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EFFECT OF IRON SALTS ON *Rhodococcus* sp. AND *Gordonia* sp. ON CAROTENOID PRODUCTION

EFECTO DE LAS SALES DE HIERRO EN *Rhodococcus* sp. Y *Gordonia* sp. EN LA PRODUCCIÓN DE CAROTENOIDES

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Abstract
Carotenoids are important pigments, due to their antioxidant activity. The purpose of this research was to observe the effect of GYEA media with $FeSO_4$ and $FeCl_2$ on the total carotenoid content (TCC) and the carotenoid profile of *Rhodococcus* sp. and *Gordonia* sp. In the biomass of *Rhodococcus* sp., with 0.025% $FeSO_4$ a TCC of 14.91±1.66 μg/g was calculated, and two carotenoids were observed; the Carotenoid 2, identified as an astaxanthin dirhamnoside like carotenoid increased from 13.45±0.52% in GYEA media without salts to 24.21±1.48% with 0.025% $FeSO_4$ (T value of 11.81, α 0.05). With the biomass of *Gordonia* sp. in the presence of 0.025% $FeCl_2$ a TCC of 28.66±8.21 μg/g was calculated and three carotenoids were observed, no significant increase was determined for these carotenoids. The formation of carotenoid-iron complexes was observed with UV-Vis.
Keywords: marine actinobacteria, carotenoids, iron salts, TCC, carotenoid profile.

Resumen
Los carotenoides son pigmentos importantes por su actividad antioxidante. El objetivo de esta investigación fue observar el efecto del medio GYEA con $FeSO_4$ y $FeCl_2$ en el contenido total de carotenoides (TCC) y en el perfil carotenoideo de *Rhodococcus* sp. y *Gordonia* sp. En la biomasa de *Rhodococcus* sp. con 0.025% $FeSO_4$ se calculó un TCC de 14.91±1.66 μg/g y se observaron dos carotenoides; el Carotenoide 2, identificado como un carotenoide similar al dirhamnósido de astaxantina incrementó de 13.45±0.52% en medio GYEA sin sales a 24.21±1.48% con 0.025% $FeSO_4$ (valor de T de 11.81, α 0.05). Con la biomasa de *Gordonia* sp. con 0.025% $FeCl_2$ se calculó un TCC de 28.66±8.21 μg/g y se observaron tres carotenoides, no se detectó un incremento significativo para éstos carotenoides. Se observó por UV-Vis la formación de complejos carotenoides-hierro.
Palabras clave: actinobacterias marinas, carotenoides, sales de hierro, TCC, perfil de carotenoides.

1 Introduction

Carotenoids are pigments with biological importance due to their antioxidant activity related to the prevention of diseases such as cancer (lung, colorectal, breast), Alzheimer and Parkinson (Candelas-Cadillo *et al*., 2005; Yahia and Ornelas-Paz, 2010; Vílchez *et al*., 2012). They are a natural alternative to synthetic colorants in pharmaceutical, chemical, cosmetic and food industries (Arredondo-Figueroa *et al*., 2003; Maldonade *et al*., 2007; Querellou *et al*., 2010; Joshi and Rana, 2011; Flores-Miranda *et al*., 2015). Owing to the toxicity detected on some artificial colorants, the demand for natural alternatives has increased.

Carotenoids can be obtained from plants, fruits, vegetables, microalgae, yeasts and bacteria (Chilkov, 2011; Delgado-Vargas *et al*., 2000). Marine microorganisms are becoming important because they can synthesize new metabolites (Joshi *et al*., 2003;

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Lordan *et al*., 2011). In this regard, actinobacteria are important because of their capacity to produce a wide variety of biologically active compounds, among these are the carotenoids (Bull *et al*., 2000; Lam, 2006; Subramani and Aalbersberg, 2012).

The biotechnological production of bacterial carotenoid pigments is an important alternative technique for their large-scale production, because the growth of bacteria is season independent, sustainable and highly productive (Kitaoka *et al*., 1997; Das *et al*., 2007; Mandelli *et al*., 2012). Bacterial carotenoid production depends on factors like temperature, the presence of light and nutrient availability, such as carbon and nitrogen sources as well as mineral salts (Ma and Chen, 2001; Bhosale, 2004).

There is evidence that ferrous ion induces oxidative stress to the bacteria (Ma and Chen, 2001; den Hengst and Buttner, 2008), enhancing the carotenogenesis process as an antioxidant defense mechanism (Groves and Lucana, 2010). On the other hand, the presence of metal ions is relevant since they are cofactors in the carotenoid biosynthesis, especially at translational level and interaction with synthases (Weinberg, 1990; Khodaiyan *et al*., 2007a; Gharibzahedi *et al*., 2013). The effect of iron salts on carotenoid production has been widely studied in microalgae (Mojaat *et al*., 2008) and in cyanobacteria (Chakilam, 2012), while marine actinobacteria have remained less studied (Khodaiyan *et al*., 2007b; Nasri Nasrabadi and Razavi, 2010).

Rhodococcus and *Gordonia* genera, belonging to the class Actinobacteria, have a biotechnological relevance in water and soil bioremediation (Álvarez *et al*., 2008; Marques *et al*., 2011) and recently have been studied as potential carotenoid sources (Jeon *et al*., 2012; Zheng *et al*., 2013). In this research were studied two marine actinobacteria strains, *Rhodococcus* sp. and *Gordonia* sp. isolated from sediments of the Gulf of México, under control conditions (GYEA medium) and in the presence of $FeSO_4$ and $FeCl_2$, to evaluate the influence of iron salts on the total carotenoid content (TCC) and carotenoid profile produced by the two microorganisms. Chávez-Castilla and Aguilar (2015) performed a study of the influence of mineral salts on prodigiosin pigment production by *Serratia marcescens* BS303.

2 Materials and methods

Rhodococcus sp. and *Gordonia* sp. were isolated from sediments from the Gulf of México obtained during an oceanographic campaign (Maldonado *et al*., 2009).

2.1 Production of biomass

2.1.1 Growth of strains on Petri dishes

Rhodococcus sp. and *Gordonia* sp were grown in GYEA medium (control) and GYEA medium supplemented with 0.025, 0.25, 0.5 and 1.00% $FeSO_4$ or $FeCl_2$ respectively (Moraleda-Muñoz *et al*. 2005, Frantz 2009, Nasri Nasrabadi and Razavi 2010) at 28ºC. Growth was monitored for 45 days.

2.1.2 Growth of strains on liquid medium

In an Erlenmeyer flask containing 250 mL of liquid GYEA medium with and without iron salts at 0.025%, strains were inoculated with *Rhodococcus* sp. or *Gordonia* sp. and incubated at 28ºC, 150 rpm for 6 days.

2.2 Carotenoid extraction

The culture media of *Rhodococcus* sp. or *Gordonia* sp. was centrifuged at 4500 rpm for 15 minutes; the biomass was separated and freeze-dried. The extraction of carotenoid pigment was repeated 5 times according to Romero *et al*. (2012) with a mixture of dichloromethane: methanol: acetone (1:1:2) until the discoloration of the biomass, the solvent was removed under reduced pressure, and extracts were stored in refrigeration until further use.

2.3 Assessment of TCC in extracts by UV-Vis spectrometry

The extract was reconstituted in ethanol, the absorbance was measured at 450 nm and TCC was calculated as Baskar *et al*. (2010) using Eq. (1).

$$TCC(\mu g/g) = \frac{AxVx10^4}{A^{1\%}_{1cm}xM} \quad (1)$$

Where A is the absorbance of the sample, V is the volume used to dissolve the sample, $A^{1\%}_{1cm}$ is the molar absorptivity of a carotenoid reference (β-carotene $A^{1\%}_{1cm}$=2620) (Rodríguez-Amaya, 2001) and M is the weight of the sample. Each measure was in triplicate and ANOVA analysis was performed for each strain to determine significant differences, followed by a Dunnett test compared to control.

2.4 *Analysis of the extract by high performance liquid chromatography (HPLC)*

The extracts were analyzed by HPLC under the conditions reported by Islas (2010) using an Agilent 1260 chromatograph, equipped with a diode arrangement detector (DAD), a Phenomenex Luna C18(2) column (150x3mm, 5 μm), using a mixture of methanol: acetonitrile: ethyl acetate: water (80:10:5:5) as mobile phase, the chromatograms were acquired at 450 nm. The peak area % was used to compare the carotenoid profiles with and without iron salts, an independent samples T-test was used to assess differences.

UV-Vis spectra was recorded (190-700 nm) during the chromatographic analysis, the maximum absorption wavelength (λmax) was determined, and if the spectra showed more than one λmax, proportion of λmax III in λmax II (%III/II) was calculated according to Rodríguez-Amaya (2001).

2.5 *Analysis of the extract by mass spectrometry (MS)*

The extracts were analyzed by direct injection using an ESI-MS under the same conditions as Ortega Cabello *et al.* (2016). Molecular weights were compared against Lipid bank and Carotenoid DB databases.

3 Results and discussion

3.1 *Effect of $FeSO_4$ and $FeCl_2$ on microbial growth*

Rhodococcus sp. and *Gordonia* sp. were grown on GYEA media (control) and GYEA supplemented with 0.025% of $FeCl_2$ or $FeSO_4$. After 10 days it was observed, a large amount of colonies in the case of *Rhodococcus* sp. on both salts comparable with the control. *Gordonia* sp. a minor growth compared to control was observed with both salts, however growth with $FeCl_2$ was better than with $FeSO_4$, this behavior is because according to Ivshina *et al.* (2013) *Rhodococcus* genera is more tolerant to the presence of heavy metals than *Gordonia* genus (Fig. 1).

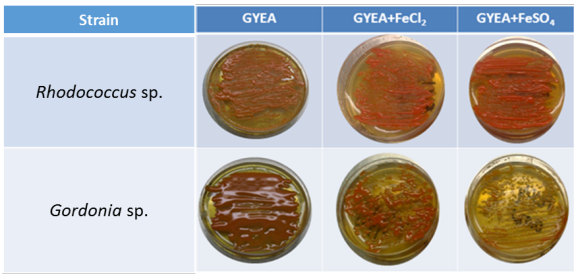

Fig. 1. Comparison of the strains after 10 days of growth under control conditions and in the presence of 0.025% iron salts.

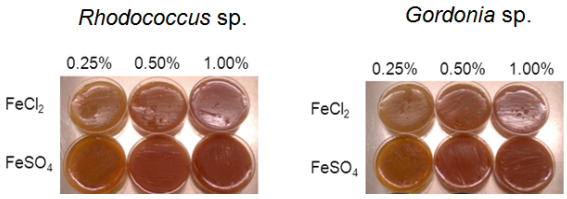

Fig. 2. Growth of strains after 45 days in the presence of iron salts, concentrations from 0.25 to 1%.

However, with the concentrations of 0.25, 0.5 and 1% of the iron salts the growth was extremely low, even after 45 days only a few colonies were observed in both strains, the intensity of coloration of the agar is because of iron salts concentration (Fig. 2). The minimal growth could have been because concentrations of iron salts above 0.025% were toxic for *Rhodococcus* sp. and *Gordonia* sp. (Schmidt *et al.*, 2005).

The coloration of the colonies was different in the presence of 0.025% of iron salts to that observed with control media (Fig. 1). In the case of *Rhodococcus* sp. the color of the colonies in the control was orange and changed to coral in the presence of iron salts; for *Gordonia* sp. the change was from orange to red. The change in the color of the colonies could be an evidence of the carotenoids activity in biological binding of metal ions (Hernández-Marin *et al.*, 2012; Ivshina *et al.*, 2013). According to Schmidt *et al.* (2005) and Ivshina *et al.* (2013), to maintain the homeostasis within the cell, microorganisms must keep the reactive heavy metals at an optimal sub-toxic level, by the formation of intracellular metal non-harmful complexes (Wang *et al.*, 2013).

3.2 *Effect of the iron salts on TCC*

After 10 days of growth on Petri dishes, *Rhodococcus* sp. and *Gordonia* sp. were inoculated on liquid control media and GYEA enriched with 0.025% of iron salts, incubated for 6 days.

Table 1. TCC (μg/g) determination after the 6 days fermentation of marine strains in control media and GYEA supplemented with iron salts

STRAIN	GYEA	GYEA + $FeSO_4$	GYEA + $FeCl_2$
Rhodococcus sp.	15.52±0.94	14.91±1.66	9.04±1.19*
Gordonia sp.	35.01±3.60	19.72±7.18*	28.66±8.21

* Significant difference ($p<0.01$)

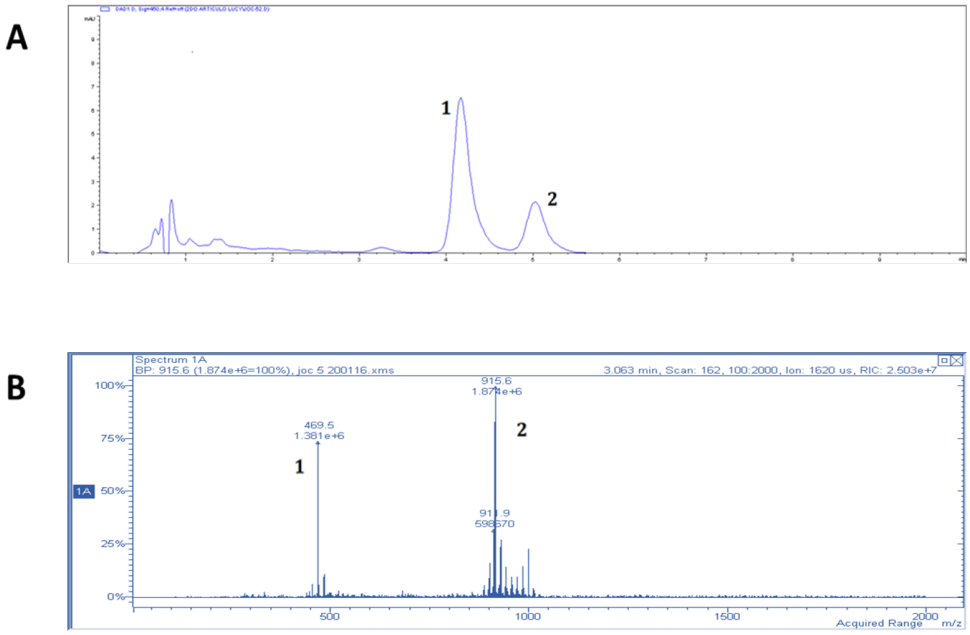

Fig. 3. Carotenoid profile of *Rhodococcus* sp. with $FeSO_4$ by HPLC (A) and ESI-MS of *Rhodococcus* sp. in control media (B).

The TCC in the extract obtained from *Gordonia* sp. with control media was more than double (35 μg/g, Table 1) than that from *Rhodococcus* sp. (15.52 μg/g, Table 1). This means that *Gordonia* sp. is a better carotenoid producer than *Rhodococcus* sp.

The production of carotenoids by *Rhodococcus* sp. and *Gordonia* sp. was dependent on the type of iron salt. The TCC in the extract from *Rhodococcus* sp., with $FeSO_4$ (14.91±1.66 μg/g) was similar to the control (15.52±0.94 μg/g), whereas with $FeCl_2$ the TCC was significantly lower (9.04±1.19 μg/g) 42% less than with the control (F value of 19.36, $p<0.01$, Table 1). On the contrary, the TCC in the extract from *Gordonia* sp. in the presence of $FeCl_2$ (28.66±8.21 μg/g) was similar to the control (35.01±3.60 μg/g), while with $FeSO_4$ a lower TCC was obtained (19.72±7.18 μg/g) 44% less than with the control (F value of 7.61, $p < 0.01$, Table 1). The variability observed in TCC could be due to other antioxidant mechanisms, such as the generation of superoxide dismutase (Albarracín *et al*. 2008, Schulte *et al*. 2010, de Carvalho 2012). To the best of our knowledge, this is the first report about the influence of Fe salts, $FeSO_4$ and $FeCl_2$, on the *Rhodococcus* sp. and *Gordonia* sp. growth and carotenoid production.

3.3 Analysis of carotenoids obtained with and without iron salts

The carotenoids were analyzed by HPLC, ESI-MS and UV-Vis.

Two carotenoids were detected in the extract from *Rhodococcus* sp. cultivated with control media or in the presence of 0.025% $FeSO_4$. The Carotenoid 1 had a retention time of 4.14 min at 450 nm (Fig. 3A) and a single λmax at 480 nm with UV-Vis spectra (Fig. 4).

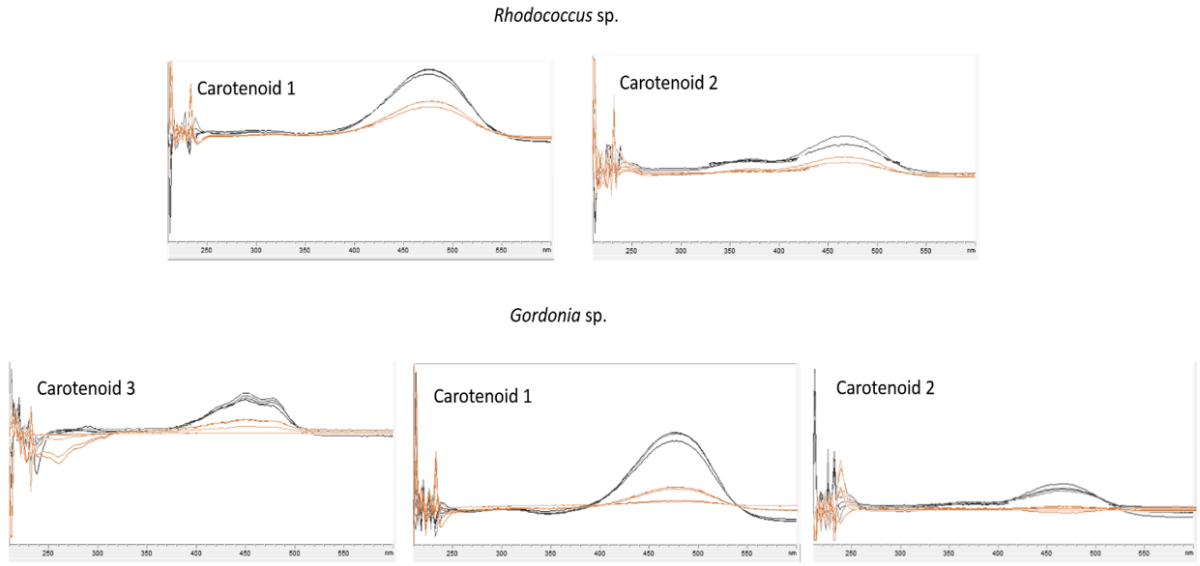

Fig. 4. UV-Vis spectra of carotenoids with control media (black line) and with iron salts (orange line).

Thus suggesting the presence of a carbonyl in conjugation with the olephinic chain, that extends the chromophore and causes a bathochromic effect on the polyene chain, with the concomitant loss in the resolution of the carotenoid UV-Vis spectra (Rodríguez-Amaya 2001). The ion mass of Carotenoid 1 was m/z = 469 (M+Na) (Fig. 3B) from the results and according to the Lipid bank and Carotenoid DB databases it could be inferred that Carotenoid 1 is an 8'-apoastaxanthinal like carotenoid (Etoh *et al*., 2012; Ortega Cabello *et al*., 2016). Carotenoid 2 had a retention time of 4.99 minutes at 450 nm (Fig. 3A), showed a single λmax at 470 nm in the UV-Vis spectra, that is in agreement with the presence of carbonyl group in the structure, as mentioned above (Fig. 4). The ion mass of Carotenoid 2 was 915.1 m/z (M+Na), according to Lipid bank and Carotenoid DB databases it could be inferred that is a carotenoid glycoside, an astaxanthin dirhamnoside like carotenoid (Asker *et al*., 2009; Ortega Cabello *et al*., 2016). Carotenoid glycosides are uncommonly molecules of recent discovery in bacteria, which may have a potential antibiotic activity and can have a role as biosurfactants (Háda *et al*., 2012; Martínez-Trujillo *et al*., 2015), which makes this *Rhodococcus* strain interesting on the production of such compounds. With these results, it can be proposed that carotenoids present in *Rhodococcus* sp. are xanthophylls (Ortega Cabello *et al*., 2016).

Even though the TCC determined in the extracts from *Rhodococcus* sp. with control media and with $FeSO_4$ is similar (Table 1), the carotenoid profile by HPLC was different. The production of carotenoids was modified; the relative quantity of Carotenoid 1 was 86.54±0.53% and was reduced to 75.78±1.48%. Whereas the relative quantity of Carotenoid 2 significantly increased from 13.45±0.52 to 24.21±1.48% (T value of 11.81, α 0.05). The increase of the carotenoid glycoside could have been because carotenoid glycosides provide more stability to the bacteria (Pfander, 1976; Háda *et al*., 2012).

Gordonia sp. produced three carotenoids with control media and in the presence of 0.025% $FeCl_2$. Retention time of Carotenoid 3 was of 3.26 minutes (Fig. 5A), the UV-Vis spectra shows three λmax of 427, 450 and 480 nm (Fig. 4) and a %III/II of 25%. That suggests the presence of hydroxyl groups on its molecule because this group does not affect the absorption of the polyene chain, and could have a cyclic ending at the end of the molecule, as Rodríguez-Amaya (2001) correlated with the λmax at 427 nm. The ion mass of Carotenoid 3 was 301.0 m/z, which suggests that, is another apocarotenoid with hydroxyl groups (Lipid Bank, Carotenoid DB) (Fig. 5B). Carotenoid 3 is the reduced form of a 4-oxo retinaldehyde like carotenoid reported by Ortega Cabello *et al*. (2016). The other two carotenoids with retention times of 4.14 and 4.99 minutes are the same as carotenoids present in *Rhodococcus* sp. Carotenoids 1 and 2.

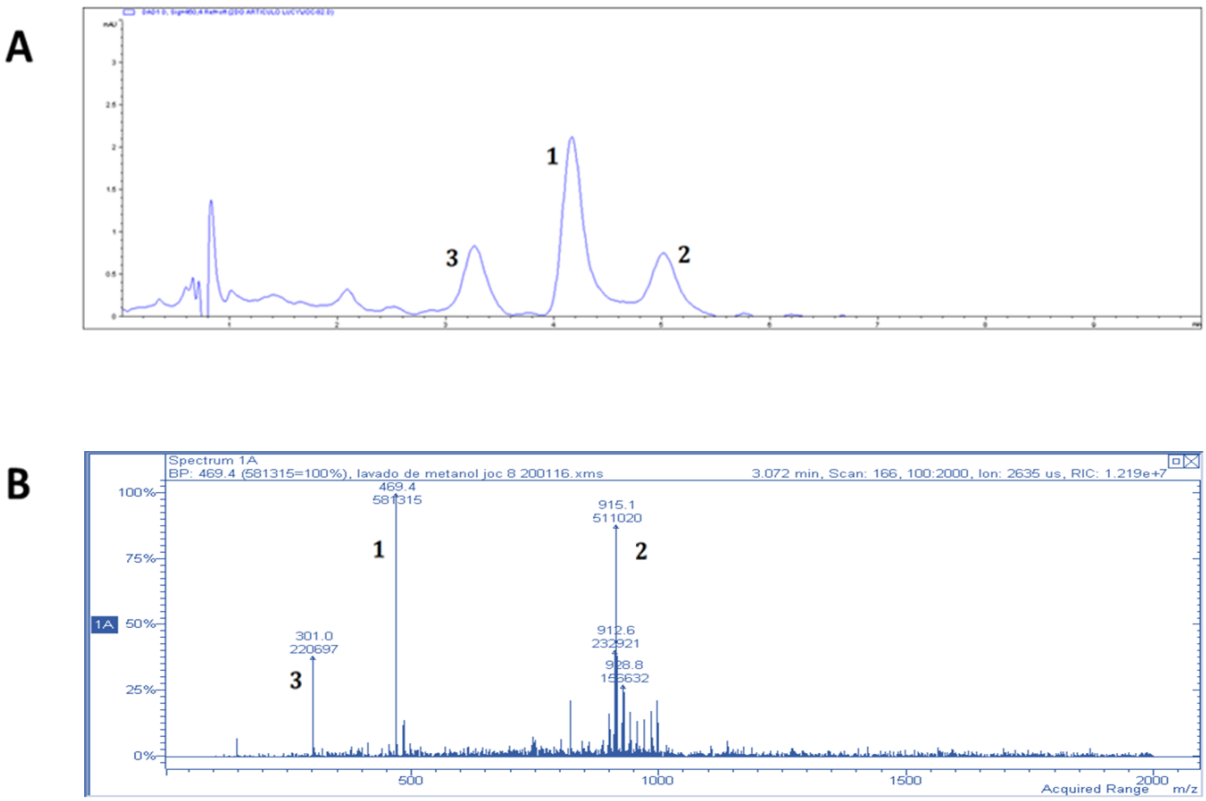

Fig. 5. Carotenoid profile of *Gordonia* sp. with $FeCl_2$ by HPLC (A) and ESI-MS of *Gordonia* sp. in control media (B).

The carotenoid profile by HPLC of *Gordonia* sp. with $FeCl_2$ showed a decrease in the UV-Vis absorption compared to the control, however it was not detected a significant change in the relative quantity for any of the three carotenoids. This behavior, could be explained because there is an antioxidant interaction between Carotenoid 3 and Carotenoid 2 as protection in an oxidative environment (Ortega Cabello *et al*. 2016).

3.4 UV-Vis spectra of carotenoids obtained with and without iron salts

A possible mechanism of bacteria to reach homeostasis in the presence of iron salts, is by the formation of non-harmful complexes between xanthophylls and iron (Polyakov *et al*., 2010; Hernández-Marín *et al*., 2012; Ivshina *et al*., 2013). These complexes can also affect the UV-Vis spectra of carotenoids changing the λmax (Polyakov *et al*., 2010), as was observed in UV-Vis spectra of Carotenoid 2 of *Rhodococcus* sp. and differences in absorption as were seen in every spectra of Fig. 4.

The chelating properties of carotenoids is an ability conferred to xanthophylls because these kind of carotenoids divert the excitation energy when such complex is formed, with concomitant changes in coloration and absorption as discussed earlier (Schmidt *et al*., 2005; Polyakov *et al*., 2010; Hernández-Marin *et al*., 2012).

3.5 Importance of carotenoid-iron complexes

Even though the TCC was not improved in the presence of iron salts, carotenoid iron complexes have important biological activity. The antioxidant activity of carotenoid-iron complexes cannot be measured by conventional methods because they give higher measures (Prior *et al*., 2005; Ozgen *et al*., 2006). Hernández-Marín *et al*. (2012) demonstrated that this complex could be a good electron donor/acceptor of superoxide radical; Polyakov *et al*. (2010) proposed that these complexes might have an important role

in photoprotection. Hess *et al*. (2005), Hurrell and Egli (2010) and Etcheverry *et al*. (2012) have also suggested a possible link between carotenoid and iron intake in the induction of erithropoyetic processes, as an immunomodulation mechanism of carotenoids, although it remains unclear.

Conclusions

The growth of *Rhodococcus* sp. with iron salts was similar to control media and with *Gordonia* sp. was lower with iron salts. Concentrations higher than 0.025% of iron salts showed to be harmful on both strains. The increase on TCC of both strains was not significant in presence of 0.025% of iron salts. In the carotenoid profile of *Rhodococcus* sp., an increase on the astaxanthin dirhamnoside like carotenoid (Carotenoid 2) was determined because it provides more stability to *Rhodococcus* sp. with a concomitant decrease of carotenoid similar to 8'-apoastaxanthinal (Carotenoid 1), thus observing the effect of iron salts on this strain. No significant increase was determined for any of the three carotenoids present on *Gordonia* sp, therefore there was no effect of iron salts. The formation of carotenoid-iron complexes was observed, these complexes can be biologically important, as better free radical scavenging agents or to increase iron absorption, thus improving the immune system of users; however further studies need to be done in order to confirm the biological importance of such complexes. A carotenoid glycoside (astaxanthin dirhamnoside like carotenoid) was characterized in both strains with probable biological relevance as antibiotic and surfactant.

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References

Albarracín, V. H., Ávila, A. L., Amoroso, M. J. and Abate, C. M. (2008). Copper removal activity by Streptomyces strains with dissimilar growth patterns and endowed with cupric reductase activity. *FEMS Microbiology Letters 288*, 141-148.

Alvarez, V. M., Santos, S. C., Casella, R. C., Vital, R. L., Sebastin, G. V. and Seldin, L. (2008). Bioremediation potential of a tropical soil contaminated with a mixture of crude oil and production water. *Journal of Microbiology and Biotechnology 18*, 1966-1974.

Arredondo-Figueroa, J. L., Pedroza-Islas, R., Ponce-Palafox, J. T. and Vernon-Carter, E. J. (2003). Pigmentation of pacific White shrimp (*Litopenaeus vannamei*, Boone 1931) with esterified and saponified carotenoids from red chili (*Capsicum annuum*) in comparison to astaxanthin. *Revista Mexicana de Ingeniería Química 2*, 101-108.

Asker, D., Amano, S., Morita, K., Tamura, K., Sakuda, S., Kikuchi, N., Furihata, K., Matsufuji, H., Beppu, T. and Ueda, K. (2009). Astaxanthin dirhamnoside, a new astaxanthin derivative produced by a radio-tolerant bacterium, *Sphingomonas astaxanthinifaciens. The Journal of Antibiotics 62*, 397-399.

Baskar, V., Madhanraj, P., Kanimozhi, K. and Panneerselvam, A. (2010). Characterization of carotenoids from *Streptomyces* sp. of marine and fresh water environment. *Annals of Biological Research 2*, 380-388.

Bhosale, P. (2004). Environmental and cultural stimulants in the production of carotenoids from microorganisms. *Applied Microbiology and Biotechnology 63*, 351-361.

Bull, A. T., Ward, A. C. and Goodfellow, M. (2000). Search and discovery strategies for biotechnology: the paradigm shift. *Microbiology and Molecular Biology Reviews 64*, 573-606.

Candelas-Cadillo, M. G., Alanís-Guzmán, M. G. J., Bautista-Justo, M., Del Río-Olague, F. and García-Díaz G. (2005). Contenido de licopeno en jugo de tomate secado por aspersión. *Revista Mexicana de Ingeniería Química 4*, 299-307.

Carotenoid DB. (2016). Available from: http://carotenoiddb.jp/list1.html.Accesed: May 3rd 2016.

Chakilam, S. (2012). Metal effects on carotenoid content of cyanobacteria. *International Journal of Botany 8*, 192-197.

Chávez-Castilla, L., Aguilar, O. (2015). Effect of mineral supplementation and type of starch on the production of prodigiosin from a culture of *Serratia marcescens* BS303. *Revista Mexicana de Ingeniería Química 14*, 641-652.

Chilkov, N. (2011). Benefits of carotenoids: what colors are on your plate? Available from: http://www.huffingtonpost.com/nalini-chilkov/carotenoidsbenefits_b_889586.html. Accessed: March 19th 2016.

Das, A., Yoon, S-H., Lee, S-H., Kim, J-Y., Oh, D-K. and Kim, S-W. (2007). An update on microbial carotenoid production: application of recent metabolic engineering tools. *Applied Microbiology and Biotechnology 77*, 505-512.

Delgado-Vargas, F., Jiménez, A. R. and Paredes-López, O. (2000). Natural pigments: carotenoids, anthocyanins, and betalains-characteristics, biosynthesis, processing, and stability. *Critical Reviews in Food Science and Nutrition 40*, 173-289.

de Carvalho C. C. C. R. (2012). Adaptation of *Rhodococcus erythropolis* cells for growth and bioremediation under extreme conditions. *Research in Microbiology 163*, 125-136.

den Hengst, C. D. and Buttner, M. J. (2008). Redox control in actinobacteria. *Biochimica et Biophysica Acta (BBA)-General Subjects 1780*, 1201-1216.

Etcheverry, P., Grusak, M. A. and Fleig, L. E. (2012). Application of *in vitro* bioaccessibility and bioavailability methods for calcium, carotenoids, folate, iron, magnesium, polyphenols, zinc, and vitamins B_6, B_{12}, D, and E. *Frontiers in Physiology 3*, 1-22.

Etoh, H., Suhara, M., Tokuyama, S., Kato, H., Nakahigashi, R., Maejima, Y., Ishikura, M., Terada, Y. and Maoka, T. (2012). Auto-oxidation products of astaxanthin. *Journal of Oleo Science 61*, 17-21.

Flores-Miranda, G. A., Valencia del Toro, G. and Yáñez-Fernández, J. (2015). Stability evaluation of β-carotene nanoemulsions prepared by homogenization-emulsification process using stearic acid as oil phase. *Revista Mexicana de Ingeniería Química 14*, 667-680.

Frantz, C. M. (2009). *Miscellaneous Experiments with Phototrophic Communities*. Marine Biological Laboratory, USA.

Gharibzahedi, S. M. T., Razavi, S. H. and Mousavi, S. M. (2013). Microbial canthaxanthin: Perspectives on biochemistry and biotechnological production. *Engineering in Life Sciences 13*, 408-417.

Groves, M. R. and Lucana, D. (2010). Adaptation to oxidative stress by Gram-positive bacteria: the redox sensing system HbpS-SenS-SenR from *Streptomyces reticuli*. In *Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology* (A. Méndez-Vilas, ed), Pp. 33-42. Formatex Research Center, SPA.

Háda, M., Nagy, V., Deli, J. and Agócs, A. (2012). Hydrophilic carotenoids: recent progress. *Molecules 17*, 5003-5012.

Hernández-Marin, E., Barbosa, A. and Martínez, A. (2012). The metal cation chelating capacity of astaxanthin. Does this have any influence on antiradical activity? *Molecules 17*, 1039-1054.

Hess, S. Y., Thurnham, D. I. and Hurrell, R. F. (2005). *Influence of provitamin A carotenoids on iron, zinc, and vitamin A status*. Harvest Plus, USA.

Hurrell, R. and Egli, I. (2010). Iron bioavailability and dietary reference values. *The American Journal of Clinical Nutrition 91*, 1461s-1467s.

Islas, R.P. (2010). *Tratamiento microbiano de residuos de camarón para obtención de quitina y astaxantina*. Tesis de Maestría en Biotecnología, UAM-Iztapalapa, México.

Ivshina, I., Kuyukina, M.and Kostina, L. (2013). Adaptive mechanisms of nonspecific resistance to heavy metal ions in alkanotrophic actinobacteria. *Russian Journal of Ecology 44*, 123-130.

Jeon, B. Y., Kim, B. Y., Jung, I. L. and Park, D. H. (2012). Metabolic roles of carotenoid produced by non-photosynthetic bacterium *Gordonia alkanivorans* SKF120101. *Journal of Microbiology and Biotechnology 22*, 1471-1477.

Joshi, V. K., Attri, D., Bala, A. and Bhushan, S. (2003). Microbial pigments. *Indian Journal of Biotechnology 2*, 362-369.

Joshi, V. K. and Rana, N. S. (2011). Optimization of apple pomace based medium and fermentation conditions for pigment production by *Sarcina* sp. *Indian Journal of Natural Products Resources 2*, 421-427.

Kitaoka, M., Tsubokura, A. and Kiyota, T. (1997). Process for extraction of carotenoids from bacterial cells. *Google Patents*, US5591343 A.

Khodaiyan, F., Razavi, S. H., Emam-Djomeh, Z. and Mousavi, S. (2007a). Optimization of canthaxanthin production by *Dietzia natronolimnaea* HS-1 using response surface methodology. *Pakistan Journal of Biological Sciences 10*, 2544-2552.

Khodaiyan, F., Razavi, S. H., Emam-Djomeh, Z., Mousavi, S. M. A. and Hejazi, M. A. (2007b). Effect of culture conditions on canthaxanthin production by *Dietzia natronolimnaea* HS-1. *Journal of Microbiology and Biotechnology 17*, 195-201.

Lam, K. S. (2006). Discovery of novel metabolites from marine actinomycetes, *Current Opinion in Microbiology 9*, 245-251.

Lipid bank. (2007). Available from: http://lipidbank.jp/cgi-bin/main.cgi?id=VCA. Accessed: March 14th 2016.

Lordan, S., Ross, R. P. and Stanton, C. (2011). Marine bioactives as functional food ingredients: potential to reduce the incidence of chronic diseases. *Marine Drugs 9*, 1056-1100.

Ma, R. and Chen, F. (2001). Induction of astaxanthin formation in the green microalga *Chlorococcum* sp. by reactive oxygen species (ROS) under mixotrophic conditions of growth. In *Algae and their Biotechnological Potential* (F. Chen and F. Jiang, eds), Pp. 121-126. Springer, USA.

Maldonade, I. R., Scamparini, A. R. P. and Rodríguez-Amaya, D. B. (2007). Selection and characterization of carotenoid-producing yeasts from Campinas region, Brazil. *Brazilian Journal of Microbiology 38*, 65-70.

Maldonado, L. A., Fragoso-Yáñez, D., Pérez-García, A., Rosellón-Druker, J. and Quintana, E. T. (2009) Actinobacterial diversity from marine sediments collected in México. *Antonie Van Leeuwenhoek 95*, 111-120.

Mandelli, F., Yamashita, F., Pereira, J. L. and Mercadante, A. Z. (2012). Evaluation of biomass production, carotenoid level and antioxidant capacity produced by Thermus filiformis using fractional factorial design. *Brazilian Journal of Microbiology 43*, 126-134.

Marques, V., Montezano, J., Korenblum, E. and Seldin, L. (2011). Comparative bioremediation of crude oil-amended tropical soil micrococosms by natural attenuation, bioaugmentation, or bioenrichment. *Applied and Environmental Soil Science*, 1-10. http://dx.doi.org/10.1155/2011/156320.

Martínez-Trujillo, Ma. A., Membrillo Venegas, I., Vigueras-Carmona, S. E., Zafra-Jiménez, G., García-Rivero, M. (2015). Optimization of a bacterial biosurfactant production. *Revista Mexicana de Ingeniería Química 14*, 355-362.

Mojaat, M., Pruvost, J., Foucault, A. and Legrand, J. (2008). Effect of organic carbon sources and Fe^{2+} ions on growth and β-carotene accumulation by *Dunaliella salina*. *Biochemical Engineering Journal 39*, 177-184.

Moraleda-Muñoz, A., Pérez, J., Fontes, M., Murillo, F. J. and Muñoz-Dorado, J. (2005). Copper induction of carotenoid synthesis in the bacterium Myxococcus xanthus. *Molecular Microbiolog 56*, 1159-1168.

Nasri Nasrabadi, M. and Razavi, S. (2010). Enhancement of canthaxanthin production from *Dietzia natronolimnaea* HS-1 in a fed-batch process using trace elements and statistical methods. *Brazilian Journal of Chemical Engineering 27*, 517-529.

Ortega Cabello, L., Pérez Méndez, H. I., Manjarrez Alvarez, N., Maldonado Manjarrez, L. A., Cassani Hernández, J., Quintana Cano, E. T. and López Luna, A. (2016). Characterization and antioxidant activity of carotenoid mixtures present in *Rhodococcus* sp. and *Gordonia* sp. *Journal of Chemical and Pharmaceutical Research 8*, 879-888.

Ozgen, M., Reese, R. N., Tulio, A. Z., Scheerens, J. C. and Miller, A. R. (2006). Modified 2, 2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method to measure antioxidant capacity of selected small fruits and comparison to ferric reducing antioxidant power (FRAP) and 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) methods. *Journal of Agricultural and Food Chemistry 54*, 1151-1157.

Pfander, H. (1976). Carotenoid glycosides. *Pure and Applied Chemistry 47*, 121-128.

Polyakov, N. E., Focsan, A. L., Bowman, M. K. and Kispert, L. D. (2010). Free radical formation in novel carotenoid metal ion complexes of astaxanthin. *The Journal of Physical Chemistry B 114*, 16968-16977.

Prior, R. L., Wu, X. and Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry 53*, 4290-4302.

Querellou, J., Børresen, T., Boyen, C., Dobson, A., Höfle, M., Ianora, A., Jaspars, M., Kijjoa, A., Olafsen, J. and Rigos, G. (2010). *Marine Biotechnology: A New Vision and Strategy for Europe*. European Science Foundation, EU, Marine Board. Available from: http://www.marinebiotech.eu/sites/marinebiotech.eu/files/public/library/MBT%20publications/2010%20ESF%20Position%20Paper.pdf

Rodríguez-Amaya, D. B. (2001). *A Guide to Carotenoid Analysis in Foods*. ILSI press, USA.

Romero, F., Fernández-Chimeno, R. I., de la Fuente, J. L. and Barredo, J. L. (2012). Selection and taxonomic identification of carotenoid-producing marine actinomycetes. In *Microbial Carotenoids from Bacteria and Microalgae: Methods and Protocols* (J. L. Barredo, ed), Pp. 75-83. Springer Science+Bussiness Media, USA.

Schmidt, A., Haferburg, G., Sineriz, M., Merten, D., Büchel, G. and Kothe, E. (2005). Heavy metal resistance mechanisms in actinobacteria for survival in AMD contaminated soils. *Chemie der Erde-Geochemistry 65*, 131-144.

Schulte, C., Arensköter, M., Berekaa, M. M., Arensköter, Q. and Priefert H. and Steinbüchel, A. (2008). Possible involvement of an extracellular superoxide dismutase (SodA) as a radical scavenger in Poly(cis-1,4-isoprene) degradation. *Applied and Environmental Microbiology 74*, 7643-7653.

Subramani, R. and Aalbersberg, W. (2012). Marine actinomycetes: An ongoing source of novel bioactive metabolites. *Microbiological Research 167*, 571-580.

Vilchez, C., Forjan, E., Cuaresma, M., Bedmar, F., Garbayo, I. and Vega, J. M. (2011). Marine carotenoids: biological functions and commercial applications. *Marine Drugs 9*, 319-333.

Wang, Y., Liu, Z. and Qin S. (2013). Effects of iron on fatty acid and astaxanthin accumulation in mixotrophic *Chromochloris zofingiensis. Biotechnology Letters 35*, 351-357.

Weinberg, E. D. (1990). Roles of trace metals in transcriptional control of microbial secondary metabolism. *Biology of Metals 2*, 191-196.

Yahia, E. M. and Ornelas-Paz, J. J. (2010). Chemistry, stability, and biological actions of carotenoids. In *Fruit and Vegetable Phytochemicals: Chemistry, Nutritional Value and Stability*, (L. De la Rosa, E. Álvarez-Parrilla. and G. González-Aguilar, eds.), Pp. 177-222.Willey-Blackwell Publishing, USA.

Zheng, Y-T., Toyofuku, M., Nomura, N. and Shigeto, S. (2013). Correlation of carotenoid accumulation with aggregation and biofilm development in *Rhodococcus* sp. SD-74. *Analytical Chemistry 85*, 7295-7301.