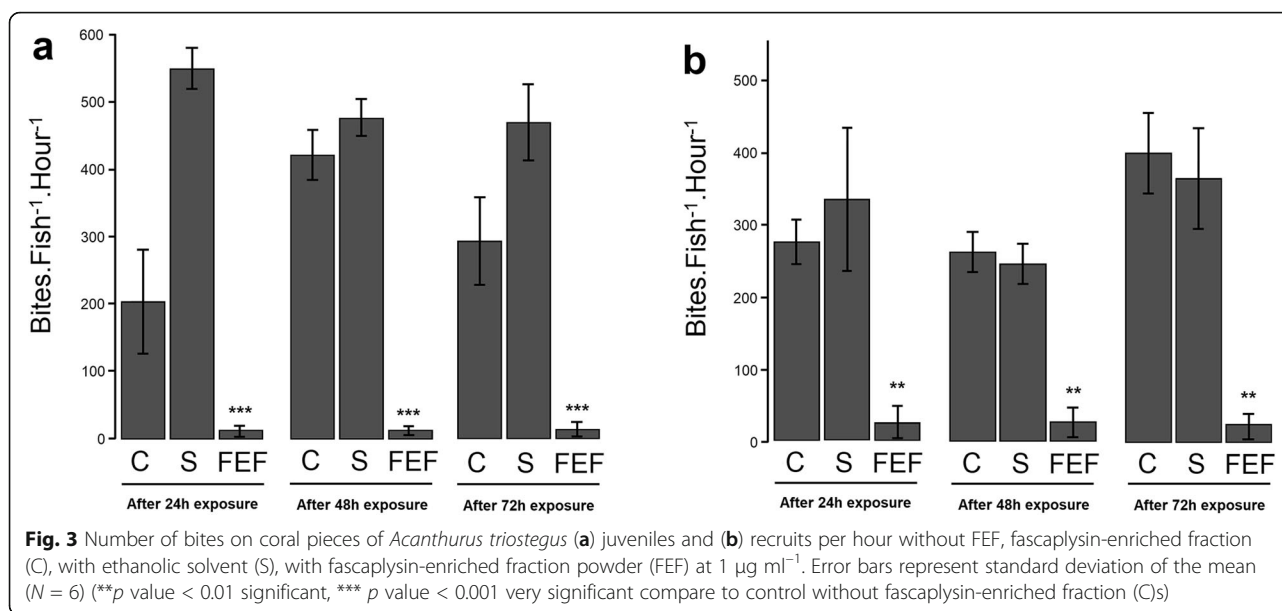
The isolation of palauolide (1) and the major compound faspaplysin (2) from the French Polynesian *F. cf reticulata* extracts is similar to those results obtained by Sullivan and Faulkner (1982) on Palauan sponges.

The QSI potential of the French Polynesian sponge *F. cf reticulata* against the QS-dependent phenotypic expression in *V. harveyi* was demonstrated for the first

time. Palauolide (1) revealed a potential as QSI by inhibiting *V. harveyi* luminescence at  $26 \mu\text{M}$ . In quantitative analysis, palauolide (1) delayed the activation of bioluminescence expression to 50 min of *V. harveyi* BB120. The *V. harveyi* growth rate was also significantly increased ( $p$  value < 0.05). The boosted growth rate of *V. harveyi* with palauolide (1) can be interpreted as a consequence of QS inhibition, because the expression of bioluminescence slows down bacterial growth rate to save energy (Nackerdien et al. 2008). The present data corroborates well with the result obtained previously on the QSI at  $23 \mu\text{M}$  of isonaamidine A isolated from the sponge *Leucetta chagosensis* (Mai et al. 2015). Other studies compared bioluminescence data at a time  $t$ , to determine the inhibition of QS (Brackman et al. 2008; Teasdale et al. 2009; Natrah et al. 2011). For example, Brackman et al. (2008) showed inhibition of *V. harveyi* bioluminescence with cinnamaldehyde and derivatives at  $100 \mu\text{M}$ , 6 h after the addition of compounds (Brackman et al. 2008). Skindersoe et al. (2008) found that manoolide, a compound of similar structure to palauolide (1), inhibits QS at  $\text{IC}_{50} = 0.66 \mu\text{M}$ . The better bioactivity of manoolide compared to palauolide (1) could be explained from the sensitivity of the intracellular bioassay used by authors.

The mode of action of palauolide (1) on the inhibition of QS has potential as an antibiotic alternative in aquaculture for *Vibrio* species. Our bioassay on *V. harveyi* double mutants JAF 375, JMH 597, and JMH 612 highlighted interference of palauolide (1) on *V. harveyi* QS, specifically with the acyl-homoserine lactone: HAI-1. Quorum sensing regulates bioluminescence and virulence factors of bacteria through autoinducers (Henke and Bassler 2004a) such as HAI-1 used for intraspecies communication (Waters and Bassler 2005; Yang et al. 2011). Acyl-homoserine lactone molecules are found in the family *Vibrionaceae* (Yang et al. 2011). Palauolide (1) can therefore interfere with *Vibrio* species QS through HAI-1 pathway and then be used as an antivirulent against *Vibrio* species as antagonist of AIs. Most of antagonists of QS sensors are small molecules (Swem et al. 2008; Gamby et al. 2012) with structural similarities to AIs such as brominated furanone derivatives (Givskov et al. 1996; Rasch et al. 2004; Steenackers et al. 2010). Palauolide (1) is a sesterterpene composed by a  $\delta$ -hydroxybutenolide moiety and a carbon skeleton. The potential of palauolide as a competitor of HAI-1 is most likely due to its small structure and the moderate polarity of its chemical structure. This enables palauolide (1) to cross over the external membrane lipid of bacteria and to bind on the periplasmic sensors Lux N (Swem et al. 2008). Further research would indicate if there is an antagonist effect of palauolide (1) on the HAI-1 sensor, such as testing against additional *V. harveyi* mutants (Swem et al. 2008; Blair and Doucette 2013).





Faspaplysin (2) supplies a broad range of biological activity within *F. cf reticulata*. First, as other β-carboline alkaloids as dysideanin (20 µg) and didemnolines A-D (100 µg), faspaplysin is a strong antibiotic (0.25 µg) (Charan et al. 2002; Hamilton 2014). In the sponge, faspaplysin (2) is the major compound which represents 0.02% of the lyophilized sponge weigh. It exhibits many biological activities including cytotoxicity against tumoral cells (Segraves et al. 2004; Shafiq et al. 2012; Hamilton 2014; Cells et al. 2015; Kumar et al. 2015), antimicrobial activities (Roll et al. 1988), and the inhibition of acetylcholinesterase (Bharate et al. 2012; Manda et al. 2016). For microbial disease treatments in aquaculture, faspaplysin (2) is not ideal. Despite its antibiotic activity against marine pathogens *V. harveyi* (Table 2) and *T. maritimum*, faspaplysin (2) is toxic toward both fresh and saltwater fish, *P. reticulata* and *A. triostegus*, respectively. Indeed, faspaplysin (2) modified fish behavior and displayed an anorexic effect. The AchE inhibition properties of faspaplysin (Bharate et al. 2012) could explain both its toxicity (Bocquené and Galgani 2004; Modesto and Martinez 2010; Assis et al. 2012) and its effect on loss of appetite of fish (Schneider 2000).

The toxicity of palauolide (1) on fish was not tested in this study because previous work highlighted a weaker cytotoxic activity of palauolide (1) compared to faspaplysin (2) (Charan et al. 2002; Hamilton 2014). However, we recommend performing additional toxicity bioassays of palauolide (1) on fish before using it as alternative of antibiotic in fish farming.

### Conclusion

In conclusion, the presence of palauolide (1) and faspaplysin (2) in *F. cf reticulata*, with QS inhibition and antibiotic properties, respectively, could act as complementary where

QSI help and increase antibiotic action on biofilm formation (Brackman et al. 2011). However, the toxicity on fish of the major compound of *F.cf reticulata* faspaplysin (2) (yield 0.02% w/w) prevents the use of the sponge extract in fish farming context. We recommend in future research to test toxicity of the cyclohexanic fraction of the sponge and palauolide (1) on fish before concluding on the potential of the cyclohexanic fraction and palauolide (1) as an alternative to antibiotics in fish farming.

### Acknowledgements

This work is part of TM's PhD thesis. We acknowledge ANR, Netbiome, French Polynesian authorities, and Délégation à la Recherche-France in Tahiti for their grant, help, and support, IRD and GOPS for funding the field trips aboard R/V ALIS, her crew and IRD's diving team for their on-field help, and Laboratoire d'Excellence LabEx CORAIL, 66100 Perpignan, France. We would also like to thank Johanna Loacker and Christina Piotrowski from Calacademy for their help with the loan of some comparative material.

### Author's contribution

CD supervised sponge sampling and TM's PhD thesis. SP supervised the setup of the extract library for biological screening. ME, KH, and DE identified sponge samples. TM and MAB carried out chemical experiments. JT, CD, MB, and DL carried out fish toxicity bioassay, and TM, EA, DS, and CD carried out antibiotic and quorum sensing bioassays. SVW was in charge of statistical analyses. TM wrote the manuscript. All authors reviewed and approved the manuscript.

### Funding

This research was supported by the research grant ANR-11-EBIM-006-1 (Project: POMARE) from ANR and Netbiome, and the grant 113-163 (Project: Biopolyval) from French Polynesian authorities and Délégation à la Recherche-France in Tahiti.

### Availability of data and materials

Not applicable.

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

**Competing interests**

The authors declare that they have no competing of interest.

**Author details**

<sup>1</sup>EIO, ILM, IFREMER, IRD, UPF, BP 6570, 98702 FAA'A, Tahiti, French Polynesia. <sup>2</sup>IRD, Univ. Brest, CNRS, IFREMER, LEMAR, F-29280 Plouzane, France. <sup>3</sup>Institut de Recherche pour le Développement, UMR 9220 ENTROPIE (Institut de Recherche Pour le Développement, Université de la Réunion, Centre National de la Recherche Scientifique), Nouméa, New Caledonia. <sup>4</sup>LEAD-NC, 98848 Nouméa, New Caledonia. <sup>5</sup>PSL Research University: EPHE-UPVD-CNRS, USR 3278 CRIOBE, BP 1013-Moorea, French Polynesia. <sup>6</sup>Observatoire Océanologique de Banyuls-sur-Mer, Université Pierre et Marie Curie Paris, Banyuls-sur-Mer, France. <sup>7</sup>Queensland Museum, PO Box 3300, South Brisbane BC, Queensland 4101, Australia. <sup>8</sup>School of Biological Sciences, University of Queensland, St Lucia, Queensland 4072, Australia. <sup>9</sup>Department of Earth and Environmental Sciences & GeoBio-Center, Ludwig-Maximilians-Universität München, Richard-Wagner Str. 10, 80333 Munich, Germany. <sup>10</sup>Biocis, Equipe "Pharmacognosie-Chimie des substances naturelles", CNRS, Université de Paris Sus, Univ. Paris-Saclay, Faculté de pharmacie, 5 rue J-B Clément, 92293 Châtenay-Malabry cedex, France.

Received: 31 July 2019 Accepted: 26 November 2019

Published online: 19 December 2019

**References**

- Aly SM, Albutti A. Antimicrobials use in aquaculture and their public health impact. *J Aquac Res Dev*. 2014;5(4):1000247. <https://doi.org/10.4172/2155-9546.1000247>.
- Assis CR, Linhares AG, Oliveira VM, França RC, Carvalho EV, Bezerra RG, De Carvalho LB Jr. Comparative effect of pesticides on brain acetylcholinesterase in tropical fish. *Sci Total Environ*. 2012;441:141–50. <https://doi.org/10.1016/j.scitotenv.2012.09.058>.
- Bassler BL, Greenberg EP, Stevens AM. Cross-species induction of luminescence in the quorum-sensing bacterium *Vibrio harveyi*. *J Bacteriol*. 1997;179(12):4043–5.
- Bauer A, Kirby W, Sherris J, Turck M. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol*. 1966;45(4):493–6.
- Bharate SB, Manda S, Joshi P, Singh B, Vishwakarma RA. Total synthesis and anti-cholinesterase activity of marine-derived bis-indole alkaloid faspaplysin. *Med Chem Commun*. 2012;3(9):1098–103. <https://doi.org/10.1039/c2md20076g>.
- Blair JMA, Webber MA, Baylay AJ, Ogbolu DO, Piddock LJV. Molecular mechanisms of antibiotic resistance. *Nat Rev Microbiol*. 2015;13(1):42–51. <https://doi.org/10.1038/nrmicro3380>.
- Blair WM, Doucette GJ. The *Vibrio harveyi* bioassay used routinely to detect AI-2 quorum sensing inhibition is confounded by inconsistent normalization across marine matrices. *J Microbiol Methods*. 2013;92:250–2.
- Blunt JW, Copp BR, Munro MHG, Northcote PT, Prinsep MR. Marine natural products. *Nat. Prod. Rep*. 2005;22:15–61. <https://doi.org/10.1039/b415080p>.
- Bocquené G, Galgani F. Les marqueurs biologiques des effets polluants: l'acétylcholinestérase. Ifremer: France; 2004.
- Brackman G, Cos P, Maes L, Nelis HJ, Coenye T. Quorum sensing inhibitors increase the susceptibility of bacterial biofilms to antibiotics *in vitro* and *in vivo*. *Antimicrob Agents Chemother*. 2011;55(6):2655–61. <https://doi.org/10.1128/AAC.00045-11>.
- Brackman G, Defoirdt T, Miyamoto C, Bossier P, Van Calenberg S, Nelis H, Coenye T. Cinnamaldehyde and cinnamaldehyde derivatives reduce virulence in *Vibrio* spp. by decreasing the DNA-binding activity of the quorum sensing response regulator LuxR. *BMC Microbiol*. 2008;8:149. <https://doi.org/10.1186/1471-2180-8-149>.
- Cells H, Kumar S, Guru SK, Pathania AS, Manda S, Kumar A, Bharate SB, Vishwakarma RA, Malik F. Faspaplysin induces caspase mediated crosstalk between apoptosis and autophagy through the inhibition of PI3K/AKT/mTOR signaling cascade in Human Leukemia HL-60 Cells. 2015; 997(January):985–97. (doi:<https://doi.org/10.1002/jcb.25053>)
- Charan RD, McKee TC, Boyd MR. Thorectandrols C, D, and E, new sesterterpenes from the marine sponge *Thorectandra* sp. *J Nat Prod*. 2002;65(4):492–5. <https://doi.org/10.1021/np010439k>.
- Chen X, Zhang L, Zhang M, Liu H, Lu P, Lin K. Quorum sensing inhibitors: a patent review (2014–2018). *Expert Opin Ther Pat*. 2018;28(12):849–65. <https://doi.org/10.1080/13543776.2018.1541174>.
- Debitus C. TUAM 2011. 2011. <http://dx.doi.org/https://doi.org/10.17600/11100010>.
- Dobretsov S, Teplitski M, Bayer M, Gunasekera S, Proksch P, Paul VJ. Inhibition of marine biofouling by bacterial quorum sensing inhibitors. *Biofouling*. 2011; 27(8):893–905. <https://doi.org/10.1080/08927014.2011.609616>.
- Editorials. The antibiotic alarm. *Nature*. 2013;495:141.
- El-Demerdash A, Moriou C, Toullec J, Besson M, Soulet S, Schmitt N, Petek S, Lecchini D, Debitus C, Al-Mourabit A. Bioactive bromotyrosine-derived alkaloids from the Polynesian sponge *Suberea ianthelliformis*. *Mar Drugs*. 2018;16(5):146. <https://doi.org/10.3390/md16050146>.
- Feng DQ, Qiu Y, Wang W, Wang X, Ouyang PG, Ke CH. Antifouling activities of hymenialdisine and debromohymenialdisine from the sponge *Axinella* sp. *Int Biodeterior Biodegrad*. 2013;85:359–64. <https://doi.org/10.1016/j.ibiod.2013.08.014>.
- Freeman J, Bassler L. A genetic analysis of the function of LuxO, a two-component response regulator involved in quorum sensing in *Vibrio harveyi*. *Mol Microbiol*. 1999;31(2):665–77.
- Gamby S, Roy V, Guo M, Smith JAI, Wang J, Stewart JE, Wang X, Bentley WE, Sintim HO. Altering the communication networks of multispecies microbial systems using a diverse toolbox of AI-2 analogues. *ACS Chem. Biol*. 2012;7(6):1023–30. <https://doi.org/10.1021/cb200524y>.
- Givskov M, DE Nys R, Manefield M, Gram L, Maximilien R, Eberl L, Molin S, Steinberg PD, Kjelleberg S. Eukaryotic interference with homoserine lactone-mediated prokaryotic signalling. *J Bacteriol*. 1996;178(22):6618–22.
- Hamilton G. Cytotoxic effects of faspaplysin against small cell lung cancer cell lines. *Mar Drugs*. 2014;12:1377–89. <https://doi.org/10.3390/md12031377>.
- Hatosy S, Martiny A. The ocean as an antibiotic resistance gene reservoir. *Appl Environ Microbiol*. 2015;81:7593–9. <https://doi.org/10.1128/AEM.00736-15>.
- Henke JM, Bassler BL. Three parallel quorum-sensing systems regulate gene expression in *Vibrio harveyi*. *J Bacteriol*. 2004a;186(20):6902–14. <https://doi.org/10.1128/JB.186.20.6902>.
- Henke JM, Bassler BL. Quorum sensing regulates type III secretion in *Vibrio harveyi* and *Vibrio parahaemolyticus*. *J Bacteriol*. 2004b;186(12):3794–805. <https://doi.org/10.1128/JB.186.12.3794>.
- Johnson FH, Shunk IV. An interesting new species of luminous bacteria. *J Bacteriol*. 1936;31(6):585–93.
- Kalia VC. Quorum sensing inhibitors: an overview. *Biotechnol Adv*. 2013;31(2):224–45. <https://doi.org/10.1016/j.biotechadv.2012.10.004>.
- Karunasagar I, Pai R, Malathi GR, Karunasagar I. Mass mortality of *Penaeus monodon* larvae due to antibiotic-resistant *Vibrio harveyi* infection. 1994; 128(3–4):203–9. (doi:[https://doi.org/10.1016/0044-8486\(94\)90309-3](https://doi.org/10.1016/0044-8486(94)90309-3))
- Kingsland S. The refractory model: the logistic curve and the history of population ecology. *Quarterly Rev Biol*. 1982;57:29–52.
- Kumar S, Guru SK, Pathania AS, Manda S, Kumar A, Bharate SB, Vishwakarma RA, Malik F, Bhushan S. Faspaplysin induces caspase mediated crosstalk between apoptosis and autophagy through the inhibition of PI3K/AKT/mTOR signaling cascade in Human Leukemia HL-60 Cells. *J Cell Biochem*. 2015;116(6):985–97. <https://doi.org/10.1002/jcb.25053>.
- Lecchini D, Dufour V, Carleton J, Strand S, Galzin A. Estimating the patch size of larval fishes during colonization on coral reefs. *J Fish Biol*. 2004;65:1142–6. <https://doi.org/10.1111/j.1095-8649.2004.00493.x>.
- Lulijwa R, Rupia EJ, Alfaro AC. Antibiotic use in aquaculture, policies and regulation, health and environmental risks: a review of the top 15 major producers. *Rev Aquac*. 2019;In press:1–24. (doi:<https://doi.org/10.1111/raq.12344>)
- Mai T, Tintillier F, Lucasson A, Moriou C, Bonno E, Petek S, Magre K, Al Mourabit A, Saulnier D, Debitus C. Quorum sensing inhibitors from *Leuconia chagosensis* Dendy, 1863. *Lett Appl Microbiol*. 2015;61:311–7. <https://doi.org/10.1111/lam.12461>.
- Manda S, Sharma S, Wani A, Joshi P, Kumar V, Guru SK, Bharate SS, Bhushan S, Vishwakarma RA, Kumar A, Bharate SB. Discovery of a marine-derived bis-indole alkaloid faspaplysin, as a new class of potent P-glycoprotein inducer and establishment of its structure-activity relationship. *Eur J Med Chem*. 2016;107:1–11. <https://doi.org/10.1016/j.ejmech.2015.10.049>.
- Manefield M, Harris L, Rice SA, De Nys R, Kjelleberg S. Inhibition of luminescence and virulence in the black tiger prawn (*Penaeus monodon*) pathogen *Vibrio harveyi* by intercellular signal antagonists. *Appl Environ Microbiol*. 2000;66(5):2079–84. <https://doi.org/10.1128/AEM.66.5.2079-2084.2000>. Updated.
- Martinez JL. Environmental pollution by antibiotics and by antibiotic resistance determinants. *Environ Pollut*. 2009;157(11):2893–902. <https://doi.org/10.1016/j.envpol.2009.05.051>.
- Modesto KA, Martinez CBR. Roundup causes oxidative stress in liver and inhibits acetylcholinesterase in muscle and brain of the fish *Prochilodus*

- lineatus*. Chemosphere. 2010;78(3):294–9. <https://doi.org/10.1016/j.chemosphere.2009.10.047>.
- Moloney MG. Natural products as a source for novel antibiotics. Trends Pharmacol Sci. 2016;37(8):689–701. <https://doi.org/10.1016/j.tips.2016.05.001>.
- Müller WEG, Wang X, Proksch P, Perry CC, Osinga R, Gardères J, Schröder HC. Principles of biofouling protection in marine sponges: a model for the design of novel biomimetic and bio-inspired coatings in the marine environment? Mar Biotechnol (NY). 2013;15(4):375–98. <https://doi.org/10.1007/s10126-013-9497-0>.
- Nackerdien ZE, Keynan A, Bassler BL, Lederberg J, Thaler DS. Quorum sensing influences *Vibrio harveyi* growth rates in a manner not fully accounted for by the marker effect of bioluminescence. PLoS One. 2008;3(2):e1671. <https://doi.org/10.1371/journal.pone.0001671>.
- Natrath FMI, Kenmegne MM, Wiyoto W, Sorgeloos P, Bossier P, Defoirdt T. Effects of micro-algae commonly used in aquaculture on acyl-homoserine lactone quorum sensing. 2011;317(1–4):53–7. (doi:<https://doi.org/10.1016/j.aquaculture.2011.04.038>)
- Pande GSJ, Scheie AA, Benneche T, Wille M, Sorgeloos P, Bossier P, Defoirdt T. Quorum sensing-disrupting compounds protect larvae of the giant freshwater prawn *Macrobrachium rosenbergii* from *Vibrio harveyi* infection. 2013;406–407(July):121–4. (doi:<https://doi.org/10.1016/j.aquaculture.2013.05.015>)
- Pérez-Sánchez T, Mora-Sánchez B, Balcázar JL. Biological approaches for disease control in aquaculture: advantages, limitations and challenges. Trends Microbiol. 2018;26(11):896–903. <https://doi.org/10.1016/j.tim.2018.05.002>.
- Peters L, Ko GM, Wright AD, Stackebrandt E, Eberl L, Riedel K. Secondary metabolites of *Flustra foliacea* and their influence on bacteria. Appl Environ Microbiol. 2003;69(6):3469–75. <https://doi.org/10.1128/AEM.69.6.3469>.
- Quévrain E, Domart-Coulon I, Bourguet-Kondracki ML. Chemical defense/chemical communication in sponges and corals. In: Osbourn A, Goss RJ, Carter GT, editors. Natural Products; 2014. p. 39–66. <https://doi.org/10.1002/9781118794623.ch3>.
- Rasch M, Buch C, Austin B, Slierendrecht WJ, Ekmann KS, Larsen JL, Johansen C, Riedel K, Eberl L, Givskov M, Gram L. An inhibitor of bacterial quorum sensing reduces mortalities caused by Vibriosis in rainbow trout (*Oncorhynchus mykiss*, Walbaum). Syst Appl Microbiol. 2004;27(3):350–9. <https://doi.org/10.1078/0723-2020-00268>.
- Reverter M, Saulnier D, David R, Bardon-Albaret A, Belliard C, Tapissier-Bontemps N, Lecchini D, Sasal P. Fish & Shell fish immunology effects of local Polynesian plants and algae on growth and expression of two immune-related genes in orbicular bat fish (*Platax orbicularis*). Fish Shellfish Immunol. 2016;58:82–8. <https://doi.org/10.1016/j.fsi.2016.09.011>.
- Roll DM, Ireland CM, Lu HSM, Clardy J. Fascaplysin, an unusual antimicrobial pigment from the marine sponge *Fascaplysinopsis* sp. J Org Chem. 1988; 53(14):3276–8. <https://doi.org/10.1021/jo00249a025>.
- Saurav K, Costantino V, Venturi V, Steindler L. Quorum sensing inhibitors from the sea discovered using bacterial N -acyl-homoserine. Mar Drugs. 2017;15:53. <https://doi.org/10.3390/md15030053>.
- Schluter J, Schoech AP, Foster KR, Mitri S. The evolution of quorum sensing as a mechanism to infer kinship. PLoS Comput Biol. 2016;12(4):e1004848. <https://doi.org/10.1371/journal.pcbi.1004848>.
- Schneider L. A critical review of cholinesterase inhibitors as a treatment modality in Alzheimer's disease. Dialogues Clin Neurosci. 2000;2(2):111–28.
- Segraves NL, Robinson SJ, Garcia D, Said SA, Fu X, Schmitz FJ, Pietraszkiewicz H, Valeriote FA, Crews P. Comparison of fascaplysin and related alkaloids : a study of structures, cytotoxicities, and sources. J Nat Prod. 2004;67:783–92. <https://doi.org/10.1021/hp049935+>.
- Shafiq MI, Steinbrecher T, Schmid R. Fascaplysin as a specific inhibitor for CDK4: insights from molecular modelling. PLoS One. 2012;7(8):e42612. <https://doi.org/10.1371/journal.pone.0042612>.
- Shah SQA, Cabello FC, L'Abée-lund TM, Tomova A, Godfrey HP, Buschmann AH, Sørum H. Antimicrobial resistance and antimicrobial resistance genes in marine bacteria from salmon aquaculture and non-aquaculture sites. Environ Microbiol. 2014;16:1310–20. <https://doi.org/10.1111/1462-2920.12421>.
- Shrestha P, Cooper BS, Coast J, Oppong R, Do Thi Thuy N, Phodha T, Cellhay O, Guerin PJ, Wertheim H, Lubell Y. Enumerating the economic cost of antimicrobial resistance per antibiotic consumed to inform the evaluation of interventions affecting their use. Antimicrob Resist Infect Control. 2018;7(98): 1–9. <https://doi.org/10.1186/s13756-018-0384-3>.
- Skindersoe ME, Ettinger-Epstein P, Rasmussen TB, Bjarnsholt T, de Nys R, Givskov M. Quorum sensing antagonism from marine organisms. Mar Biotechnol (NY). 2008;10(1):56–63. <https://doi.org/10.1007/s10126-007-9036-y>.
- Spellberg B, Gilbert DN. The future of antibiotics and resistance: a tribute to a career of leadership by John Bartlett. Clin Infect Dis. 2014;59:S71–5. <https://doi.org/10.1093/cid/ciu392>.
- Steenackers HP, Levin J, Janssens JC, De Weerd A, Balzarini J, Vanderleyden J, De Vos DE, De Keersmaecker SC. Structure-activity relationship of brominated 3-alkyl-5-methylene-2(5H)-furanones and alkylmaleic anhydrides as inhibitors of *Salmonella* biofilm formation and quorum sensing regulated bioluminescence in *Vibrio harveyi*. Bioorg Med Chem. 2010;18(14):5224–33. <https://doi.org/10.1016/j.bmc.2010.05.055>.
- Sullivan B, Faulkner DJ. An antibacterial sesterterpene from a palauan sponge. Tetrahedron Lett. 1982;23:907–10. <https://doi.org/10.1017/CBO9781107415324.004>.
- Swem LR, Swem DL, Wingreen NS, Bassler BL. Deducing receptor signaling parameters from *in vivo* analysis: LuxN/AI-1 quorum sensing in *Vibrio harveyi*. Cell. 2008;134(3):461–73. <https://doi.org/10.1016/j.cell.2008.06.023>.
- Teasdale ME, Liu J, Wallace J, Akhlaghi F, Rowley DC. Secondary metabolites produced by the marine bacterium *Halobacillus salinus* that inhibit quorum sensing-controlled phenotypes in gram-negative bacteria. Appl Environ Microbiol. 2009;75(3):567–72. <https://doi.org/10.1128/AEM.00632-08>.
- Tello E, Castellanos L, Duque C. Synthesis of cembranoid analogues and evaluation of their potential as quorum sensing inhibitors. Bioorg Med Chem. 2013;21(1):242–56. <https://doi.org/10.1016/j.bmc.2012.10.022>.
- Thomas T, Moitinho-silva L, Lurgi M, Bjo JR, Easson C, Astudillo-garci C, Olson JB, Erwin PM, Lopez S, Luter H, Chaves-fonnegra A, Costa R, Schupp PJ, Steindler L, Erpenbeck D. Diversity, structure and convergent evolution of the global sponge microbiome. Nat Commun. 2016;7:11870. <https://doi.org/10.1038/ncomms11870>.
- Waters CM, Bassler BL. Quorum sensing cell-to-cell communication in bacteria. Annu Rev Cell Dev Biol. 2005;21:319–46. <https://doi.org/10.1146/annurev.cellbio.21.012704.131001>.
- Wester PW, Vos JG. Toxicological pathology in laboratory fish : an evaluation with two species and various environmental contaminants. 1994;3(1):21–44. (doi: <https://doi.org/10.1007/BF00121386>)
- WHO, FAO, OIE. Antimicrobial use in aquaculture and antimicrobial resistance. Geneva: WHO press; 2006.
- Yang Q, Han Y, Zhang X-H. Detection of quorum sensing signal molecules in the family Vibrionaceae. J Appl Microbiol. 2011;110(6):1438–48. <https://doi.org/10.1111/j.1365-2672.2011.04998.x>.

## Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

**Ready to submit your research? Choose BMC and benefit from:**

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

**At BMC, research is always in progress.**

Learn more [biomedcentral.com/submissions](https://www.biomedcentral.com/submissions)

