Egyptian Journal of Aquatic Research (2015) **41**, 49–56

HOSTED BY

ELSEVIER

National Institute of Oceanography and Fisheries

Egyptian Journal of Aquatic Research

http://ees.elsevier.com/ejar
www.sciencedirect.com

FULL LENGTH ARTICLE

Bioactive phthalate from marine *Streptomyces ruber* EKH2 against virulent fish pathogens

Khouloud Mohamed Barakat *, **Ehab Aly Beltagy**

Microbiology Lab., Marine Environment Division, National Institute of Oceanography and Fisheries, El-Anfoushy, Qayet Bay, Alexandria, Egypt

Received 21 January 2015; revised 4 March 2015; accepted 4 March 2015
Available online 3 April 2015

KEYWORDS

Phthalate;
Marine *Streptomyces ruber* EKH2;
Fish pathogens;
GC–MS;
Toxicity

Abstract Marine *Streptomyces ruber* EKH2 isolated from sediments of Bardawil Lake, Egypt, was found as a promising strain for producing bioactive metabolite(s) working against some virulent fish pathogens. Some biochemical and morphological characterizations of marine *S. ruber* EKH2 were carried out. Cell free culture showed activities against the tested pathogens ranging from 15 to 30 mm. Optimized conditions for maximum activities were observed at neutrality and temperature 28 °C against the tested strains. Two grams of the ethyl acetate crude extract from 10 L culture supernatant was chromatographically separated into three fractions and bioassayed. One major antibacterial compound was separated exhibiting MIC average 12.5 μg/ml. Phthalic acid was structurally suggested on the basis of gas chromatography–mass spectrum (GC–MS) and infrared spectrum (IR). Phthalate activities were compared with known standard antibiotics used in fish therapy and found to be superior. A slight toxicity of phthalate against brine shrimp (LC_{50} = 2800 μg/ml) was observed. Dealing with pan-drug resistant bacteria in fish therapy, this study confirmed that marine *S. ruber* EKH2 is potentially used for extracting phthalic acid as a novel bioactive and non-toxic agent for treating bacterial fish infections.

© 2015 National Institute of Oceanography and Fisheries. Hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Introduction

Over the last decades, world aquaculture industry has obviously grown. This development has been represented by a transition that involves a lot of work in farming methods which encourages an increased profitability (Food, 2012). Facing the fact, an increased threat of many diseases caused by a vast number of virulent microbes resulted in considerable economical losses. In order to develop rapid diagnostic tests and effective disease precautions, all research activities have been carried out with prevention strategies (Frans et al., 2013).

Recently, new therapeutic agents have entered the clinical area, with side effects (Rajan and Kannabiran, 2014). Side effects of existing drugs and drug resistance have become serious public health problems for both human and animals which require the development of new antimicrobial agents (Urban et al., 2003). Many scientists are working on new antimicrobial drugs, mainly of actinomicetal origin (Oskay et al., 2004; Paterson et al., 2004), where unique chemical structures obtained from actinomycetes are considered as

* Corresponding author. Tel.: +20 3 4801553; fax: +20 3 4801174.
E-mail address: kh2m2@yahoo.com (K.M. Barakat).

Peer review under responsibility of National Institute of Oceanography and Fisheries.

http://dx.doi.org/10.1016/j.ejar.2015.03.006

1687-4285 © 2015 National Institute of Oceanography and Fisheries. Hosting by Elsevier B.V.
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

antimicrobial, anti-parasitic, antiviral, antitumor activities and cytotoxic metabolites (Kekuda et al., 2010). Until recently, actinomycetes isolated from marine sediments, as the least explored resources, showed to be a source of bioactive compounds production; however, today it became one of the more promising sources.

Species of *Streptomyces* as versatile producers of new secondary metabolites from different biosynthetic pathways, originate from different ecological niches and could be used to hunt for novel bioactive compounds (You et al., 2013). Marine ecosystems with a large diversity of actinomycetes have the power of producing bioactive secondary metabolites (Olano et al., 2009a). Regarding secondary metabolic production from microorganisms isolated from the deep sea, it has been found to remain normally dormant or weakly expressed under laboratory conditions, which was critical for metabolite development prior to extensive chemical investigation (Ochi and Hosaka, 2013).

Bioactive compounds from marine actinomycetes of unique structure and obvious effect have been obtained; however, how to tap these treasurous compounds? (Li et al., 2013). Olano et al. (2009b) suggested that *Streptomyces* isolates from marine sediments are valuable for the production of antibiotics. Compounds synthesized by most *Streptomyces* species with broad spectrum features, including antibiotics, pesticides, herbicides, enzyme inhibitors and anti parasitic, are more importance to tend healthily and commercially effective drugs, and occupy approximately one third of the known isolated metabolites from *Streptomyces* (Balagurunathan et al., 2010).

Therefore, the purpose of this study is to evaluate, *in vitro*, the antagonistic activity of a bioactive metabolite from marine *Streptomyces ruber* EKH2 against some virulent fish pathogens. Moreover, the study would extend to separate, analyze and characterize the crude extract using thin layer chromatography, and be identified by gas chromatography–mass spectral (GC–MS) and infrared spectroscopic analysis.

Materials and methods

Isolation and characterization of S. ruber ekh2

Marine *S. ruber* EKH2 was isolated from Bardawil Lake sediment samples, Sinai Peninsula, Egypt, and identified by Xcelris Genomics labs in India. Luria–Bertani medium (LB), used for isolation, consisted of (g/l sea water); tryptone, 10; yeast extract, 5 and sodium chloride, 10, agar 20; pH adjusted at 7 and incubation temperature at 30 °C (Kandpal et al., 2012). Microscopic and morphological examinations were noted with respect to type of cells, aerial mycelium color, nature of colony and reverse side plate color.

Fish pathogens

The fish pathogens including *Aeromonas hydrophila*, *Edwardsiella tarda, Pseudomonas aeruginosa* and *Vibrio ordalii* employed for *in vitro* antimicrobial assay were kindly provided from the Department of Poultry and Fish Diseases, Faculty of Veterinary Medicine, Alexandria University. The tested pathogens were stored in glycerol at −20 °C.

Fermentation condition and Bioactivity

One hundred milliliter of the production LB broth medium was inoculated by 2×10^7 CFU/ml spores suspension. Flasks were kept for 5 days at 120 rpm shaker speed and 30 °C (Deepika and Kannabiran, 2010). Fermented medium was collected and centrifuged from cell debris for further use as the crude extract.

Bioactivities were screened using disc diffusion method on tryptic soya agar (TSA) (Bauer et al.,1966). Twenty-five milliliter of sterilized TSA medium were mixed with 400 µl of over- night culture of tested organism (10^4 CFU/ml), then poured into three sterile Petri-dishes, as replicates, and allowed to solidify. Sterile plain discs (5 mm) were immersed in free cell filtrate of the cultivated *S. ruber* EKH2, and placed on the prepared plates then incubated at 30 °C overnight.

Extraction of bioactive compounds

For the extraction of the bioactive metabolites from fermented broth, different organic solvents (50 ml each) from polar (butanol, diethyl ether and ethyl acetate) to non-polar (benzene, hexane and petroleum ether) were tried for product recovery from 50 ml fermented broth in a 250 ml separating funnel. The mixture was shaken vigorously for 20 min and kept in stationary condition for another 20 min to separate the solvent from aqueous phase (Atta and Ahmad, 2009).

Minimal inhibitory concentrations (MIC) of the ethyl acetate extracted compound(s) were determined according to Richard et al. (2007). For maximum conditional activity, MIC was recorded at different temperatures (28, 37 and 45 °C) and pHs (6, 7, 8) in three replicates using representative response surface plot curves, STATISTICA (ver. 8, ed. 2006).

Thin layer chromatography

The culture broth (10 L) was extracted with ethyl acetate (1:1 v/v) stepwise and concentrated by rotary evaporator at 50 °C to yield 2 g of brown crude residue. The residue was chromatographed on silica gel preparative slides using different solvent systems: acetone, hexane and ethyl acetate, separately, or combined solvent systems; acetone:ethyl acetate (1:2), acetone:ethyl acetate. (2:1), hexane: ethyl acetate (1:2), and hexane: ethyl acetate (2:1). The starting crude spots were observed for migration and separation by the previously prepared mobile phases. R_f values of the obtaining colored and non-colored spots with the aid of visible and UV lamps were recorded (Usha et al., 2010). Using silica gel plates (20×20 cm dimensions and 0.50 mm thickness of 60GF254 fine grade), the active bands were gathered, dissolved in ethyl acetate and concentrated to dryness in the vacuum. Bioactivity of the selected bands was examined against the four pathogens using the broth dilution bioassay.

Spectral analysis

Gas chromatography–mass spectrum

The active fraction was analyzed using the SHIMADZU GC–MS-QP5050A with programm CLASS 5000 in the Central Lab of the Higher Institute of Public Health.

Identification was performed using WILEY MASS SPECTRAL DATA BASE Library (Aravamuthan et al., 2010).

Infrared

One mg sample of extracted crude was subjected to IR-spectral analyses using Infrared Spectrophotometer (Perkin Elmer, Spectrum). Mid IR region of 400–4000 cm^{-1} was used for sample analysis. A mixture of spectroscopic pure KBr was in the ratio of 5:95; pellets were fixed in sample holder.

Antibiogram

Fish pathogens were screened for their sensitivity towards standard antibiotics used in fish therapy including amikacin (AN, 30 µg/ml), ciprofloxacin (CIP, 30 µg/ml), florfenicol (KF, 50 µg/ml) and rifamycin (RD, 50 µg/ml). MIC values of these antibiotics compared with the active fraction were performed using serial dilution method. 0.5 McFarland standards were used to adjust spectrophotometric concentrations of the tested pathogens (Zhang et al., 2012).

Biotoxicity

The biomarker brine shrimp *''Artemia salina''* was used for toxicity assay according to Meyer et al. (1982). Two hundred mg of the active fraction was dissolved in 2 ml dimethylsulfoxide (DMSO). In glass vials, varied crude concentrations (10, 20, 50, 100, 150, 200, 400 and 600 µg/ml) were added to 20 ml of sterilized brackish water. Ten *A. salina* nauplii were transferred to each vial. Biomarker viable count was recorded after 24 h of application. Using probit analysis method, the mortality percentage and half lethal concentration (LC50) of examined nauplii were determined (Reish and Oshida, 1987).

Results and discussion

S. ruber EKH2 isolated from marine sediment was found to produce active phthalate for treating bacterial fish infections. Culture free cell bioassay and optimized conditions for maximum activities were determined against the tested pathogens. Phthalate as the major active and non-toxic compound was separated and structurally suggested using GC–MS and IR spectral analysis.

Characterization of S. ruber EKH2 and bioassay

S. ruber EKH2 was morphologically examined with respect to the color of aerial mycelium, nature of colony and reverse side color; and biochemically by its assimilation of various carbon sources as shown in Table 1. Spore surface and chain morphology are shown in Fig. 1 using scanning electron microscope.

Cell free culture showed broad spectrum activities against *A. hydrophila, E. tarda P. aeruginosa* and *V. ordalii*; 30, 27, 22 and 15 mm, respectively, (Fig. 2). Compared to our results, a marine *Streptomyces* strain showed the highest activities 26, 23 and 28 mm against *P. aeruginosa*, *Staphylococcus aureus and Escherichia coli,* respectively (Nandhini et al., 2013); whereas, the bioactive metabolite produced by *Streptomyces aureofaciens* showed antibacterial activity ranging 10–12 mm for *Staphylococcus* and 13–15 mm for *Pseudomonas* spp. (Vennila and Krishnaveni, 2013). Marine *Lactobacillus murinus* AU06 showed broad spectrum activities against some fish pathogens with ranges of 24 mm for *S. aureus*, 22 mm for *P. aeruginosa,* 18 mm for *E. coli* but no activity for *Vibrio* sp. (Elayaraja et al., 2014).

Table 1 Morphological and biochemical characteristics of *S. ruber* EKH2.

Characteristics		Utilization of sole carbon sources	
Color of aerial mycelium	Greenish-white	Fructose	+
Reverse color	Faint cream	Inositol	+
Diffusible pigment	Pale yellow	Mannitol	+
Spore chain morphology	Spiral	Sucrose	+
Spore surface	Smooth	Xylose	+

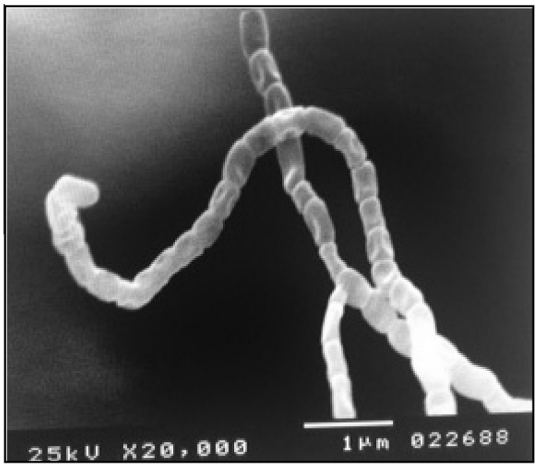

Figure 1 Scanning electron micrograph of *S. ruber* EKH2 showing smooth spore surface. The bar represents 1 µm.

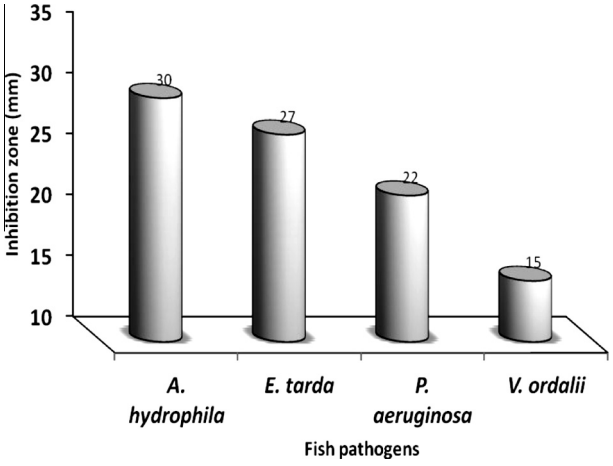

Figure 2 Antimicrobial activities of cell free culture *S. ruber* EKH2, against some bacterial fish pathogens.

Extraction and optimized conditions

The best solvent for extraction process was ethyl acetate showing the highest values of inhibition zone at 35, 30, 28 and 12 mm against *A. hydrophila*, *E. tarda*, *P. aeruginosa* and *V. ordalii*, respectively. Other polar and non-polar solvents showed different tendencies for the antimicrobial activity against the four tested pathogens (Table 2). Anusha et al. (2014) reported that the ethyl acetate extracts from herbs showed the maximum antibacterial activity of 18 mm against the virulent fish pathogenic strain *A. hydrophila* CMST. Therefore, ethyl acetate solvent was selected for further testing.

Optimum pH and temperature were recorded at 7 and 28 °C showing the highest value of MIC at 4, 12, 24 and 48 μg/ml against *A. hydrophila, E. tarda, P. aeruginosa* and *V. ordalii*, respectively (Table 3). The interaction of the initial pH values and incubation temperatures was graphically illustrated in Fig. 3 using response surface plot.

Different pHs indicated the tendency of the bioactive metabolite production to increase in acidic rather than basic condition against the four tested pathogens. The maximum bioactivity was lower than 13.7 μg/ml against *A. hydrophila* within 25 and 30 °C. Moreover, *S. ruber* EKH2 bioactivities recorded as 18.5 and 27.9 μg/ml against *E. tarda* and *P. aeruginosa* respectively, revealed a wide tolerance of temperature above 30 °C. On the other hand, the lower activity against *V. ordalii* (38.0 μg/ml) was reported below 30 °C. In comparison to our results, initial pH and temperature of the medium appropriate to induce *Streptomyces philanthi* RM-1-138 for the highest antifungal activity were pH 7.5 and 30 °C (Boukaew and Prasertsan, 2014). Rajan and Kannabiran (2014) showed the highest activity (8 μg/ml) of marine *Streptomyces* sp. VITBRK2 at 28 °C and pH 7.4. On the other hand, *Streptomyces bangladeshiensis* activity was stable within alkaline pH 8.5 medium at 32.5 °C (Al-Bari et al., 2006). The optimum temperature for the *Streptomyces* strain varied from 30 to 40 °C with maximum productivity of 6.2 μg/ml and pH range from 5 to 9 (Nandhini and Selvam, 2013).

Table 2 Bioactivity showing the appropriate solvent(s) for extraction of the active agents.

Solvents	Inhibition zone diameter (mm)			
	A. hydrophila	*E. tarda*	*P. aeruginosa*	*V. ordalii*
Butanol	10	8	9	0
Diethyl ether	17	21	15	0
Ethyl acetate*	**35**	**30**	**28**	**12**
Benzene	14	15	17	0
Hexane	11	12	0	0
Petroleum ether	24	25	20	8

* Potent solvent for extraction.

Table 3 Effect of different pHs and temperatures on the bioactivity of S. *ruber* EKH2, against the four tested pathogens.

Parameters		MIC (μg/ml)			
Temp. (°C)	pH	*Tested pathogens*			
		A. hydrophila	*E. tarda*	*P. aeruginosa*	*V. ordalii*
28	6	12	24	48	64
28	7*	**4**	**8**	**12**	**48**
28	8	32	32	48	124
37	6	24	32	64	64
37	7	32	16	24	48
37	8	32	48	64	64
45	6	64	64	64	128
45	7	64	64	64	64
45	8	64	64	128	128

* Optimized conditions for bioactivity.

Bioassay of partially purified extracts

Two grams of ethyl acetate crude extract was obtained from 10 L of fermented broth. One mg of crude was partially purified through preparative silica gel plate using solvent system composed of acetone: ethyl acetate (1:2). Three bands were observed having different R_f values; band (1) 0.96 with brown color, while, bands (2) and (3) 0.68 and 0.45, respectively, with UV-Transilluminator. Fractions from TLC plate were collected, weighed and resuspended in ethyl acetate for disc diffusion assay. Significant activities were observed against *A. hydrophila* as the most sensitive pathogens (Fig. 4); while, low or no activities for the other three tested organisms. Similarly, the partially purified crude extract from marine *Acinetobacter calcoaceticus* showed significant inhibition zones against some fish pathogens such as *A. hydrophila, V. alginolyticus, V. parahaemolyticus* and lower activity against *V. harveyi* and *P. fluorescens* (Gopi et al., 2012).

A. hydrophila was the most sensitive stain showing MIC 4 μg/ml using the ethyl acetate crude extract. This was followed by 16 μg/ml as moderate sensitivity toward *E. tarda* and *P. aeruginosa*. The most resistant pathogen was *V. ordalii*. Fraction 1, considered the major compound within the extracted crude, showed the highest activities against the target pathogens (2, 8, 8 and 32 μg/ml, *A. hydrophila*, *E. tarda* and *P aeruginosa* and *V. ordalii,* respectively) compared to the other two separated fractions (Table 4). Similarly, extractable metabolite from marine *Streptomyces* sp. Merv8102 showed antibacterial activities with MICAVG of 2–8 μg/ml against Gram-positive and Gram-negative bacteria respectively (El-Gendy et al., 2008). Arasu et al. (2013), recorded different MIC values from marine *Streptomyces* sp. AP-123 against *Bacillus subtilis*, *E. coli, P. aeruginosa* and *S. aureus* were 25 and 37.5 of 50 and 37.58 μg/ml, respectively. Another marine *Streptomyces sundarbansensis* showed selective activity against methicillin-resistant *S. aureus* (MIC = 24 μg/ml) (Djinni et al., 2013). Extractable compounds from *S. bangladeshiensis* showed different MICs against *B. subtilis* and *Salmonella typhi* (16 μg/ml), *S. aureus* and *Shigella dysenteriae* (32 μg/ml) (Al-Bari et al., 2006). Marine extractable product from *Streptomyces* sp. showed its highest activities against MRSA 32 μg/ml (Kumar and Rao, 2012). Bioactive compounds produced by marine *Streptomyces* strain showed maximum MIC 16 μg/ml against *Klebsiella pneumonia*, *E. coli* and *P. aeruginosa*, 32 μg/ml against *S. aureus* and 64 μg/ml against *B. subtilis* (Nandhini and Selvam, 2013).

Spectral analysis

Spectral analyses for the potent compound using GC–MS; GC showed compound with retention time at 21 min 83 s and with

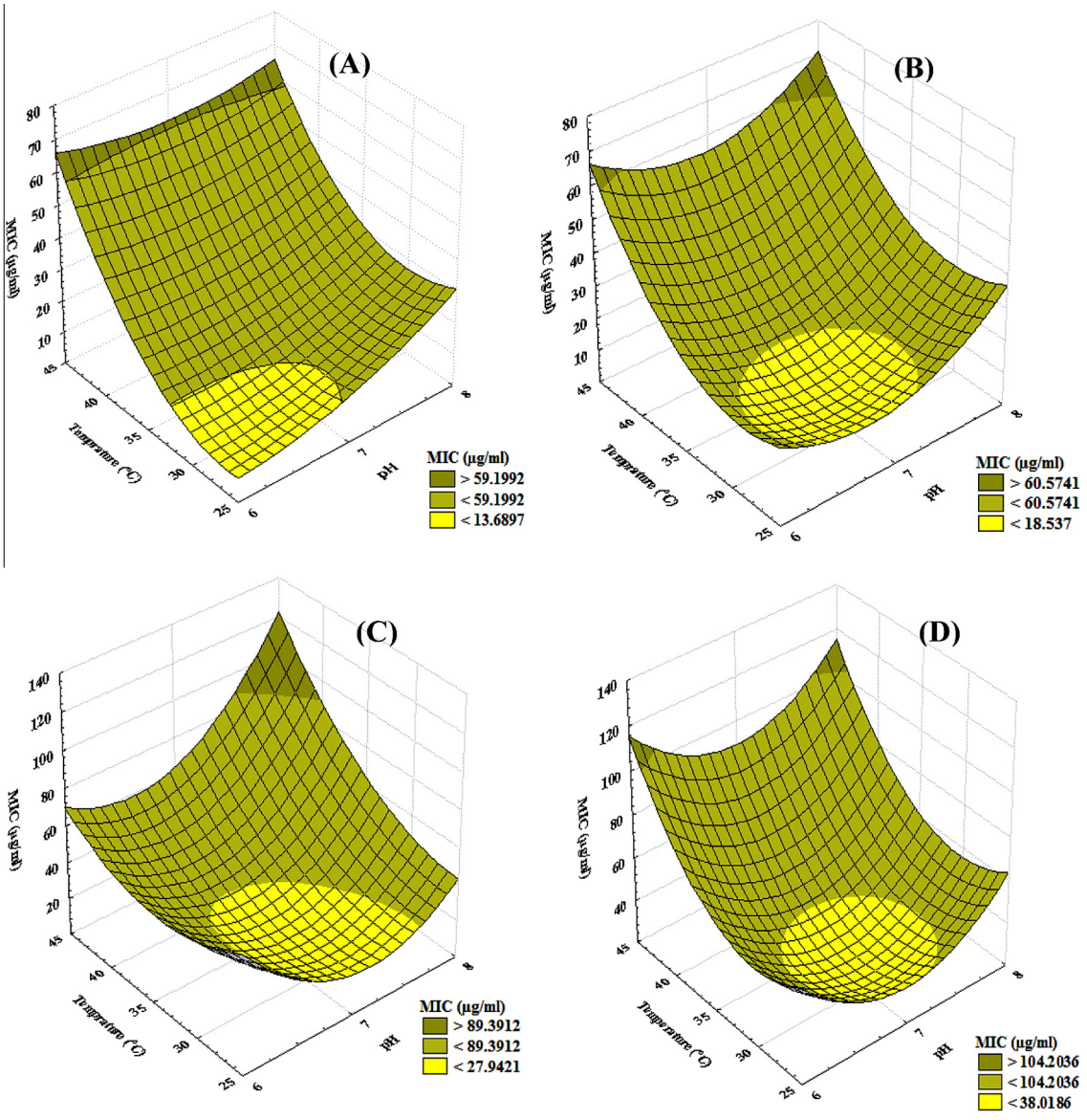

Figure 3 Effect of the interaction between pH and temperature (°C) on the bioactivity of *S. ruber* EKH2 against *Aeromonas hydrophila* (A), *Edwardsiella tarda* (B), *Pseudomonas aeruginosa* (C) and *Vibrio ordalii* (D) using response surface plot.

relative abundance of 91% (Fig. 5a). Mass spectral analysis suggested MS at $m = z$ 149 of compound. It was represented in this data that this compound corresponds to a molecular formula of C24H38O4, a dibasic phthalic acid (1,2-benzenedicarboxylic acid) (Fig. 5b). IR spectra showed peaks at 2850 (CH2), 1717 (C═O), 1457 (C═C), 1030 (C–O) and 818 (C–H) cm^{-1} (Fig. 6). Natural production of phthalate from *S. bangladeshiensis* showed antimicrobial activity against some pathogenic Gram positive bacteria (Al-Bari et al., 2006). Dibutyl phthalate, the bioactive compound produced by *Streptomyces albidoflavus* 321.2, was reported by Roya et al. (2006). So far, characterization of cytotoxic phthalate from marine sediment derived actinomycete *Streptomyces avidinii* strain SU4 was reported (Sudha and Masilamani, 2012). Terephthalic acid had been isolated from marine *Streptomyces* sp. YIM66017, and determined by spectral analysis (Zhou et al., 2014). Based on the NIST search database, the spectral peak of the ethyl acetate extract from herbs was confirmed as two phthalate derivatives: diethyl phthalate and dibutyl phthalate (Anusha et al., 2014). Structure elucidation of many bioactive metabolites from a marine – *Streptomyces* sp. was recently reported (Lee et al., 2014; Rajan and Kannabiran, 2014; Zhang et al., 2015).

Antibiogram of phthalate

It is interesting to note that phthalic acid obtained from *S. ruber* EKH2 exhibited a higher anti-virulent activity as shown before (2, 8, 8 and 32 µg/ml against *A. hydrophila*, *E. tarda*, *P. aeruginosa* and *V. ordalii*, respectively) compared to the standard antibiotics used in fish therapy. MICAVG of phthalic acid was 12.5 µg/ml higher than amikacin (AN) and ciprofloxacin (CIP), 22 and 24 µg/ml, respectively; and more superior to florfenicol (KF) and rifamycin (RD) with MICAVG 34 and

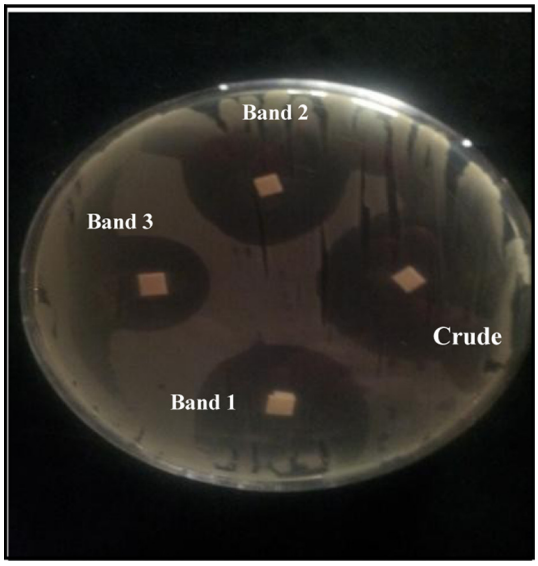

Figure 4 Agar disc diffusion assay of *S. ruber* EKH2 crude extract and fractions of TLC against *A. hydrophila* (most sensitive pathogen).

44 μg/ml, respectively, (Fig. 7). Phthalic acid derivative from *S. bangladeshiensis* had a good potency against *B. subtilis*, *S. aureus*, *S. dysenteriae* and *S. typhi* with MICAVG 24 μg/ml comparing with amoxycillin trihydrate of MICAVG 23 μg/ml (Al-Bari et al., 2006). A marine *Streptomyces* SCSIO 01127 isolated from the South China Sea sediment produced two new lobophorins showing antibacterial activities against *S. aureus* and *Enterococcus faecalis* with MICAVG of 8 μg/ml and were better than spiramycin antibiotic that showed MICAVG of 16 μg/ml (Niu et al., 2011).

Phthalate toxicity

Different concentrations of phthalic acid from 100 to 9000 μg/ml were tested for its toxicity limit toward *A. salina*. After 24 h, the mortality percentage of nauplii was estimated. The obtaining results pointed to the low toxicity with maximum concentration of phthalic acid at 2800 μg/ml and the estimated LC50 was 3.4 ppm (Table 5). Slight toxicity of phthalic acid obtained from *S. ruber* EKH2 against brine shrimp compared to phthalate ester from *Synechococcus lividus* extended the survival of newly hatched brine shrimp (*A. salina*) larvae at maximum acute toxicity LC50 = 2715 μg/ml (Acey et al., 2002). From this result, we may conclude that this compound is a natural

Table 4 Antimicrobial activities of the crude extract and its fractions against fish pathogens.

Bacterial pathogens	MIC (μg/ml)			
	E.A.* crude extract	Frac.1	Frac.2	Frac.3
Aeromonas hydrophila	4	2	256	>256
Edwardsiella tarda	16	8	256	>256
Pseudomonas aeruginosa	16	8	256	>256
Vibrio ordalii	32	32	>256	>256

* E.A.: ethyl acetate.

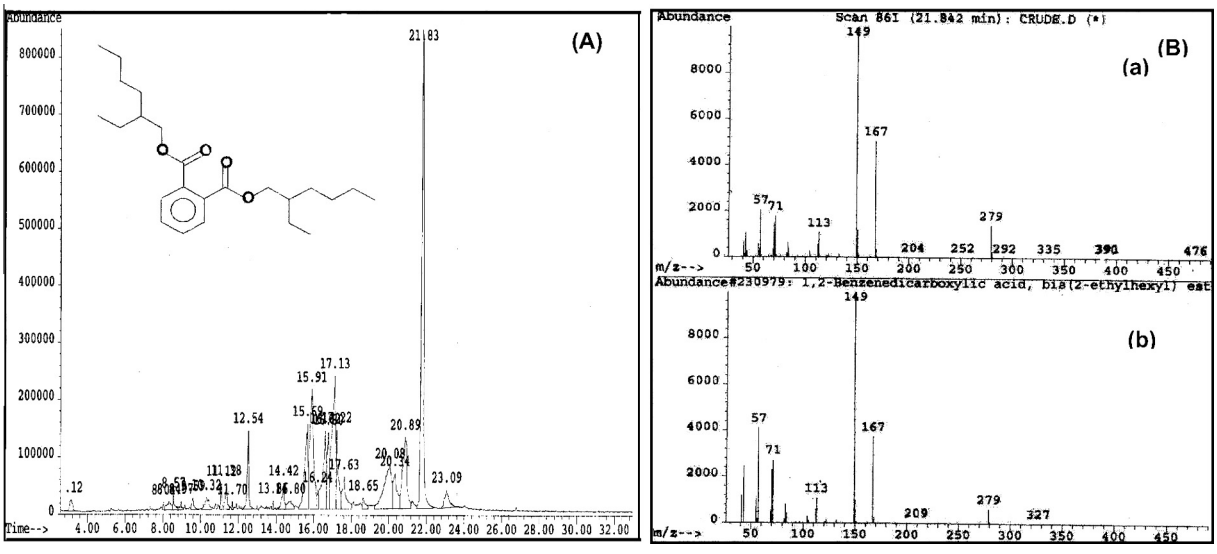

Figure 5 Gas chromatogram (GC) spectral analysis showing the major peak of the active compound at specific retention time (A), mass-spectral analysis (MS) of phthalic acid in the crude [a] and phthalic acid authentic sample [b] (B).

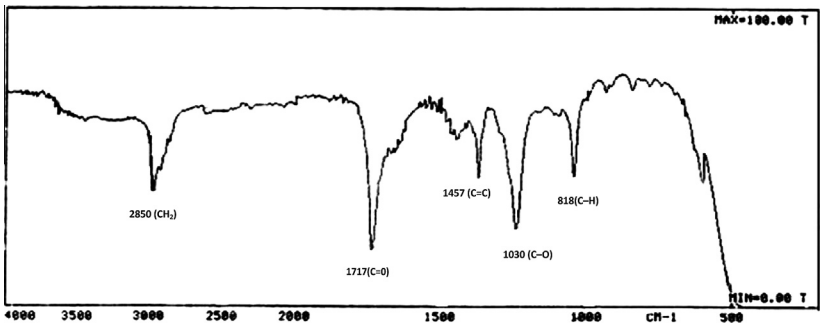

Figure 6 IR spectrum of phthalic acid derivatives with its related function groups.

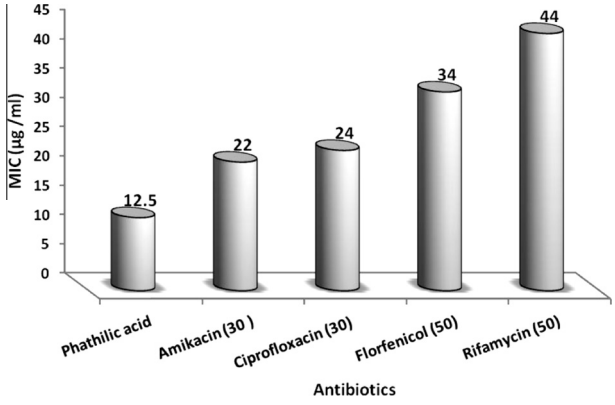

Figure 7 Averages of Minimum Inhibitory Concentrations (MICs) of phthalic acid compared with some antibiotics applied in fish therapy.

Table 5 Toxicity of different phthalate concentrations using *Artemia salina*.

Phthalate concentrations (µg/ml)	Log (Phthalate concentration) (ppm)	Mortality percent after 24 h brine shrimp (*Artemia salina*)
100	2.0	0
500	2.7	0
1000	3.0	20
2000	3.3	30
4000	3.6	55
6000	3.8	60
8000	3.8	80
9000	4.0	100

compound with minimum side effects unlike synthetic or semisynthetic antibiotics.

Conclusion

It can be concluded that phthalate is a new nontoxic bioactive dibasic acid that could be isolated from marine *S. ruber* EKH2. For treating excessive septicemic fish diseases, as a promising candidate, the effects of phthalate derivative on mechanical stress and inflammation in association with hemorrhage and congestion behaviors will be examined in the next stage of research.

References

Acey, R.A., Baileya, S., Healya, P., Joa, C., Ungera, T.F., Hudsonb, R.A., 2002. Butyrylcholinesterase in the early development of the brine shrimp (*Artemia salina*) larvae: a target for phthalate ester embryotoxicity? Biochem. Biophys. Res. Commun. 299, 659–662.

Al-Bari, M.A.A., Sayeed, M.A., Rahman, M.S., Mossadik, M.A., 2006. Characterization and antimicrobial activities of a phthalic acid derivative produced by *Streptomyces bangladeshiensis*. A novel species collected in Bangladesh. Res. J. Med. Sci. 1, 77–81.

Anusha, P., Thangaviji, V., Velmurugan, S., Michaelbabu, M., Citarasu, T., 2014. Protection of ornamental gold fish *Carassius auratus* against *Aeromonas hydrophila* by treating *Ixora coccinea* active principles. Fish Shellfish Immunol. 36, 485–493.

Arasu, A.V., Duraipamdiyan, V., Ignacimuthu, S., 2013. Antibacterial and antifungal activities of polyketide metabolite from marine *Streptomyces* sp. AP-123 and its cytotoxic effect. Chemosphere 90, 479–487.

Aravamuthan, N., Kumar, G., Karthik, L., Rao, K.V., 2010. *In vitro* antagonistic activity of soil actinobacteria against multi drug resistant bacteria. Pharmacology 2, 507–516.

Atta, H.M., Ahmad, M.S., 2009. Antimycin-A antibiotic biosynthesis produced by *Streptomyces* sp. AZ-AR-262: taxonomy, fermentation, purification and biological activities. Aust. J. Basic Appl. Sci. 3, 126–135.

Balagurunathan, R., Masilamani, M., Kathiresan, K., 2010. Bioprospecting of mangrove rhizosphere actinomycetes from pichavaram with special reference to antibacterial activity. J. Pharm. Res. 3, 909–911.

Bauer, A.W., Kirby, W.M.M., Sherris, J.C., Turck, M., 1966. Antibiotic susceptibility testing by standardized single disc method. Tech Bull. Regist. Med. Technol. 36, 49–52.

Boukaew, S., Prasertsan, P., 2014. Factors affecting antifungal activity of *Streptomyces philanthi* RM-1-138 against *Rhizoctonia solani*. World J. Microbiol. Biotechnol. 30, 323–329.

Deepika, L., Kannabiran, K., 2010. Biosurfactant and heavy metal resistance activity of *Streptomyces* spp. isolated from saltpan soil. Br. J. Pharmacol. Toxicol. 1, 33–39.

Djinni, I., Defant, A., Kecha, M., Mancini, I., 2013. Antibacterial polyketides from the marine alga-derived endophitic *Streptomyces sundarbansensis*: a study on hydroxypyrone tautomerism. Mar. Drugs 10, 124–135.

Elayaraja, S., Annamalai, N., Mayavu, P., Balasubramanian, T., 2014. Production, purification and characterization of bacteriocin from *Lactobacillus murinus* AU06 and its broad antibacterial spectrum. Asian Pac. J. Trop. Biomed. 4, 305–311.

El-Gendy, M.M.A., Shaaban, M., Shaaban, K.A., El-Bondkly, A.M., Laatsch, H.A., 2008. Essramycin: a first triazolopyrimidine antibiotic isolated from nature. J. Antibiot. 61, 149–157.

Food and Agriculture Organization of the United Nations: FAO, 2012. The State of World Fisheries and Aquaculture, Rome.

Frans, I., Dierckens, K., Crauwels, S., Van Assche, A., Leisner, J., Larsen, M.H., Michiels, C.W., Willems, K.A., Lievens, B., Bossier, P., Rediers, H., 2013. Does virulence assessment of *Vibrio anguillarum* using sea bass (*Dicentrarchus labrax*) larvae correspond with genotypic and phenotypic characterization? PLoS One 6, 8(8):e70477.

Gopi, M., Kumar, T.T.A., Balagurunathan, R., Vinoth, R., Dhaneesh, K.V., Rajasekaran, R., Balasubramanian, T., 2012. Phylogenetic study of sponge associated bacteria from the *Lakshadweep archipelago* and the antimicrobial activities of their secondary metabolites. World J. Microbiol. Biotechnol. 28, 761–766.

Kandpal, K.C., Jain, D.A., Kumar, U., Tripathi, R., Kumar, T.S., 2012. Isolation and screening of endophytic actinomycetes producing antibacterial compound from *Citrus aurantifolia* fruit. Eur. J. Exp. Biol. 2, 1733–1737.

Kekuda, P., Shobha, K.S., Onkarappa, R., 2010. Fascinating diversity and potent biological activities of actinomycete metabolites. Pharm. Res. 3, 250–256.

Kumar, S.S.R., Rao, K.V.B., 2012. *In-vitro* antimicrobial activity of marine actinobacteria against multidrug resistance *Staphylococcus aureus*. Asian Pac. J. Trop. Biomed. 2, 787–792.

Lee, J., Kim, H., Lee, T.G., Yang, I., Won, D.H., Choi, H., Nam, S.-J., Kang, H., 2014. Anmindenols A and B, inducible nitric oxide synthase inhibitors from a marine- derived *Streptomyces* sp. J. Nat. Prod. 77, 1528–1531.

Li, X.-G., Tang, X.-M., Xiao, J., Ma, G.-H., Xu, L., Xie, S.-J., Xu, M.-J., Xiao, X., Xu, J., 2013. Harnessing the potential of halogenated natural product biosynthesis by mangrove- derived actinomycetes. Mar. Drugs 11, 3875–3890.

Meyer, B.N., Ferringni, N.R., Putnam, J.E., Jacobsen, L.B., Nichols, D.E., Melaughlin, J.L., 1982. Brine shrimp: a convenient general bioassay for active plant constituents. Planta Medica 45, 31–34.

Nandhini, S.U., Selvam, M.M., 2013. Bioactive compounds produced by *Streptomyces* strain. Intern. J. Pharm. Pharm. Sci. 5, 176–178.

Niu, S., Li, S., Chen, Y., Tian, X., Zhang, H., Zhang, G., Zhang, W., Yang, X., Zhang, S., Ju, J., Zhang, C., 2011. Lobophorins E and F, new spirotetronate antibiotics from a South China Sea-derived *Streptomyces* sp. SCSIO 01127. J. Antibiot. 64, 711–716.

Ochi, K., Hosaka, T., 2013. New strategies for drug discovery: activation of silent or weakly expressed microbial gene clusters. Appl. Microbiol. Biotechnol. 97, 87–98.

Olano, C., Mendez, C., Salas, J.A., 2009a. Antitumor compounds from marine actinomycetes. Mar. Drugs 7, 210–248.

Olano, C., Méndez, C., Salas, J.A., 2009b. Antitumor compounds from actinomycetes: from gene clusters to new derivatives by combinatorial biosynthesis. Nat. Prod. Rep. 26, 628–660.

Oskay, M., Tamer, A.U., Azeri, C., 2004. Antibacterial activity of some actinomycetes isolated from farming soils of Turkey. Afr. J. Biotechnol. 3, 441–446.

Paterson, D.L., Ko, W.C., Von Gottberg, A., 2004. International prospective study of *Klebsiella pneumoniae* bacteremia: implications of extended-spectrum beta-lactamase production in nosocomial infections. Ann. Intern. Med. 140, 26–32.

Rajan, B.M., Kannabiran, K., 2014. Extraction and identification of antibacterial secondary metabolites from marine *Streptomyces* sp. VITBRK2. Intern. J. Mol. Cell. Med. 3, 130–137.

Reish, D.J., Oshida, A.S., 1987. Manual of methods in aquatic environment research, part (10) short-term static bioassay. FAO Fish 247, 1–62.

Richard, S., Lynn, S.-M., Avery, C.G., 2007. Antimicrobial Susceptibility Testing Protocols. CRC Press, New York, USA.

Roya, R.N., Laskarb, S., Sena, S.K., 2006. Dibutyl phthalate, the bioactive compound produced by *Streptomyces albidoflavus* 321.2. Microbiol. Res. 161, 121–126.

Sudha, S., Masilamani, S.M., 2012. Characterization of cytotoxic compound from marine sediment derived actinomycete *Streptomyces avidinii* strain SU4. Asian Pac. J. Trop. Biomed. 2, 770–773.

Urban, C., Segal-Maurer, S., Rahal, J.J., 2003. Considerations in control and treatment of nosocomial infections due to multidrug-resistant *Acinetobacter baumannii*. Clin. Infect. Dis. 36, 1268–1274.

Usha, R., Ananthaselvi, P., Venil, C.K., Palaniswamy, M., 2010. Antimicrobial and antiangiogenesis activity of *Streptomyces parvulus* KUAP106 from mangrove soil. Eur. J. Biol. Sci. 2, 77–83.

You, Z.-Y., Wang, Y.-H., Zhang, Z.-G., Xu, M.-J., Xie, S.-J., Han, T.-S., Feng, L., Li, X.-G., Xu, J., 2013. Identification of two novel anti-fibrotic benzopyran compounds produced by engineered strains derived from *Streptomyces xiamenensis* M1-94P that originated from deep-sea sediments. Mar. Drugs 11, 4035–4049.

Vennila, S., Krishnaveni, M., 2013. Bioactive compound produced from *Streptomyces aureofaciens* KF532950 and its antimicrobial activity. Int. J. Chem. Anal. Sci. 4, 153–155.

Zhang, D., Satake, M., Fukuzawa, S., Sugahara, K., Niitsu, A., Shirai, T., Tachibana, K., 2012. Two new indole alkaloids, 2-(3,3-dimethylprop-1-ene)-costaclavine and 2-(3,3- dimethylprop-1-ene)-epicostaclavine, from the marine-derived fungus *Aspergillus fumigatus*. J. Nat. Med. 66, 222–226.

Zhang, S.M., Zhang, H.Y., Yang, S.X., Qu, C.L., Xie, Z.P., Pescitelli, G., 2015. Isolation, stereochemical study, and cytotoxic activity of isobenzofuran derivatives from a marine *Streptomyces* sp. Chirality 27, 82–87.

Zhou, H., Yang, Y., Peng, T., Li, W., Zhao, L., Xu, L., Ding, Z., 2014. Metabolites of *Streptomyces* sp., an endophytic actinomycete from *Alpinia oxyphylla*. Nat. Prod. Res. 28, 265–267.