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Article

# Evaluation of antimycobacterial activity from marine and freshwater microalgae

Evaluación de la actividad antimicobacteriana de microalgas dulceacuícolas y marinas

**Duahmet A. Ruiz-Güereca[1,3], Alexei F. Licea-Navarro[2] and M. del Pilar Sánchez-Saavedra[3*]**

[1]Departamento de Microbiología, Centro de Investigación Científica y de Educación Superior de Ensenada, Baja California, México
[2]Departamento de Innovación Biomédica, Centro de Investigación Científica y de Educación Superior de Ensenada, Baja California, México
[3]Departamento de Acuicultura, Centro de Investigación Científica y de Educación Superior de Ensenada, Carretera Tijuana-Ensenada #3918, Fraccionamiento Zona Playitas, Zip code 22860, Ensenada, Baja California, México
*Corresponding author: psanchez@cicese.mx

**Resumen.-** La tuberculosis es considerada una de las enfermedades epidemiológicas más importantes a nivel mundial. El tratamiento actual para la tuberculosis consiste en la combinación y uso de fármacos antituberculoso por al menos 9 meses. En la actualidad, la búsqueda de nuevos agentes farmacológicos para el tratamiento de la tuberculosis es una tarea urgente. Las microalgas producen una amplia variedad de moléculas que poseen actividades biológicas de interés farmacéutico. Por lo tanto, el objetivo de este trabajo fue evaluar la actividad antimicobacteriana de los extractos crudos hexánicos obtenidos de 6 microalgas: *Chlamydomonas mexicana*, *Porphyridium cruentum*, *Isochrysis galbana*, *Rhodomonas* sp., *Aphanocapsa marina* y *Nitzschia palea*. Todos los extractos crudos inhibieron al menos el 90% del crecimiento de *Mycobacterium tuberculosis* H37Rv a una concentración de 100 µg $ml^{-1}$. El extracto crudo obtenido de *I. galbana* presentó la mayor actividad antimicobacteriana con un porcentaje de inhibición igual al del fármaco antituberculoso isoniazida y con una concentración inhibitoria mínima de 50 µg $ml^{-1}$. Estos hallazgos demuestran que las microalgas son una excelente fuente para la búsqueda de nuevos compuestos antimicobacterianos.

**Palabras clave:** Tuberculosis, microalgas, extracto crudo, ensayo en microplaca con sal de tetrazolio, actividad antimicobacteriana

**Abstract.-** Tuberculosis is considered one of the most important epidemiological diseases worldwide. The current treatment for tuberculosis is a combination of antituberculosis drugs for at least 9 months. Nowadays, the search for new pharmacological agents for the treatment of tuberculosis is an urgent endeavor. Microalgae produce a wide variety of molecules that possess biological activities of pharmaceutical interest. Therefore, the objective of this work was to evaluate the antimycobacterial activity of hexane crude extracts of 6 microalgae: *Chlamydomonas mexicana*, *Porphyridium cruentum*, *Isochrysis galbana*, *Rhodomonas* sp., *Aphanocapsa marina,* and *Nitzschia palea*. All of the hexane extracts inhibited at least 90% of the growth of *Mycobacterium tuberculosis* H37Rv at a concentration of 100 µg $ml^{-1}$. The hexane crude extract of *I. galbana* had the highest antimycobacterial activity displaying a percentage inhibition that was equal to that of the antituberculosis drug isoniazid and having a minimum inhibitory concentration of 50 µg $ml^{-1}$. These findings demonstrate that microalgae are an excellent source for the search of novel antimycobacterial compounds.

**Key words:** Tuberculosis, microalgae, crude extract, tetrazolium microplate assay, antimycobacterial activity

## Introduction

Tuberculosis (TB), acquired immune deficiency syndrome (HIV/AIDS), and malaria are considered the most important epidemiological diseases worldwide (Sotgiu & Migliori 2014). TB is a systemic, chronic disease that primarily affects the respiratory system; the main causative agent of TB in humans is *Mycobacterium tuberculosis* (Mtb). In 2015, it was estimated that one-third of the world's population was infected with Mtb and that 10% of these subjects could develop TB (WHO 2016). In Mexico, approximately 15,000 new cases of TB and 2,000 deaths due to this disease are registered annually (INDRE 2014). The morbidity rate varies among states of Mexico, the highest of which occurs in the state of Baja California, with 42 cases of TB per 100,000 habitants per year (INDRE 2014). The current treatment of TB is a combination of antituberculosis drugs over 9 to 12 months to ensure the total eradication of mycobacteria (World Health Organization 2016). This extended period of medication is one of the main factors of nonadherence, which favors the appearance of drug-resistant Mtb strains (Migliori *et al*. 2007). Certain infectious diseases that are caused by drug-resistant bacteria can be treated with alternative drugs (Powers 2004). However, this approach cannot be applied to TB, because the drugs that are used

to treat drug-resistant Mtb strains are more expensive, less effective, and more toxic (Sotgiu & Migliori 2014). Due to the need for more efficacious antituberculosis drugs, namely those with a shorter treatment duration, few side effects and that do not require being combined with other drugs and an increase in drug-resistant Mtb strains, the search for new pharmacological agents for the treatment of TB is a critical issue worldwide (Hoagland *et al*. 2016).

Microalgae, *s. ampl.* considering cyanobacteria as microalgae, are cosmopolitan microorganisms, because they are found in various aquatic and terrestrial habitats, even in extreme environments, such as hypersaline waters, frozen lakes, and hydrothermal vents (Whitton & Potts 2000). To survive in such inhospitable environments, microalgae produce a wide variety of compounds. These compounds have demonstrated to display a range of bioactivities, such as antiviral, anticancer, antifungal, and antibacterial, which has fascinated researchers, based on their incredible pharmaceutical potential (Pulz & Gross 2004).

Different microalgae strains (46) were studied to evaluate their potential antimicrobial and antimycobacterial activities related to strain diversity. Results showed that extracts of two diatoms, *Skeletonema costatum* and *Chaetoceros pseudocurvisetus* have anti-tuberculosis activity and were only active when cultured in the control and phosphate starvation conditions. The organic and water extracts were also tested, and we found that only the organic extracts of both diatoms were active. The chemical characterizations of the compounds are related to chlorophyll breakdown and lipids (Lauritano *et al*. 2018).

Antimycobacterial activity has been found in methanolic extracts of cyanobacteria for the genus *Oscillatoria*, *Spirulina*, *Anabaena*, *Scytonema* and *Hapalisiphon* (Rao *et al.* 2007). A potent antimycobacterial effect of anthraquinone derivatives has also been reported from *Eucapsia* sp. (Sturdy *et al*. 2010). Two new compounds (carbamidocyclofanes) have been discovered for the cyanobacteria *Nostoc* sp. with antimycobacterium activity (Luo *et al.* 2014). The hexanic extracts from the cyanobacteria *Mycrocystis aeruginosa* inhibit the growth of *Mycobacterium tuberculosis*, *M. terrae*, *M. chelonae* and *M. kansasii*. This cytotoxic effect is related to their microcystins content (monocyclic heptapeptides toxins that act as inhibitors or serine/threonine protein phosphatases) (Dawson 1998, Fernandes-Ramos *et al*. 2015). Our research group, has satisfactorily screened diverse groups of microalgae for antibacterial activity (Sánchez-Saavedra *et al*. 2010, González-Davis *et al*. 2012, Molina-Cárdenas & Sánchez-Saavedra 2017). Thus, this work was focused on the evaluation of the antimycobacterial activity of hexanic crude extracts obtained from six different phyla of microalgae.

## Materials and methods

### Microalgal material

Microalgal strains examined in this work (Table 1) were *Chlamydomonas mexicana* (NIES-2218), *Porphyridium cruentum* (NIES-2138), *Isochrysis galbana* (CCMP 1323), and *Rhodomonas* sp. (NIES-1005). Additionally, the cyanobacteria *Aphanocapsa marina* and the diatom *Nitzschia palea*, isolated earlier by our group (Fierro-Reséndiz 2006, Jiménez-Valera & Sánchez-Saavedra 2016) were also examined. These microalgal strains were grown in monospecific, nonaxenic batch cultures in Fernbach flasks that contained 2 L of "f" medium (Guillard & Ryther 1962). The microalgal cultures were kept at 20 °C under continuous light that was provided by cold white light lamps

**Table 1. List of microalgae strains, the type of environment and, the strain bank or place of isolation from which they were obtained** / Lista de las microalgas utilizadas, el tipo de ambiente y el lugar de aislamiento o en su caso el cepario del que provienen

| Microalgae strain | Environment | Strain bank or place of isolation |
|---|---|---|
| Cyanophyta | | |
| *Aphanocapsa marina* | Marine | Ensenada, Baja California, México |
| Chlorophyta | | |
| *Chlamydomonas mexicana* | Freshwater | National Institute for Environmental Studies (NIES) |
| Bacillariophyta | | |
| *Nitzschia palea* | Freshwater | Mexicali, Baja California, México |
| Criptophyta | | |
| *Rhodomonas* sp. | Marine | National Institute for Environmental Studies (NIES) |
| Haptophyta | | |
| *Isochrysis galbana* | Marine | National Center for Marine Algae and Microbiota (BIGELOW) |
| Rhodophyta | | |
| *Porphyridium cruentum* | Marine | National Institute for Environmental Studies (NIES) |

(F40D-EX, General Electric®) at 100 µmol photons $m^{-2}$ $s^{-1}$. Growth curves of each microalgal strain were generated to identify their stationary growth phase. To this end, 1 ml of culture sample was taken daily, and its optical density (OD) was measured at 680 nm on a spectrophotometer (6000 UV-VIS, Hach®). The stationary growth phase was defined as the stage in which the OD did not change considerably over 4 consecutive days. The OD values were transformed to a logarithmic scale and plotted. Then, microalgae biomass was harvested during the stationary growth phase by centrifuging the cultures at 3,500 g and 5 °C for 15 min. Then, the supernatant was discarded, the cell pellet was lyophilized. After the lyophilization process, samples were weighed out in order to obtain the yield of the biomass produced from each microalgal strain. Lastly, the lyophilized biomass was stored at -20 °C.

### Preparation of microalgal crude extracts

Lipophilic extracts of the lyophilized biomass were obtained from each microalgal strain using hexane (ACS grade, Fermont®). Briefly, 50 mg of lyophilized biomass was rehydrated with 500 µl of sterile distilled water, frozen in liquid nitrogen, and thawed at room temperature; this process was performed 3 times to lyse the cells in the lyophilized biomass. The biomass was then transferred to amber flasks, to which 50 ml hexane was then added. The biomass-solvent mixture was allowed to stir in darkness at 150 rpm and 21°C overnight on an orbital shaker. Next, the sample was filtered, and the filtrate (*i.e.,* the hexane extract, was recovered). Subsequently, the resulting extracts were dried at 30 °C *in vacuo* to evaporate the hexane and avoid their toxic effect. The dried extracts were weighed out to measure the extract yield. After that, crude extracts were dissolved in 100% dimethylsulfoxide (DMSO) (ACS grade, Fermont®) to a concentration of 10 mg $ml^{-1}$ (Chlipala *et al*. 2009). Finally, the crude extracts were stored in glass amber vials at -20 °C until their use in the antimycobacterial activity assays. All the extraction procedures were performed considering the recommendations mentioned on the implementation of antimicrobial screens (Beutler 2009, Fallarero *et al.* 2015).

### Mycobacterial growth conditions and inoculum preparation

The Mtb strain that we used was H37Rv (ATCC 27294®), which is sensitive to the 5 first-line antituberculosis drugs. The mycobacteria were cultured in 10-ml test tubes with Lowenstein-Jensen medium (MacFaddin 1985) and maintained for 3 weeks at 37 °C in an incubator. At the end of the incubation, the Mtb cultures were in the exponential growth phase and thus ready for use in the antimycobacterial activity assays. The Mtb inoculum was prepared by taking loops of the mycobacteria that was cultured in Lowenstein-Jensen medium and transferring them into a tube with 10 ml sterile distilled water. Then, the Mtb clusters were disrupted and homogenized using a vortex to produce a mycobacterial suspension.

Subsequently, a volume of the mycobacterial suspension was transferred to a new tube with 10 ml sterile distilled water until reaching a turbidity that was similar to that of tube #1 of the McFarland scale, that is equivalent to 3 x $10^8$ CFU $ml^{-1}$. Finally, 0.5 ml of this suspension was added to 9.5 ml of Middlebrook 7H9 medium (Becton, Dickinson and Company®) that was supplemented with 0.5% glycerol (Sigma-Aldrich®) and 10% OADC (oleic acid, albumin, dextrose and catalase) (Becton, Dickinson and Company®) resulting in a final mycobacterial concentration of 1.5 x $10^7$ CFU $ml^{-1}$.

### Determination of the innocuous DMSO concentration

The innocuous concentration of the dimethyl sulfoxide (DMSO) was determined by a colorimetric technique with microplate and different concentrations of DMSO (0.5, 1, 2, 3, 4 and 5%) by triplicate (Galvao *et al*. 2014). The extracts were dissolving at innocuous concentration of 1% (10 mg $ml^{-1}$) with DMSO.

### Antimycobacterial activity assays

The antimycobacterial activity of the extracts was measured by tetrazolium microplate assay (TEMA) (Palomino *et al*. 2007). Briefly, to minimize evaporation from the microplate all perimeter wells were filled with 200 µl of sterile water. As a positive control, in wells B, C, and D of column 10, 50 µl of the antituberculosis drug isoniazid at 4 µg $ml^{-1}$ (Sigma-Aldrich®) and 50 µl of Middlebrook 7H9 medium supplemented with glycerol and OADC as described in the previous section were added with 100 µl of mycobacterial inoculum. In wells E, F, and G of column 10, 98 µl of Middlebrook 7H9 medium, 2 µl 100% DMSO, and 100 µl of mycobacterial inoculum were added, serving as a control for DMSO. In wells B, C, and D of column 11, 200 µl of Middlebrook 7H9 medium was added as the blank. As a negative control, in wells E, F, and G of column 11, 100 µl of Middlebrook 7H9 medium was added with 100 µl of mycobacterial inoculum. In the remaining wells, 2 µl of hexane extract was added in triplicate (*i.e.,* the same microalgal crude extract in 3 different wells with 98 µl of Middlebrook 7H9 medium). Lastly, 100 µl of the mycobacterial inoculum was added, resulting in final concentrations of microalgal crude extracts and DMSO of 100 µg $ml^{-1}$ and 1% (v/v), respectively.

The microplate was kept at 37 °C for 7 days in an incubator, after which all wells received 20 µl of the tetrazolium salt dye MTS (3-4,5-dimethylthiazol-2-yl-5-3-carboxymethoxyphenyl-2-4-sulfophenyl-2H-tetrazolium) (Celltiter 96, Promega®). Then, the microplate was incubated at 37 °C for 2 h, and the OD of the wells was

measured at 550 nm on a microplate reader (Multiskan, Thermo Scientific®). Finally, the percentage growth inhibition (%) was determined by the following formula:

$$\text{Growth inhibition (\%)} = \left[1 - \left(\frac{\text{OD of test well (extract of drug)}}{\text{mean OD of negative control}}\right)\right] \times 100$$

### Determination of the minimum inhibitory concentration (MIC)

The MIC was determined by TEMA with the necessary modifications to calculate it. Subsequently, two-fold serial dilutions were made by taking 100 µl from the wells in row B and transferring them to the wells sequentially from rows C to G. Finally, 100 µl of mycobacterial inoculum was added to all test wells resulting in concentrations for microalgal extracts from 100 to 3.1 µg $ml^{-1}$ and from 1 to 0.03 µg $ml^{-1}$ for isoniazid. The microplates were incubated at 37°C for 7 days, and the inhibition of mycobacterial growth was measured spectrophotometrically using MTS as described in the previous section. The MIC was defined as the lowest concentration of crude extract or drug that inhibited at least 90% of the mycobacterial growth (Collins & Franzblau 1997).

### Statistical analysis

One-way analysis of variance (ANOVA) was used to evaluate the differences in percentage inhibition against Mtb by the hexane crude extracts. The hypotheses underlying the one-way ANOVA were verified: the normality and the homogeneity of variance were analyzed by Shapiro-Wilks test and Brown-Forsythe test, respectively (Zar 2010). The level of significance (α) was 0.05, and when significant differences arose between treatments, Fisher's least significant difference (LSD) test was used. The statistical analyses were performed using GraphPad-Prism®, version 6.0.

## Results

### Microalgal growth and preparation of crude extracts

Four microalgal strains, *A. marina*, *Rhodomonas* sp., *I. galbana,* and *P. cruentum*, did not experience a conditioning phase with exponential growth from the beginning of the culture, whereas *C. mexicana* and *N. palea* did (Fig. 1). The cultures of *I. galbana* and *Rhodomona*s sp. were harvested at eleventh day of culture, while the remaining cultures were harvested on the twelfth day (Table 2). Differences

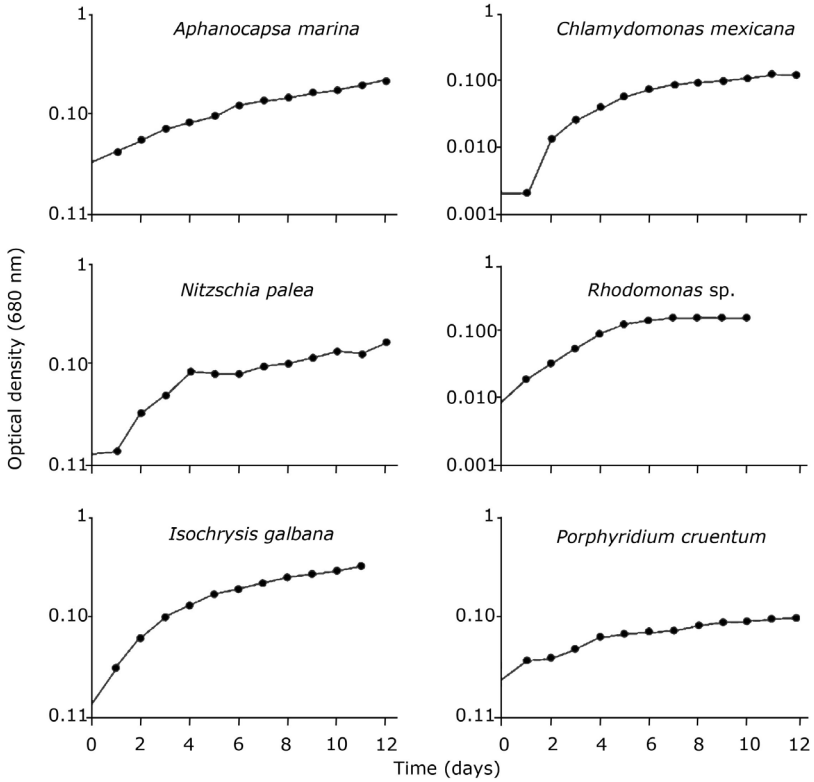


**Figure 1. Growth curves for six microalgae strains used in this study** / Curvas de crecimiento de seis cepas de microalgas utilizadas en este estudio

were observed in biomass production and the extraction yield among microalgal strains (Table 2). The highest biomass production was for *A. marina* (224.4 mg $l^{-1}$) while the lowest production was for *P. cruentum* (60.7 mg $l^{-1}$). Likewise, the highest extraction yield was for *I. galbana* (7.7%) while the lowest yield was for *A. marina* (1.0%) and *C. mexicana* (1%).

Significant differences were found ($P < 0.05$) on the percentage of inhibition of *M. tuberculosis* due DMSO concentrations (Fig. 2). DMSO at 0.5% did not affect the growth of *M. tuberculosis*, meanwhile, the concentrations of 1% of DMSO showed the lower effect on its growth. The concentrations of 2% of DMSO inhibited the growth of *M. tuberculosis* at values nearly 50%, while the concentration of 5% of DMSO completely inhibited the growth of *M. tuberculosis* (Fig. 2).

**Table 2. Biomass harvest day, dry weight values, and extraction yield values for the different microalgae strains used /** Día de cosecha de la biomasa, y valores de peso seco y de rendimiento de extracción para las diferentes microalgas utilizadas

| Microalgae strain | Harvest date (day) | Dry weight (mg $l^{-1}$) | Extraction yield (%) |
|---|---|---|---|
| Cyanophyta | | | |
| *Aphanocapsa marina* | 12 | 224.4 | 1.0 |
| Chlorophyta | | | |
| *Chlamydomonas mexicana* | 12 | 103.5 | 1.0 |
| Bacillariophyta | | | |
| *Nitzschia palea* | 12 | 143.8 | 2.2 |
| Criptophyta | | | |
| *Rhodomonas* sp. | 11 | 119.2 | 4.2 |
| Haptophyta | | | |
| *Isochrysis galbana* | 11 | 91.3 | 7.7 |
| Rhodophyta | | | |
| *Porphyridium cruentum* | 12 | 60.7 | 2.0 |

### Antimycobacterial activity evaluation

All of the hexane crude extracts of the six microalgae strains exhibited considerable antimycobacterial effect. The extracts of *P. cruentum*, *C. mexicana* and *I. galbana* inhibited mycobacterial growth with values of 99.4, 98.4 and 98.3% respectively to the same extent as isoniazid (99.4%) (Fig. 3). Meanwhile, the extracts of *A. marina* (81.6%), *Rhodomonas* sp. (89.3%), and *N. palea* (95.4%) showed about 80 to 90% of growth inhibition (Fig. 3). The lowest MIC was 50 µg $ml^{-1}$, observed for the hexane crude extract of *I. galbana* (Fig. 4). For the remaining extracts, the MIC was the initial concentration that was used in the antimycobacterial assays: 100 µg $ml^{-1}$. While, the MIC of isoniazid was 0.06 µg $ml^{-1}$ (Fig. 4).

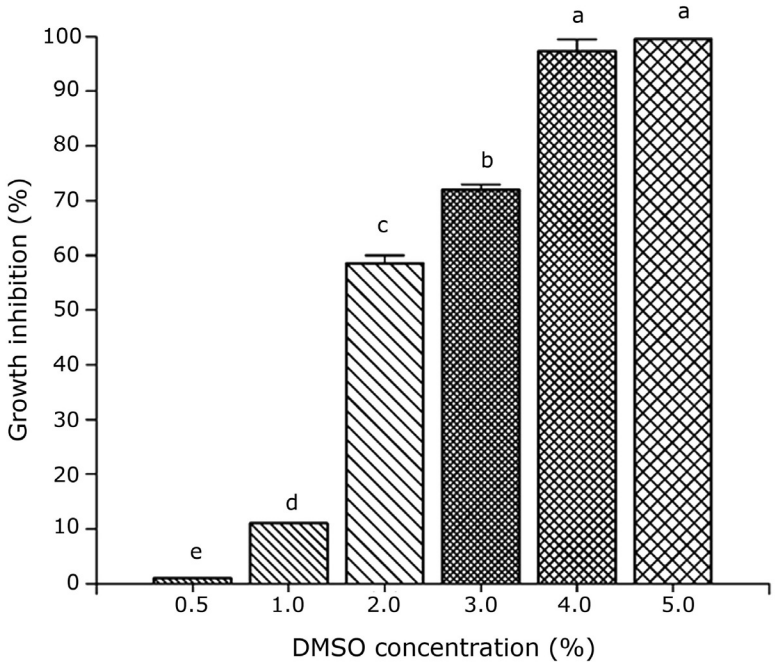


**Figure 2. Mean values and standard deviations of growth inhibition of *Mycobacterium tuberculosis* by effect of differents concentrations of dimethyl sulfoxide (DMSO). Bars with different lowercase letters are significantly different (Kruskal-Wallis one-way ANOVA and Fisher's LSD *post hoc* test; *F*= 123,6, *P* < 0,0001, a>b>c>d>e; mean values ± SD; n= 3)** / Valores promedio y desviación estándar del porcentaje de inhibición del crecimiento de *Mycobacterium tuberculosis* por efecto de diferentes concentraciones de dimetil sulfóxido (DMSO). Las barras con letras minúsculas distintas son significativamente diferentes (ANOVA de una vía por Kruskal-Wallis y prueba *post hoc* de LSD de Fisher; *F*= 126,6, *P* < 0,0001, a>b>c>d>e; los valores son el promedio ± desviación estándar; n= 3)

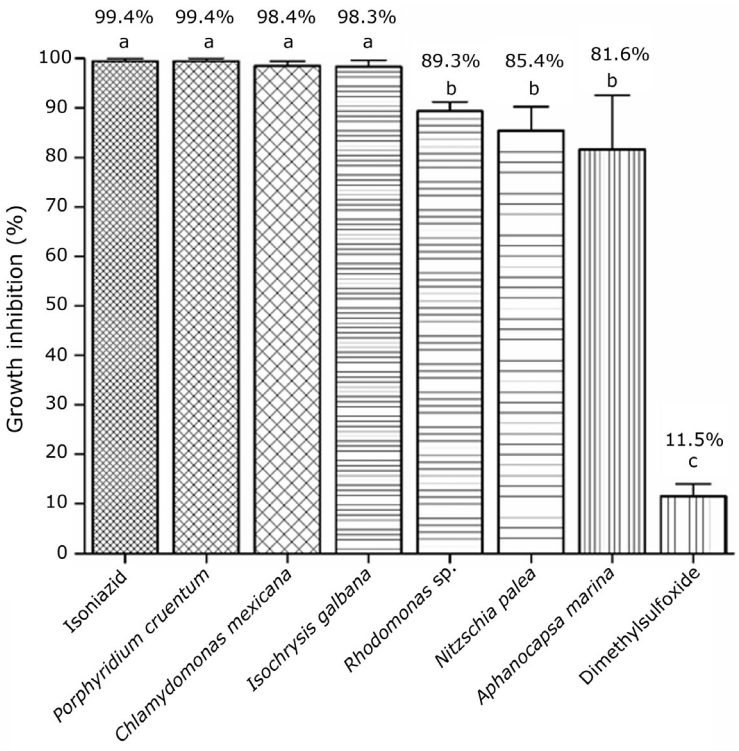


**Figure 3. Percentage of growth inhibition by the crude extracts and control compounds against 1.5 x $10^7$ CFU $ml^{-1}$ *Mycobacterium tuberculosis* H37Rv. Bars with different lowercase letters are significantly different (one-way ANOVA and Fisher's LSD *post hoc* test; *F*= 126.3, *P* < 0.0001, a>b>c; mean values ± SD; n= 3) /** Porcentaje de inhibición de extractos crudos y control en contra 1.5 x $10^7$ CFU $ml^{-1}$ de *Mycobacterium tuberculosis* H37Rv. Las barras con letras minúsculas distintas son significativamente diferentes (ANOVA de una vía y prueba *post hoc* de LSD de Fisher; *F*= 126,3, *P* < 0,0001, a>b>c; promedio ± desviación estándar; n= 3)

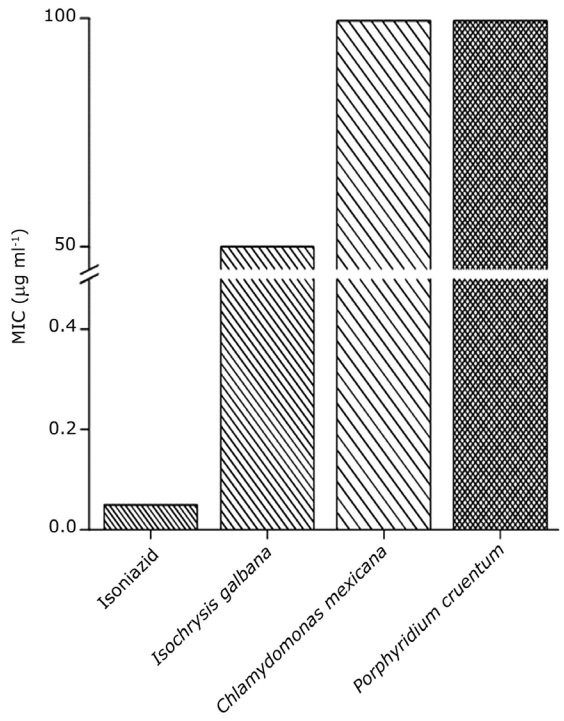


**Figure 4. Minimum inhibitory concentration (MIC) values for the antituberculosis drug isoniazid and the crude extracts that showed a percentage of inhibition of growth equal to this drug /** Valores de la concentración mínima inhibitoria (CMI) para el fármaco antituberculoso isoniazida y para los extractos crudos que tuvieron un porcentaje de inhibición del crecimiento igual a éste fármaco

## Discussion

The differences in growth pattern and biomass production could be due to the adaptability of each strain to the culture conditions and the type of nutrients that are available in the culture medium, as has been reported for various classes of microalgae (Andersen 2005). The microalgal cultures were harvested in the stationary growth phase, in as much as is known that in microalgae the secondary metabolites which commonly possess bioactivity are synthesized during this stage (Namikoshi & Rinehart 1996). When microalgae encounter unfavorable conditions, they tend to favor the adaptation and not the cell division. This adaptation elicits the production of secondary metabolites that confer an advantage during competition for the resources in the environment (Borowitzka 1995).

Methanol, ethanol, dichloromethane, chloroform and hexane are the most commonly used solvents for the extraction of bioactive metabolites from microalgae (Østensvik *et al*. 1998, Kreitlow *et al*. 1999, Sánchez-Saavedra *et al*. 2010). However, most of these studies do not report extraction yields (*i.e*., the weight of the mass obtained by evaporating the solvents). In this work the extraction percentages were calculated, in as much as the extraction yield can be good selection criteria among a group of extracts. It is known that the extraction yield depends

on several factors such as the water content in the sample, the cellular physiology (*e.g.,* whether they are coccoid or filamentous cells, cells with or without cell walls, and the polarity of the metabolites of interest) (Fastner *et al*. 1998).

The antimycobacterial activity of the hexane extracts of *C. mexicana* and *I. galbana* is consistent with other studies that have reported the antimycobacterial effects of crude extracts from Haptohyte and Chlorophyte microalgae (Prakash *et al*. 2010). On the other hand, to the best of our knowledge there are not reports about the antimycobacterial activity by microalgae belonging to phyla Rhodophyta, Cryptophyta or Bacillariophyta. Nonetheless, in this work, the hexane crude extracts from microalgae in these phyla considerably inhibited mycobacterial growth. Also, the antibacterial effects of extracts from Rhodophyta, Cryptophyta, and Bacillariophyta microalgae against various pathogenic bacteria, such as *Staphylococcus aureus*, *Listonella anguillarum*, *Vibrio alginolyticus,* and *Bacillus subtilis,* have been examined extensively (Kellam & Walker 1989, Naviner *et al*. 1999). Conversely, Cyanophyta is the phylum with the greatest number of reports in literature about the antimycobacterial activity by microalgae (Rao *et al*. 2007, Chlipala *et al*. 2009, Broniatowska *et al*. 2011). Some studies that have identified the cyanobacterial compounds responsible for the antimycobacterial activity have found that they are compounds such as terpenes, peptides, alkaloids, aromatic compounds, cyclic peptides, cyclophane, extracellular pigments, phenols, polyketides, polyphenol ethers, terpenoids and anthraquinones (Mo *et al*. 2009, Sturdy *et al*. 2010, Swain *et al.* 2017).

Many authors consider that a crude extract that presents antimycobacterial activity with an MIC $\leq 100$ μg $ml^{-1}$, has the potential for the search of antituberculosis compounds (Molina-Salinas *et al*. 2006, Chlipala *et al*. 2009, Mayer *et al.* 2017). Based on this criterion, the extracts from *I. galbana*, *C. mexicana,* and *P. cruentum* are potential sources of antimycobacterial compounds. Notwithstanding, one must be cautious when the MIC value of a crude extract is used as the criterion in evaluating extracts. That an extract has a lower MIC compared with another extract does not necessarily mean that the active principle in the first extract is more potent than that of the second, because it is possible that the active compound in the first extract is found in a greater quantity than in the second extract. Thus, the activity of the active principle in the second extract can be underestimated (Pauli *et al*. 2005).

The antimycobacterial activity of hexane crude extracts from the six microalgae strains, probably was due to the presence of lipid-nature compounds such as saturated and unsaturated fatty acids, sterols and, terpenes. This is plausible because there are many studies that have reported the antimycobacterial activity of fatty acids and terpenes (Newton *et al*. 2000, Copp 2003), and it is well established that microalgae produce these types of compounds (Hu *et al*. 2008, Pattanaik & Lindber 2015, Martínez-Francés & Escudero-Oñate 2018). One approach to mitigating drug resistance in pathogenic bacteria is the use of new compounds that are unrelated to existing antibiotics (Eom *et al*. 2012). Marine organisms are a tremendous source of novel compounds to combat Mtb drug resistance, because they produce molecules with unique structural features compared with their terrestrial analogous (Larsen *et al*. 2005). To this end, many researcher groups worldwide have performed screenings of microalgae to discover novel antimycobacterial molecules and develop them into new antituberculosis drugs (Garcia *et al*. 2012, Mayer *et al.* 2017).

In conclusion, this study is the first -to the best of our knowledge- to report the antimycobacterial activity of more than two phyla of microalgae. We will analyze the hexane extracts of *P. cruentum*, *C. mexicana,* and *I. galbana* by bioassay-guided fractionation in order to isolate and characterize their antimycobacterial compounds. Lastly, our results are an incentive to continue the search for microalgal molecules with the potential to develop new antituberculosis drugs.

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