In vitro Evaluation of Anti-microbial Activities of Marine Streptomyces against Viral Models, Bacterial and Fungal Strains

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ABSTRACT

Herpes simplex virus type 2 (HSV-2) is a dsDNA virus and it is the causative agent of genital herpes infection. The most important problem of herpes viruses is the reactivation that may lead to recurrent infection. On the other hand, Vesicular stomatitis virus (VSV) is a negative strand RNA virus that can cause many diseases to animals and rarely to human. Excess antiviral drugs use in the treatment of viral infections can induce mutagenicity and cross-resistance so searching for new source of antiviral drugs such as marine actinomycetes is required. A total of 72 Actinomycetes isolated from Qarun Lake governed to El-Fayoum-Egypt were screened for their antimicrobial activities against six bacterial strains, three fungal strains and one yeast strain. All of actinomycetes isolates were assayed for their antimicrobial activities using inhibition zone method and found that 10 isolates were active against bacteria, 3 isolates have activities against fungi and 5 isolates have both antibacterial and antifungal activities. All active isolates were tested for antiviral potentials using Cytopathic Effect (CPE) inhibition assay after determination of safe concentrations of actinomycete filtrates on Vero cells using MTT assay. Vesicular stomatitis virus (VSV) and Herpes simplex virus type 2 (HSV-2), were used as a test viruses. Nine isolates proved antiviral potentials against both viruses. Two isolates coded to Q3 and B2T were selected as the most active isolates against HSV-2 and VSV, respectively and were identified as a genus of Streptomyces. Our result gives conclusion that, marine actinomycetes still considered as a valuable source of many antimicrobial agents and can produce antiviral drugs against both DNA and RNA viruses.

Key words: Streptomyces, Vesicular stomatitis virus, Herpes simplex virus, antimicrobial agent

INTRODUCTION

Viral diseases caused by pathogenic virus infections which have high morbidity and mortality rates are still the leading cause of death in humans worldwide. A virus is a unique pathogen which is incapable of replicating without host cell. It utilizes the host cell environment and cellular factors for its propagation. These unique features of viruses make it difficult to design a treatment to attack the virus or its replication directly without any adverse effects on the infected cells (Kitazato et al., 2007).
More than one third of world is affected by *Herpes simplex virus* (HSV), family herpesviridae, HSV infection are the cause of several infectious disease such as labial herpeses, genital herpes, keratitis and encephalitis that may be life-threatening. These clinical symptoms mainly occur in neonates and immunocompromised patient population. The HSV has two different types namely HSV-1 and HSV-2 that cause oral and genital infections without notable symptoms (Cheng and Xu, 2005). A very effective treatment of herpes viruses is available since the introduction of acyclovir in 1970 and it is still the most commonly used chemotherapy (Brady and Bernstein, 2004). However, this compound is not always well tolerated and drug-resistant strains are rapidly emerging, particularly in immunocompromised patients. Therefore, the demand for antiviral drugs with novel mode of action is great (Mandal *et al*., 2008). *Vesicular stomatitis virus* (VSV) is a non segmented negative-strand RNA virus and the prototype of the rhabdovirus family. It is an arthropod-borne virus that primarily affects rodents, cattle, swine and horses but can also infect humans and other species. It is thought that VSV is spread between hoofed animals and rodents via insect vectors (Mead *et al*., 2000).

Pharmaceutical interest in marine organisms has provided thousands of new and novel compounds that have shown important biological properties, such as anticancer, antiviral, antiprotozoal and antibacterial activities (Sagar *et al*., 2010; Mayer *et al*., 2011; Uzair *et al*., 2011). In this context, marine actinomycetes have been a prolific source of diverse antiviral secondary metabolites with complex and unique structures (Sonya and Galal, 2005; Kumar *et al*., 2006; Suthindhiran *et al*., 2011).

Actinomycetes are Gram-positive bacteria showing a filamentous growth. They are a group of organisms widespread in nature and play a significant role in the future of biotechnology, because of their importance as producers of vitamins, enzymes, antitumor agents, Immunomodifying agent mainly antibiotic compounds (Lam, 2006). According to Sanglier *et al.* (1993) between 1988 and 1992 more than a hundred new molecules from actinomycetes were discovered. Approximately, 75% of these originated from the *Streptomyces* genus and at least 5000 documented bioactive compounds are known as being produced by this genus (Anderson and Wellington, 2001).

In this report we investigate the antimicrobial activities of actinomycetes isolated from marine environment against bacterial and fungal strains as well as their possible activities against HSV-2 and VSV viruses.

**MATERIALS AND METHODS**

**Study area:** Qarun lake is a closed saline lake, located in the deepest part of El-Fayoum depression at the western desert, 70 km south Cairo-Egypt between longitudes 30°24’ and 30°49’E and latitudes of 29°24’ and 29°33’, its salinity is somewhat below that of the Mediterranean Sea; 32.28% (Anonymous, 2004).

**Sampling:** Sediments samples were collected during December 2012 at ten stations (St. I-St. X) along the lake in addition to one sample at the shore (Fig. 1). First five stations represented the eastern site; the second two stations represented the middle, while the last three stations represented the western site of the lake (Table 1).

**Sample processing and isolation of actinomycetes:** Air dried (ambient room temperature for 14 day) ground and sieved sediment samples were processed as soon as possible where 10 g of dry sediment samples were mixed in a mortar with 10 g of calcium carbonate. The mixture was
incubated for 10 days at 28°C in a closed inverted sterile petri dish (El-Nakeeb and Lechevalier, 1962). The pretreated sediments were suspended in 100 mL of sea water then shaken with a rotary shaker at 200 rpm for 30 min for the detachment of spore chains. Mixtures were allowed to settle and serial dilutions up to $10^{-4}$ were prepared using sterile sea water and agitated with the vortex at maximum speed. An aliquot of 0.2 mL of each dilution was taken and spread evenly over the surface of Starch Casein Agar (SCA) plates prepared using 50% sea water, collected from Qarun lake (Thakur et al., 2007). Rifampin (5 μg L$^{-1}$) and Nystatin (50 μg L$^{-1}$) was added to the SCA to prevent the growth of bacteria and fungi (Mincer et al., 2005). Plates were incubated at 55°C for isolation of thermophiles; other group of plates were incubated at 37°C for isolation of mesophiles and monitored every day for 2 weeks. After incubation period, actinomycetes isolates were examined by eye and purified on SCA medium.
Antimicrobial activities: Pure actinomycete isolates were grown on starch casein broth medium and tested for antibacterial and antifungal activities using inhibition zone method (NCCLS, 2003). Bacterial strains used were *Escherichia coli* (ATCC 10536), *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633), *Bacillus cereus* (ATCC 10876), *Salmonella typhimurium* (ATCC 14028) and *Pseudomonas aeruginosa* ATCC 9027 (American Type Culture Collection, ATCC, USA). Fungal strains were *Aspergillus niger* 111EMCC, *Aspergillus flavus* ATCC 204304, *Penicillium chrysogenum* ATCC 10106 and *Candida albicans* 105EMCC as a yeast strain (Cairo MIRCEN, Faculty of Agriculture, Ain Shams University, Cairo-Egypt). The antimicrobial active actinomycete isolates were tested for its antiviral activities.

Cells and viruses: Vero cells (African green monkey cells, clone CCL-81 passage 124) were cultured in 75 cm² cell culture flasks (PPL-Swiss) using 199-E medium supplemented with 10% FBS; Fetal Bovine Serum (sigma-Aldrich-USA) as culture medium. *Herpes simplex* virus type 2 (HSV-2) and *Vesicular stomatitis* virus (VSV) were kindly supplied from Applied Research Sector VACSERA, Egypt.

Cytotoxicity: Cytotoxicity test was carried out using MTT staining assay according to Yasuhara-Bell et al. (2010), where, the actinomycete filtrates were filtered through 0.22 μm syringe filter (Millipore-USA). Precultured 96-well Vero cells plates (Nunc-USA) were treated with descending double fold serially diluted actinomycetes filtrates at 37°C for 24 h. Negative cell control of untreated cells was included. Residual living cells were treated with 50 μL of MTT (0.5 mg mL⁻¹) (Sigma-Aldrich-USA) at 37°C for 4 h MTT was discarded. Plates were Phosphate Buffer Saline (PBS) washed three times. The DMSO (BDH-England) was added as 50 μL well⁻¹. Plates were shacked on plate shaker (Staurt-England) for 30 min to dissolve the produced intracellular blue MTT-formazan complex. Optical densities (O.Ds) were read at 570 nm using an ELISA plate reader (Dynatech-England). Data were reported for three independent experiments. Viability percentage was calculated as follows (Chen et al., 2009):

\[
\text{Cell viability percentage} = \frac{\text{O.D of treated cells}}{\text{O.D of untreated cells}} \times 100
\]

Antiviral activities using CPE inhibition assay: The non-toxic concentrations of actinomycete filtrates were used to evaluate the antiviral activities against HSV-2 and VSV using CPE inhibition assay as well as reduction in virus titer (TCID 50 mL⁻¹) TCID50 is 50% tissue culture infective dose under *in vitro* conditions (Petricevich and Mendonca, 2003). Test viruses were titrated on non washed 24 h actinomycete filtrates pretreated cells. Virus titer was determined; the differences between the virus titers in filtrates treated and untreated cells represent the antiviral activities (Reed and Muench, 1938). Statistical differences between the virus titer in actinomycetes filtrates treated cells and its titer in untreated cells were determined using one way ANOVA. Differences at $p \leq 0.05$ were considered significant.

Identification of the actinomycete isolates: The most active actinomycetes for antiviral activity were identified according to the key proposed by Pridham and Tresner (1974). The characters of actinomycetes in this study, were determined according to the methods described by
the International *Streptomyces* Project (ISP) as described by Shirling and Gottlieb (1966). Using the differential electron microscopy, its morphological characters were determined.

The cultural characteristics of the most potent active isolate(s) were tested. The colors of mature sporulating aerial mycelium and substrate mycelium were monitored for the 7, 14 and 21 day old cultures grown on the following agar media: Inorganic salt agar medium, oat meal medium, glycerol asparagine medium, yeast extract-malt extract agar medium, C'zapeks agar medium and nutrient agar medium (Jiang et al., 2011).

The physiological and biochemical characteristics were determined according to the methods of Shirling and Gottlieb (1966) and Waksman (1967). Cultures were incubated at 37 and 55°C then examined after 7-14 days, except for gelatin liquefaction as it was tested during growth after 2, 4 and 7 days. Utilization of carbon sources was investigated using the procedure of Pridham and Gottlieb (1948), where, carbon sources were added to the basal salt medium at 1.0% (w/v). Growth and gas production were recorded after 7, 14 and 21 days.

**RESULTS AND DISCUSSION**

**Antimicrobial activities:** Marine environment is a source of interesting research for new species and promising source of pharmaceutically important compounds (Fenical and Jensen, 2006). In this context, Qarun Lake was selected to be the study area. Qarun Lake considered being an attractive source for bioactive microorganisms. Many researchers succeeded to isolate actinomycetes with antibacterial, antifungal and antiviral activities (Sonya and Galal, 2005; Rabeh and Fareed, 2008). In the present work we isolated a total of 72 actinomycete isolates from Qarun Lake (58 mesophiles, 24 thermophiles) and were tested for their antimicrobial activity against bacteria, fungi and yeast strains. Eighteen actinomycetes isolates; 11 mesophiles, named AF, AD, AA1, IA, D6, D3, Q3, Q8, QD1, QB1 and QH2 and 7 thermophiles named C2T, A2T, B2T, C1T, QA1T, QG1T and ICT showed high activity. Ten isolates were found to be active against bacteria, 3 isolates have activities against fungi and 5 isolates have both antibacterial and antifungal activities (Table 2). All of these 18 active isolates were tested for their activity against VSV and HSV-2.

**Cytotoxicity:** To evaluate antiviral activities of actinomycete filtrates, cytotoxicity test was done to test their toxicity on Vero cells. Data recorded revealed that 100% cellular viability (safe concentration) for all tested 18 filtrates were found to be ranged between 1/8 and 1/256 dilution factor (Table 3). The A2T, QD1, QH2 and QG1T isolates have less toxicity on Vero cells, gave maximum safe concentration at dilution factor 1/8, followed by AD, C2T, IA, AA1 and QA1T isolates (1/16), followed by D3 isolate (1/32), followed by D6 and QB isolates (1/64), followed by B2T, AF and Q8 isolates (1/128). The ICT, C1T and Q6 isolates was the highly toxic isolates, gave 100% viability at 1/256 dilution factor. Sacramento et al. (2004) isolated *Streptomyces* from Mata Atlantica soil able to produce a substance with non toxic concentration on HEp-2 cells (human larynx tumor cell line) at 1/10240.

**Antiviral activities:** Antiviral activities of actinomycete filtrates against HSV-2 and VSV were tested. The susceptibility of the Vero cell line to HSV-2 and VSV were evaluated by observation of CPE. It was observed that there were no CPE in uninfected cells. Initially the specific CPE developed as localized areas of areas of rounded and refractile degenerating cells. The CPEs with typical multiple vaculation, cell detachment, rounding and aggregation of numerous virus particles in the cytoplasm of both viruses infected Vero cells were observed.
Table 2: Antimicrobial activities of isolates

| Actinomycetes code | Isolate | Escherichia coli | Staphylococcus aureus | Pseudomonas aeruginosa | Bacillus subtilis | Bacillus cereus | Salmonella typhimurium | Aspergillus niger | Aspergillus flavus | Penicillium chrysogenum | Candida albicans |
|-------------------|---------|------------------|-----------------------|-----------------------|------------------|------------------|------------------------|----------------|----------------|--------------------------|----------------|----------------|
| Mesophiles        | D3      | 0.0              | 20.0                  | 15.3±0.6              | 12.3±0.6         | 0.0              | 0.0                    | 0.0             | 0.0             | 0.0                      | 16.0±1         |
|                   | D3      | 0.0              | 17.0±1                | 0.0                   | 21.0             | 22.2±0.3         | 0.0                    | 0.0             | 0.0             | 0.0                      | 0.0             |
|                   | C2T     | 0.0              | 18.0±1                | 23.8±1.3              | 0.0              | 18.7±0.6         | 0.0                    | 0.0             | 0.0             | 0.0                      | 0.0             |
|                   | A2T     | 0.0              | 0.0                   | 0.0                   | 0.0              | 12.3±0.8         | 0.0                    | 0.0             | 0.0             | 0.0                      | 0.0             |
|                   | B2T     | 0.0              | 15.3±1.5              | 0.0                   | 0.0              | 15.2±0.8         | 19.3±1.2               | 0.0             | 0.0             | 0.0                      | 0.0             |
|                   | C2T     | 0.0              | 14.7±0.6              | 0.0                   | 16.3±0.6         | 17.3±0.6         | 0.0                    | 0.0             | 0.0             | 20.0                      | 0.0             |
|                   | Q3      | 0.0              | 15.0                  | 0.0                   | 12.0             | 0.0              | 0.0                    | 0.0             | 0.0             | 0.0                      | 0.0             |
|                   | Q4      | 0.0              | 13.0                  | 0.0                   | 12.3±0.6         | 0.0              | 12.2±0.3               | 0.0             | 0.0             | 0.0                      | 20.0             |
|                   | QD3     | 0.0              | 12.0                  | 0.0                   | 0.0              | 0.0              | 0.0                    | 0.0             | 0.0             | 0.0                      | 25.3±0.6        |
|                   | QB3     | 0.0              | 0.0                   | 0.0                   | 0.0              | 0.0              | 16.0                   | 0.0             | 0.0             | 22.3±0.3                 | 15.0             |
|                   | QH3     | 0.0              | 0.0                   | 0.0                   | 0.0              | 15.0             | 12.7±0.6               | 0.0             | 0.0             | 0.0                      | 0.0             |
|                   | QA,T    | 0.0              | 0.0                   | 0.0                   | 0.0              | 0.0              | 0.0                    | 0.0             | 0.0             | 0.0                      | 0.0             |
| Thermophiles      | QB,T    | 0.0              | 0.0                   | 0.0                   | 0.0              | 0.0              | 0.0                    | 17.0            | 0.0             | 0.0                      | 0.0             |
|                   | QA,T    | 0.0              | 0.0                   | 0.0                   | 0.0              | 0.0              | 0.0                    | 0.0             | 18.0           | 0.0                      | 0.0             |
|                   | AF      | 0.0              | 16.0                  | 0.0                   | 0.0              | 0.0              | 0.0                    | 0.0             | 0.0             | 0.0                      | 0.0             |
|                   | AD      | 12.2±0.3         | 15.0                  | 0.0                   | 0.0              | 0.0              | 0.0                    | 0.0             | 0.0             | 0.0                      | 0.0             |
|                   | AA1     | 0.0              | 0.0                   | 12.2±0.3              | 16.0             | 12.7±1.5         | 0.0                    | 0.0             | 0.0             | 0.0                      | 11.0             |
|                   | IA      | 0.0              | 20.0                  | 14.7±0.6              | 15.0             | 0.0              | 0.0                    | 0.0             | 0.0             | 0.0                      | 0.0             |
|                   | ICT     | 0.0              | 18.0±0.6              | 0.0                   | 16.0             | 0.0              | 0.0                    | 0.0             | 0.0             | 0.0                      | 0.0             |

Data was expressed as mean of three independent experiments±SD
Data recorded as shown in Fig. 2 reveal that in the control Vero cells, the titer of VSV was 5.5 log TCID 50 mL⁻¹. Nine isolates decreased the VSV infectivity titer and 8 isolates have no effect on the VSV titer while one isolate named A₂T was found to increase the virus titer by 0.4 log(10). The B₂T (thermophile) isolate was the most active isolate against VSV since it showed a decrease in the virus infectivity titer by 1.6 log(10) mL⁻¹ (Fig. 2). Lee et al. (2007) were able to isolate *Streptomyces nitrosporeus* showed antiviral activities against both HSV-2 and VSV viruses. Other researchers were discovered a novel protein against RNA virus (human immunodeficiency virus) from an actinomycete, inhibits viral entry (Chiba et al., 2004).

For HSV-2 the virus titer was 6.5 log TCID50 mL⁻¹ and 9 actinomycetes showed antiviral activity while, the rest (9 isolates) have no effect. The Q₃ (mesophile) showed the higher anti HSV-2 activity (Fig. 3) with a decrease in virus titer equal to 1.9 log(10) mL⁻¹. This result was in agreement with the previous studies.

Table 3: Evaluation of cytotoxicity of actinomycete filtrates using MTT assay

| Actinomycetes codes  | Viability (%) |
|----------------------|---------------|
|                      | 1/2 | 1/4 | 1/8 | 1/16 | 1/32 | 1/64 | 1/128 | 1/256 |
| D₆                  | 7   | 12  | 13  | 48   | 69   | 100  | 100   | 100   |
| D₃                  | 40  | 45  | 65  | 96   | 100  | 99   | 100   | 100   |
| C₂T                 | 14  | 61  | 99  | 100  | 99   | 69   | 100   | 100   |
| B₂T                 | 45  | 61  | 95  | 99   | 99   | 99   | 100   | 100   |
| A₂T                 | 46  | 72  | 100 | 100  | 100  | 100  | 100   | 100   |
| AD                  | 44  | 60  | 95  | 100  | 100  | 100  | 100   | 100   |
| LA                  | 30  | 60  | 90  | 100  | 100  | 100  | 100   | 100   |
| ICT                 | 30  | 31  | 35  | 48   | 77   | 92   | 94    | 100   |
| C₂T                 | 59  | 65  | 71  | 81   | 85   | 92   | 97    | 99    |
| Q₅                  | 47  | 47  | 53  | 94   | 95   | 97   | 98    | 100   |
| AF                  | 33  | 36  | 39  | 57   | 84   | 97   | 100   | 100   |
| Q₂                  | 14  | 23  | 31  | 64   | 97   | 96   | 100   | 100   |
| AA₁                 | 21  | 25.6| 45  | 100  | 100  | 100  | 100   | 100   |
| QD₁                 | 60  | 95  | 100 | 100  | 100  | 100  | 100   | 100   |
| QH₁                 | 63  | 59  | 100 | 100  | 100  | 100  | 100   | 100   |
| QG₁T                | 85  | 99  | 100 | 100  | 100  | 100  | 100   | 100   |
| QA₁T                | 66  | 80  | 99  | 100  | 100  | 100  | 100   | 100   |
| QB₁                 | 18  | 32  | 93  | 94   | 94   | 100  | 99    | 100   |

Data was expressed as a mean of three independent experiments

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Fig. 2: Evaluation of antiviral activity of actinomycetes against *Vesicular stomatitis virus* using CPE inhibition assay. The means of virus titer induce CPE in three replicates with each filtrate, LSD 0.05 = 0.26
Virus titer (log10 m L¯)1

HSV D6 D3
CT1 CT2 BT2 AT2 Q3 Q8
QD1
QB1
QH2
QAT1
QCT1 Aa1
AD AF IC IA

with Hayashi et al. (2000), who isolated *Streptomyces* strain which gave activity against HSV-1. Regarding the above results, B₂T and Q₃ isolates were the most active isolates against VSV and HSV-2, so they were selected for the identification.

**Identification of the most active isolates:** The microscopic examination of the selected isolates Q₃ and B₂T (Fig. 4, 5) revealed that aerial mycelia bearing a long spiral spore chains, not borne in verticillate sporophores. Mature spore mass of Q₃ isolate is oval and belonging to gray color series, while, B₂T isolate spore is circular and belonging to white series with warty spores surface for both (Fig. 4, 5). In further investigation, the Cultural, physiological and biochemical characters for Q₃ and B₂T isolates were presented in Table 4.

Morphological characters showed that both strains Q₃ and B₂T are belonging to *Streptomyces* sp as they form well developed branching, non-septate, non-fragmented aerial mycelia bearing a

Fig. 3: Evaluation of antiviral activity of actinomycetes against *Herpes simple* virus using CPE inhibition assay. The means of virus titer induce CPE in three replicates with each filtrate, LSD 0.05 = 0.28

Fig. 4: Transmission electron micrograph of Q₃ isolate (8000X)
Fig. 5: Transmission electron micrograph of B₁T isolate (6000X)

Table 4: Taxonomical characters of Q₃ and B₁T isolates

| Characteristics                                      | Q₃          | B₁T         |
|------------------------------------------------------|-------------|-------------|
| **Cultural characteristics**                         |             |             |
| Color of aerial mycelium                             | Grey        | White       |
| Color of substrate mycelium                          | Colorless   | Colorless   |
| Diffusible pigments                                  | -           | -           |
| **Physiological and biochemical characteristics**     |             |             |
| Melanoid pigment production                          |             |             |
| Tyrosine broth                                       | +           | +           |
| Peptone yeast iron                                   | +           | -           |
| Tryptone yeast extract broth                         | -           | -           |
| Growth on Czapeks medium                             | +           | +           |
| Sodium chloride tolerance                            | ≤15%        | ≤3%         |
| Sensitivity to streptomycin (50 μg mL⁻¹)             | +           | -           |
| Antibacterial activity                               | +           | +           |
| Antifungal activity                                  | -           | -           |
| Nitrate reduction                                    | +           | -           |
| Starch hydrolysis                                    | +           | +           |
| Gelatin liquefaction                                 | +           | +           |
| **Hydrolysis of**                                    |             |             |
| Casein                                               | +           | +           |
| Tyrosine                                             | -           | -           |
| Xanthin                                              | -           | -           |
| Urea                                                 | +           | +           |
| **Utilization of different carbon sources**           |             |             |
| No carbon                                            | -           | -           |
| D-glucose                                            | +           | +           |
| D-xylene                                            | +           | +           |
| L-arabinose                                          | +           | -           |
| L-rhamnose                                           | +           | -           |
| D-fructose                                           | +           | +           |
| Galactose                                            | +           | -           |
| Raffinose                                            | -           | -           |
| D-mannitol                                           | +           | +           |
| Inositol                                             | +           | -           |
| Sucrose                                              | 3.15        | -           |

+: Positive result, -: Negative result

long non-motile spore chains, not borne in verticillate sporophores (Pridham and Tresner, 1974). Identification to species level is under investigation using 16S rRNA. These results confirm the fact that *Streptomyces* have more than 75% of the new bioactive compounds and at least
5000 documented bioactive ones (Anderson and Wellington, 2001). Finally, our selected isolates considered to be valuable sources for antiviral activities against DNA virus (HSV-2) and RNA virus (VSV). The main active compounds from each isolate are under investigation and their chemical and physical properties will be reported in another issue.

CONCLUSION

Qarun Lake is used as a reservoir for the drainage water of El-Fayoum province. The drainage water is loaded with salts, nutrients and pesticides that may accumulate and eventually contaminate the aquatic environment. Microorganisms that can withstand this condition may have promising challenged activities. Actinomycetes especially *Streptomyces* considered to be the most important group of actinobacteria responsible for antimicrobial agent(s) production. Further studies should be done to purify the antiviral compound(s) to be tested against the used therapies of VSV and HSV-2 viruses.

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