Int.J.Curr.Microbiol.App.Sci (2018) *7*(2): 1577-1586

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 7 Number 02 (2018)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.702.190

Morphological Changes in *Fouquieria splendens* Callus Cocultivated with Endophyte Bacteria

Daniel Corona-Álvarez[1], Verónica Azeret Salinas-Patiño[1], MaríaVictoria Hernández-Pimentel[1], Silvano Montes-Villafan[1], Marcial García-Pineda[2], Leonor Angélica Guerrero-Zúñiga[3] and Angélica Rodríguez-Dorantes[1*]

[1]Laboratorio de Fisiología Vegetal, Departamento de Botánica, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, México City 11340, México
[2]Jardín Botánico, Facultad de Estudios Superiores, Iztacala, Universidad Autónoma de México, Los Reyes Iztacala s/n, México City54090, México
[3]Instituto Mexicano del Petróleo, México City 07730, México

**Corresponding author*

Keywords

Biotization, Callus culture, Endophytes, Co-cultive

Article Info

Accepted:
15 January 2018
Available Online:
10 February 2018

A B S T R A C T

Functional associations between phytobacteria and cultured plants have often been successful; some authors suggest that co-cultivation of bacterial strains with *in vitro* cells or tissues, seems to be one of the appropriate approaches for mass propagation of plants. This study analyzed the effect on growth and development of a desert plant *Fouquieria splendens* callus, co-cultivated with an endophyte bacterium. From all the obtained results, *F. splendens* callus evaluated at 19 and 45 days showed no visible damage, discoloration and diminish of growth comparing between co-cultivated and no co-cultivated conditions. Particularly the plant regulators added to the medium and the presence of the endophyte bacteria, favored the increase in callus biomass of this plant species. Scanning electron microscopy analysis of the selected callus showed morphological changes induced in co-cultivated conditions with the development of an extracellular matrix, giving an early shoots development, as a differentiation event. In this work, the establishment of co-cultives between *F. splendens* callus and the streaked endophyte on culture medium showed that it could be a complex network of responses between the plant growth regulators present in culture medium and the release of phytohormones produced by the phytobacteria.

Introduction

The use of *in vitro* systems allows the dissection of the complex interaction between plant, soil, and microorganisms, by the analysis of stress factors that affects metabolism and specific enzymes involved in the plant's response. Golan-Goldhirsh *et al.*, (2004) mention that the advantages of plant tissue cultures can be summarized in two principal aspects: their reproducible growth during long periods and the sterile cultures allows the measurement of physiological activities that can be controlled by

environmental conditions. Attempts to create functional associations between the bacteria and cultured plants under *in vitro* conditions have often been successful (Bertrand *et al.,* 2001).

Nowak (1998), define the *in vitro* co-culture of tissue explants with beneficial microbes that induces developmental and metabolic changes as ‘‘biotization’’. Some plant growth promoting bacteria are able to enhance the growth and development of plants by interfering in the concentration of known phytohormones (Khalid *et al.,* 1997).

Parray *et al.,* (2015) mention that keeping in view the present knowledge; co-cultivation of rhizobacterial strains with *in vitro* raised cells/tissues etc. seems to be one of the appropriate approaches for mass propagation of plants.

Preininger *et al.,* (1997) notes that in the past few years, there has been an increasing interest in this approach to understand the symbiotic mechanisms in the co-culture between plant growth promoting bacteria and plant tissue cultures favoring a better propagation of plants.

Nowak (1998) also suggest that this relationship can increase the plant cell biomass and survival rate of tissue cultures. Some biotization experiments reported successfully symbiotic relationships for carrot (Varga *et al.,* 1994), strawberry (Preininger *et al.,* 1997; Vestberg *et al.,* 2004), sugarcane (Oliveira *et al.,* 2002) and *Crocus sativa* L. (Parray *et al.,* 2015). All these studies showed significant effects in morphogenetic responses towards plant growth.

This study analyzed the effect on growth and development of the desert plant *Fouquieria splendens* callus, co-cultivated with an endophyte bacterium.

Materials and Methods

Callus culture inoculant

The endophyte bacteria employed: *Staphylococcus pasteuri* strain Fs2, was isolated by Salinas (2018), from leaves of the desert plant *Fouquieria splendens*, grown in the botanical garden of the Facultad de Estudios Superiores, Iztacala (FES)-UNAM, it was classified according to Khalid *et al.,* (2004) by its *in vitro* production of indole acetic acid (IAA)as medium producer (13µg/mL). Bacterial inoculum was maintained by culturing it on plates with nutritive agar (NA) medium for 48 h at 28ºC.

Fouquieria splendens callus culture

Callus were obtained from leaves of *F. splendens* plants, these were surface-sterilized with sodium hypochlorite solution (10%) for 45 seconds, followed by several rinses in sterile distilled water. Leaf explants were obtained aseptically cutting fractions of $1cm^2$. Five explants were placed separately in baby food flasks with Magenta SIGMA caps containing 25mL of Murashige and Skoog (MS)¼ salts medium(Murashige and Skoog, 1962) supplemented with 30 g/L of sucrose, 1mg/L of naphthalene acetic acid (NAA) and 1.5mg/L of kinetin (KIN) and 3 g/L phytagel, incubated at 28°C with photoperiod of 16 h light /8 h dark, for 30 days.

Fouquieria splendens callus co-cultive with *Staphylococcus pasteuri* strain Fs2

In vitro co-cultives of *F. splendens* callus and the endophyte bacteria were established according to Sharma *et al.,* (2015), divided in two sequential phases, as follows: in the first phase, Petri dishes containing MS ¼ salts medium, with different phytohormones combination: “MS” Medium (without phytohormones), “MSB” Medium (NaH_2PO_4

42.5mg/L + 5mg/L NAA + 1mg/L KIN), "MSD" Medium (5mg/L NAA + 1mg/L KIN) and "MSE" Medium (1mg/L NAA + 1.5mg/L KIN), supplemented with 30 g/L of sucrose and 3 g/L phytagel; were equal divided in three sections, where an inoculum with calibrated loop (1/100 cells) was obtained from the endophyte strain cultured on plates with NA, streaked at the left and right sides of the central division. Control plates were considered without the streak of the endophyte bacteria. All Petri dishes were incubated for 48 h at 28°C. The second phase of the established co-cultures initiated after the incubation of the endophyte strain when colonies appeared on the medium. Four pieces ($16mm^2$, each one) of the obtained *F. splendens* callus where deposited in the central division of the plates, with or without the endophyte bacteria. Petri dishes were sealed with Parafilm to prevent water loss and incubated at 28°C with photoperiod of 16 h light /8 h dark. All the experiments were performed by triplicate and the effect of co-cultives was analyzed at 19 and 48 days.

Analysis of the co-cultive effect on *Fouquieria splendens* callus

Callus pieces were recovered and the increase of callus biomass was recorded by the measure of the area (mm^2), selected fractions were prepared for scanning electron microscopy (SEM) for the analysis of morphological changes induced in *F. splendens* callus. The samples were cut into 4 or 5mm pieces, fixed in 2.5% gluataraldehyde for 1 h at room temperature (27°C), then, washed three times in a phosphate buffer, post fixed in 1% OsO_4 for 1h at room temperature, washed and dipped into distilled water for three times. The samples were undergone a series of dehydration processes: 30 % ethanol for 10 min; 40 % ethanol for 10 min; 50 % ethanol for 10 min; 60 % ethanol for 10 min; 70 % ethanol for 10 min; 80 % ethanol for 10 min; 90 % ethanol for 10 min and finally 100 % ethanol for 10 min (three times). The material was dried in the critical point dryer apparatus, mounted and sputter-coated with gold in an ion coater for 60 seconds. Finally, the samples were ready for viewing under field scanning electron microscope JSM 5800 LV for their examination and photography.

Statistical analysis

All data obtained were analyzed by one-way analysis of variance and the mean differences were compared applying a Tukey-Kramer Method using the statistics program Graph Pad Instat Ver. 2.03. Principal Component Analysis (PCA) was done with matrix data set of all the experimental conditions of co-cultures and both times; employing the Pearson correlation, with PAST (Paleontological Statistics Software Package) Ver. 2.17b.

Results and Discussion

Growth of *F. splendens* callus culture

Figures 1 and 2, shows the progress of callus development under no co-cultivated (Fig.1) and co-cultivated (Fig. 2) conditions, where according to the rating system proposed by Souissi and Kremer (1994; 1998) for the evaluation of the phytobacteria and the absence of producing an inhibitory or promontory effect on callus growth, based on visual observations; in this study, only the callus cultured in MS medium co-cultivated or not, showed a diminished growth and browning color, categorized at the highest rating callus damage number 4, with characteristics like tissue color change, cellular leakage, callus disintegration and severe growth reduction. The rest of the callus were categorized in rating number 0, with no visible callus damage, no discoloration and an obvious not growth reduction.

Fig.1 *In vitro Fouquieria splendens* callus development, under no- co-cultive conditions, all the control callus grown in MS, MSB, MSD and MSE medium. Numbers indicate: 1= initial callus growth, 2 = 19 days and 3 = 48 days

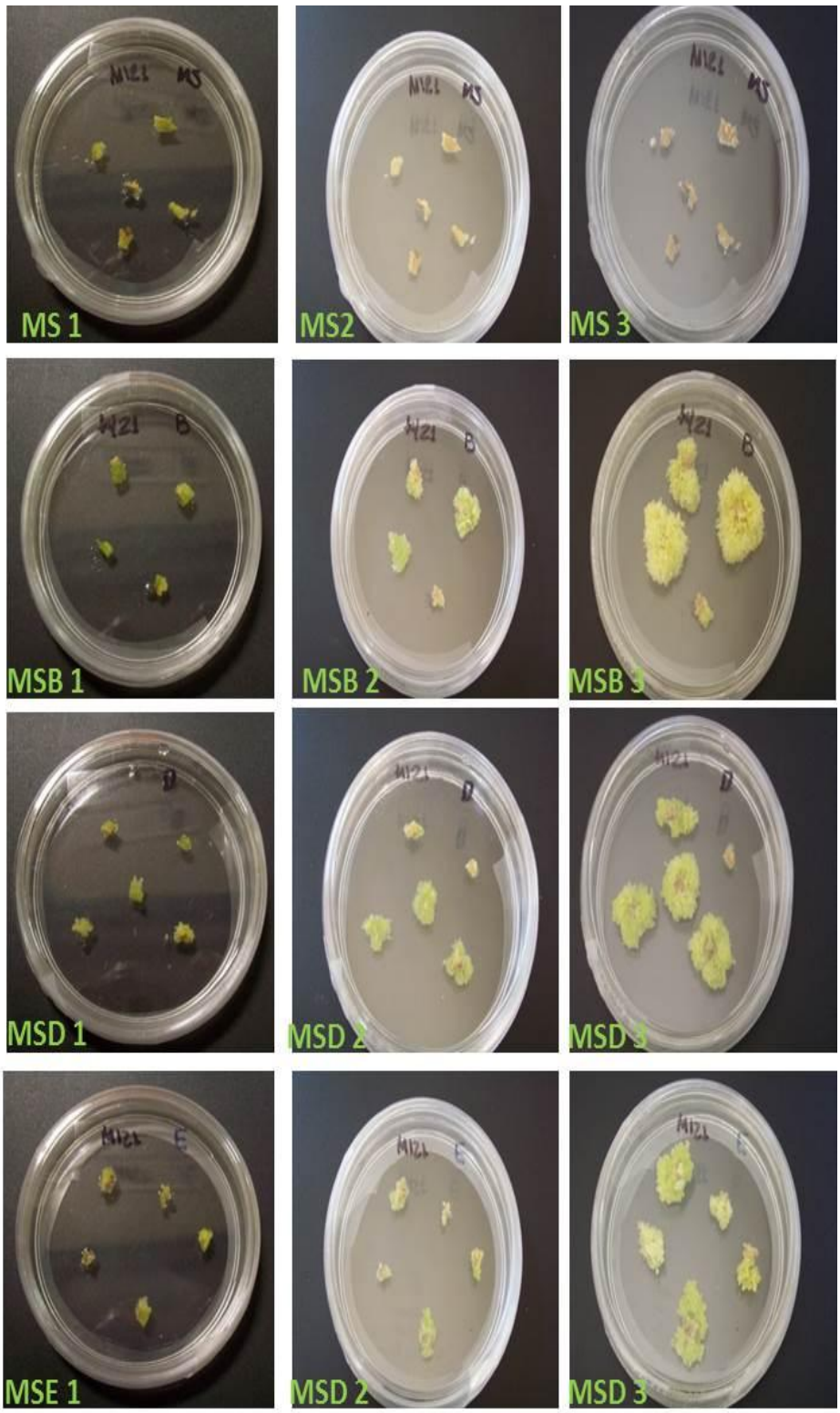

Fig.2 *In vitro Fouquieria splendens* callus development, under co-cultive conditions, all the callus grown in different culture medium and *Staphylococcus pasteuri* strain Fs2 streaked: MSFs2, MSBFs2, MSDFs2 and MSEFs2. Numbers indicate: 1= initial callus growth, 2 = 19 days and 3 = 48 days

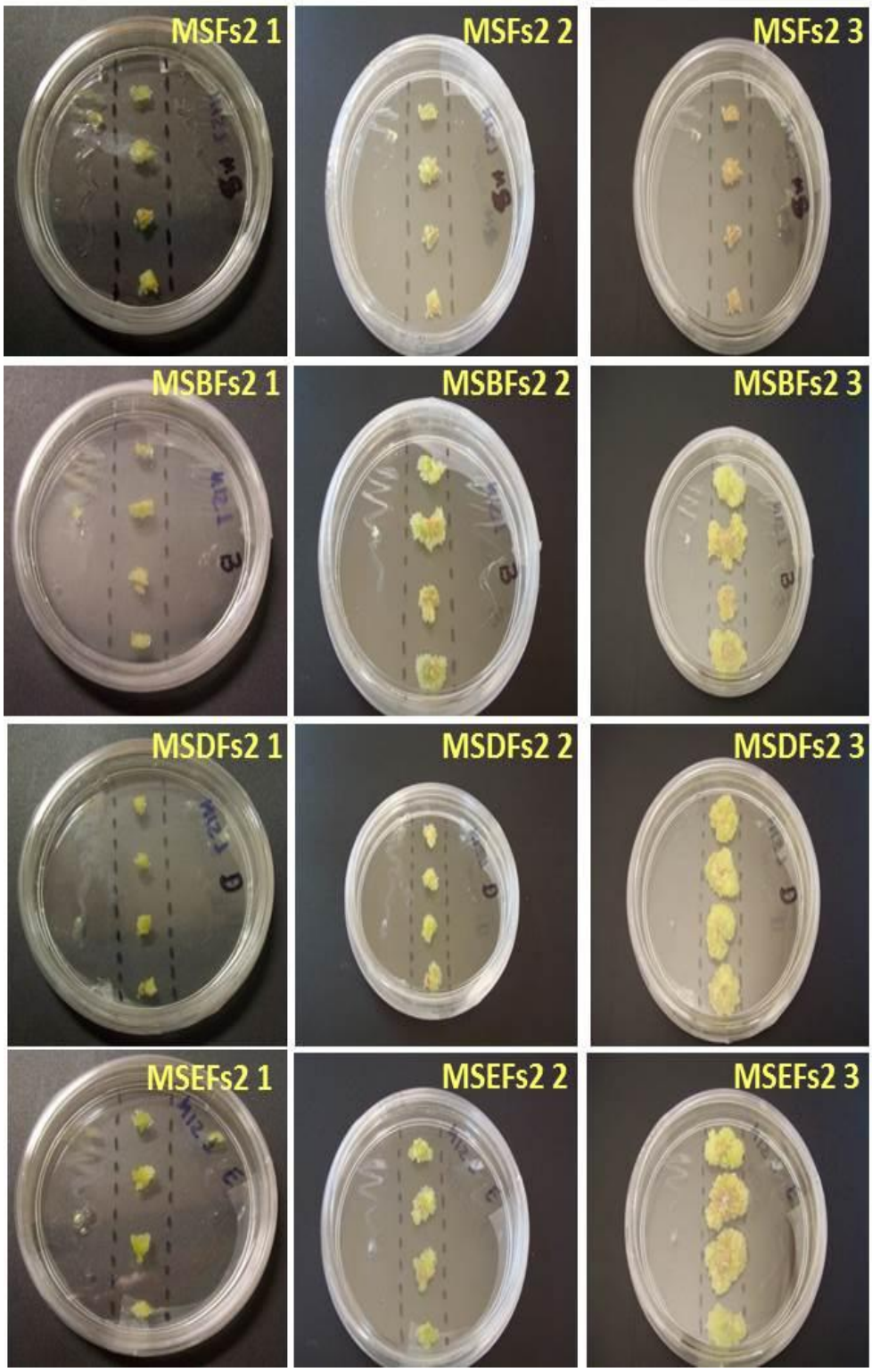

Fig.3 *Fouquieria splendens* callus growth. 3a) callus area recorded at 19 days and 3b) callus area recorded at 48 days. (n=15, the different lower-case letters shows the significant differences founded [$p < 0.01$])

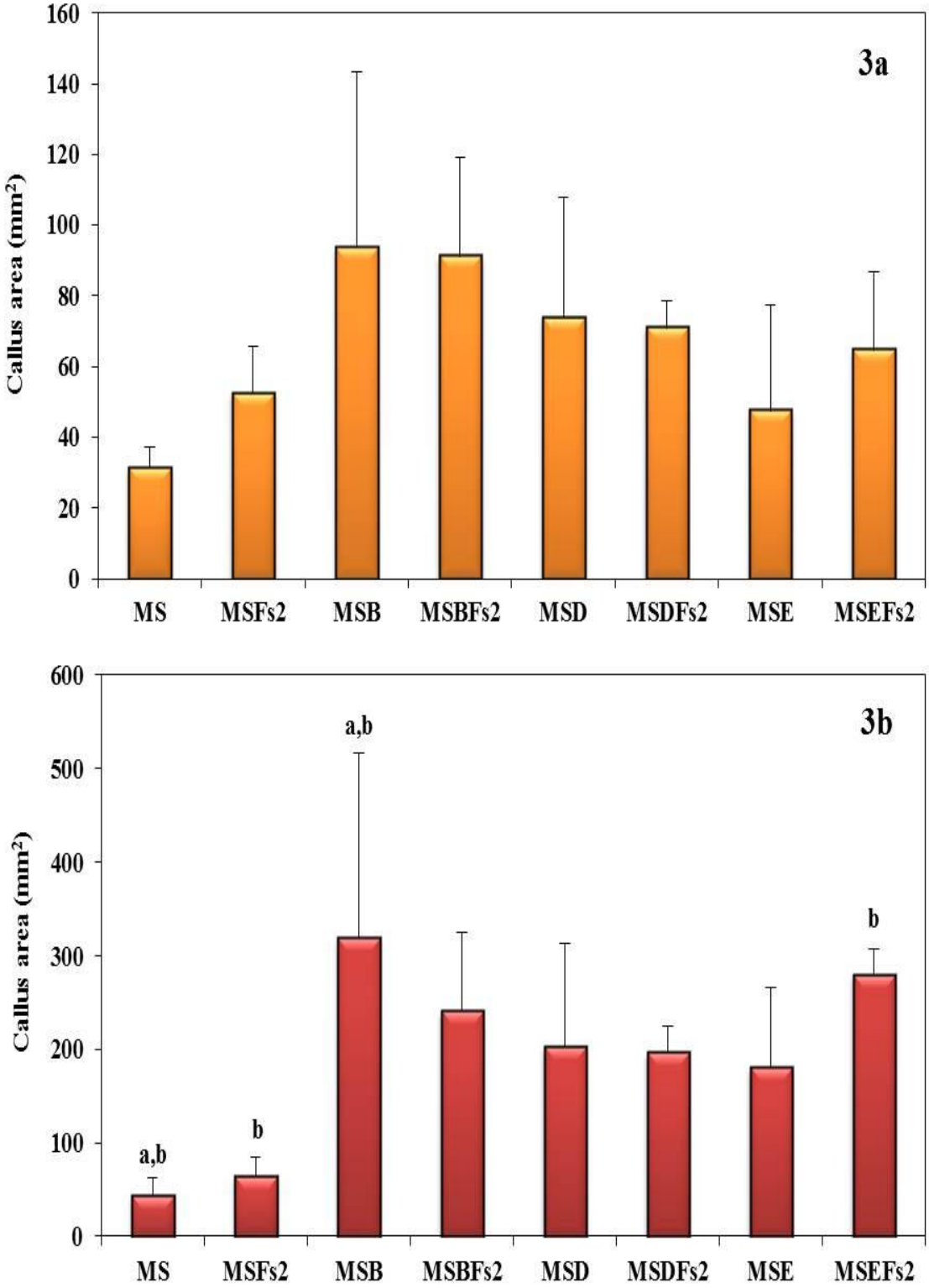

Fig.4 Principal component analysis grouping the *Foquieria splendens* callus co-cultivated with *Staphylococcus pasteuri* strain Fs2 for both recorded times

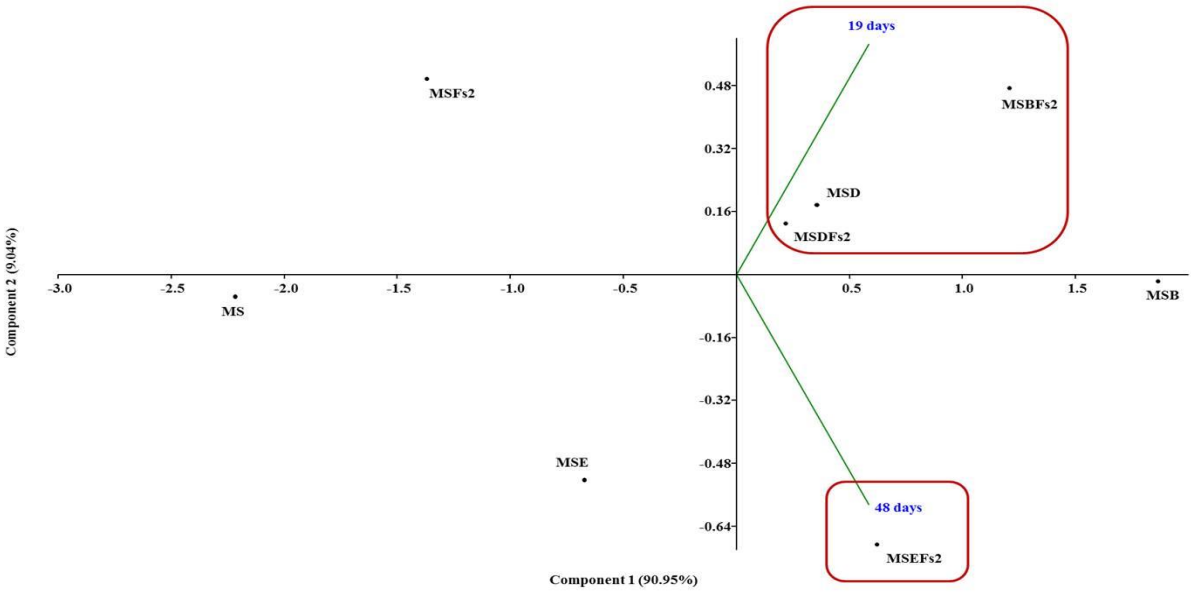

Fig.5 Scanning electronic micrographs of selected *Fouquieria splendens* callus co-cultivated with *Staphylococcus pasteuri* strain Fs2. co-cultivated in MS, MSD and MSE; numbers indicate: 1= initial callus growth, 2 = 19 days and 3 = 48 days

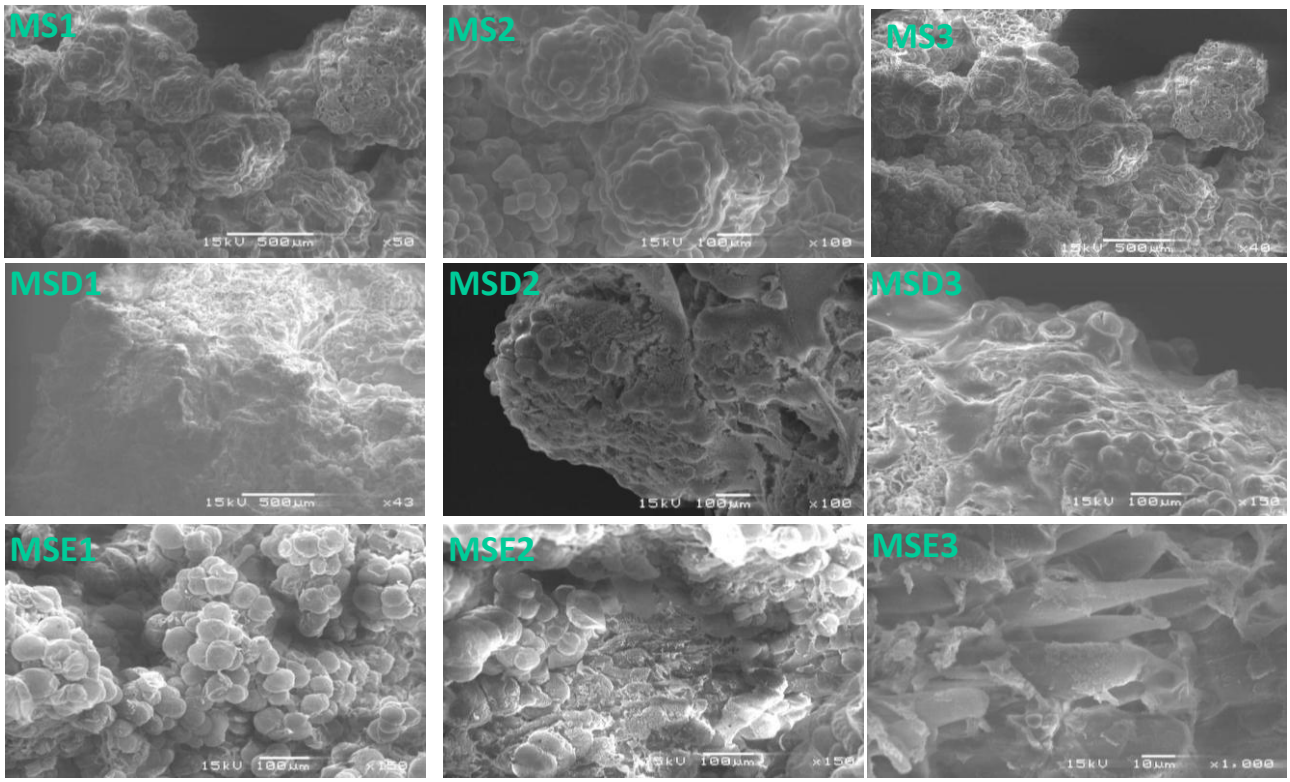

Figure 3 shows *F. splendens* callus growth at 19 (Fig. 3a) and 48 (Fig. 3b) days, comparing the co-cultivated and no co-cultivated callus. At 19 days, no significant response was obtained between all the cultures media and conditions, but particularly the concentration of phytohormones added to MSB medium and the presence of the salt, favored the increase in callus biomass of this plant species. Also this response in the presence of endophyte bacteria, followed by callus cultured in MSD medium and finally the response in MSE medium.

At the final time analyzed (48 days), the callus promoting response was significant ($p< 0.01$) in callus cultured in MSB medium and with gain of biomass in the experiments co-cultivated with *S. pasteuri* strain Fs2, as follows: MSEFs2 (280mm^2), MSBFs2 (241mm^2) and MSDFs2 (197mm^2).

As a complement of the study, Figure 4 showed a separation of co-cultive conditions tested according to the times evaluated; where negative Component 1 values (90.95%) indicates a particular relationship between 48 days and MSEFs2 condition. In this figure there is also a particularly positive association between the co-cultive conditions: MSDFs2, MSBFs2 and MSD with the analysis done at 19 days.

Smolenskaya *et al.,* (2007) reported that the presence of particularly growth regulators and their concentrations are important factors for the maintaining of callus lines in cultures; this is agree with Lakshmanan *et al.,* (2002) showing that the effect of growth regulator on tissue cultures can vary according to the chemical nature of the compound, plant species, type of culture and even the developmental state of the explant. In this study, the nature of phytohormones assayed in *F. splendens* callus in the media tested: MSB, MSD and MSE, the presence of NAA and KIN were determinant. About the employ of NAA in cell cultures, Smolenskaya *et al.,* (2007) and Meftahizade *et al.,* (2010); noted that this phytohormone is a common auxin that ensures the growth and viability of them.

Regarding to cytokinins Sharma *et al.,* (2015) mention that the effects of higher concentration of cytokinins reduce the number of shoots generated per explant.

Morphological changes induced in F. splendens callus

The scanning electron microscopy (Fig. 5) of the selected *F. splendens* callus shows the morphological changes induced in co-cultive conditions, for callus co-cultivated with *S. pasteuri* strain Fs2 grown in MS and MSD medium, there was an evidence of an extracellular matrix cover all the mass of cells, giving an early shoots development, as a differentiation of cells. This response is according to Lim *et al.,* (2016); they found the presence of an extracellular matrix and cellulose microfibril movement as a ‘‘rosette’’. Mariani and Erlangga (2014) define this rosette as a structure to identify the process of cellulose microfibril synthesized in the enzyme complex and further for the protoderm formation which only occurs during an apparent *in vitro* embryo stage. Callus of *F. splendens* developed in MSE medium co-cultivated with the endophyte bacteria do not showed this morphological change; but some cells of them change acquiring a large shape, with slightly appearance of early root hairs or roots.

It is important to note as Parray *et al.,* (2015) mention, that the effect of plant growth promoting bacteria are considered to be highly specific with respect to plant and bacterial genotypic combination and also, as Yang *et al.,* (1991) reported, another possible reason for the induction of some callus

changes is the release of certain compounds from the bacteria. These authors also mention that, besides the phytobacteria strains, there were several factors influencing the initiation and differentiation of callus; the quality and especially the age of the callus employed were of great importance; because some young callus lines are considered more resistant and contribute to the initiation and growth of callus with a great regeneration potential. And finally, the composition of the culture medium has a greatly influence on co-cultives, giving an initiation, proliferation and development of the callus tested.

In this work, the established co-cultive between *Fouquieria splendens* callus and the presence of the streaked endophyte *Staphylococcus pasteuri* strain Fs2 on culture medium, showed that it could be a complex network of responses between the plant growth regulators present in culture medium and the release of phytohormones produced by the phytobacteria; that at least showed a notably effect on the growth and differentiation response of the callus tested.

Acknowledgements

Authors are grateful to the Research Projects SIP-IPN: 20161905 and SIP-IPN: 20171598 of the Secretaría de Investigación y Posgrado del Instituto Politécnico National, for providing the facilities to carry out this work and also wish to thank for the fellowships from Comisión de Operación y Fomento de Actividades Académicas (COFAA, I.P.N.), EDI (Estímulo al Desempeño de los Investigadores, I.P.N.) and SNI-CONACYT.

References

Bertrand, H., Nalin, R., Bally, R., Marel, J.C.C., 2001. Isolation and identification of the most efficient plant growth promoting bacteria associated with canola. Biol. Fertil. Soils. 33,152-156.

Golan-Goldhirsh, L.A., Barazani, O., Nepovim, A., Soudek, P., Smrcek, S., Dufkova, L., Krenkova, S., Yrjala, K., Schröder, P., Vanek, T., 2004. Plant response to heavy metals and organic pollutants in cell culture and at whole plant. J. Soil Sediment. 4, 133-140.

Khalid, A., Arshad, M., Zahir, Z.A., 2004. Screening plant growth-promoting rhizobacteria for improving growth and yield of wheat. J. Appl. Microbiol. 96, 473-480.

Khalid, A., Arshad, M., Zahir, Z.A., Khaliq, A., 1997. Potential of plant growth promoting rhizobacteria for enhancing wheat yield. J. Anim. Plant Sci. 7, 53-56.

Lakshmanan, P., Danesh, M., Taji, A., 2002. Production of four commercially cultivated *Echinacea* species by different methods of *in vitro* regeneration. J. Hort. Sci. Biotech. 77, 58-163.

Lim, S.L., Subramaniam, S., Zamzuri, I., Amir, H.G., 2016. Biotization of *in vitro* calli and embryogenic calli of oil palm (*Elaeis guineensis* Jacq.) with diazotrophic bacteria *Herbaspirillum seropedicae* (Z78). Plant Cell Tiss. Organ. Cult. 127, 251-262.

Mariani, T.S., Erlangga, B.P., 2014. SEM study on early stages of oilpalm (*Elaeis guineensis* Jacq.) somatic embryos. Asian J. Appl. Sci. 2, 167-171.

Meftahizade, H., Lotfi, M., Moradkhani, H., 2010. Optimization of micropropagation and establishment of cell suspension culture in *Melissa officinalis* L.Afr. J. Biotech.9, 4314-4321.

Murashige, T., Skoog, F., 1962. Arevised medium for rapid growth and bioassays with tobacco tissues cultures. Physiol. Plant. 15, 473-497.

Nowak, J., 1998. Benefits of *in vitro*

‘‘biotization’’ of plant tissue cultures with microbial inoculants. In Vitro Cell Dev. Biol. Plant. 34, 122-130.

Oliveira, A.D., Urquiaga, S., Döbereiner, J., Baldani, J.I., 2002. The effect of inoculating endophytic N_2-fixing bacteria on micropropagated sugarcane plants. Plant Soil. 242, 205-215.

Parray, J.A., Kamili, A.N., Reshi, Z.A., Qadri, R.A., Jan, S., 2015. Interaction of rhizobacterial strains for growth improvement of *Crocus sativus* L. under tissue culture conditions. Plant Cell Tiss. Organ. Cult. 121, 325-334.

Preininger, Ė., Zatyko, J., Szucs, P., Koranyi, P., Gyurjan, I., 1997. *In vitro* establishment of nitrogen-fixing strawberry (*Fragaria ananassa*) via artificial symbiosis with *Azomonas insignis*. In Vitro Cell Dev. Biol. Plant. 33,190-194.

Salinas, P.V.A., 2018. Caracterización de endófitos de *Fouquieria splendens* y su relación como promotores del crecimiento vegetal. Tesis de Licenciatura, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, México.

Sharma, V., Kamal, B., Srivastava, N., Negi, Y., Dobriyal, A.K., Jadon, V.S., 2015. Enhancement of *in vitro* growth of *Swertia chirayita* Roxb. ExFleming co-cultured with plant growth promoting rhizobacteria. Plant Cell Tiss. Organ. Cult. 121, 215-225.

Smolenskaya, I.N., Reshetnyak, O.V., Smirnova, Y.N., Chernyak, N.D., Nosov, A.M., Nosov, A.V., 2007. Opposite effects of synthetic auxins, 2,4-dichlorophenoxyacetic acid and 1-naphthalene acetic acid on growth of true ginseng cell culture and synthesis of ginsenosides. Russian J. Plant Physiol. 54, 215-223.

Souissi, T., Kremer, R.J., 1994. Leafy spurge (*Euphorbia esula*) cell cultures for screening deleterious rhizobacteria. Weed Sci. 42, 310-315.

Souissi, T., Kremer, R.J., 1998. A rapid microplate callus bioassay for assessment of rhizobacteria for biocontrol of leafy spurge (*Euphorbia esula* L.). Biocontrol Sci. Techn. 8, 83-92.

Varga, S.S., Koranyi, P.A., Preininger, E., Gyruran, I., 1994. Artificial associations between *Daucus* and nitrogen-fixing *Azotobacter* cells *in vitro*. Plant Physiol. 90, 789-790.

Vestberg, M., Kukkonen, S., Saari, K., Parikka, P., Huttunen, J., Tainio, L., Devos, N., 2004. Microbial inoculation for improving the growth and health of micropropagated strawberry. Appl. Soil Ecol. 27, 243-258.

Yang, Y.S., Wada, K., Goto, M., Futsuhara, Y., 1991. *In vitro* formation of nodular calli in soybean (*Glycine max* L.) induced by cocultivated *Pseudomonas maltophilia*. Japan J. Breed. 41, 595-604.

How to cite this article:

Daniel Corona-Álvarez, Verónica Azeret Salinas-Patiño, MaríaVictoria Hernández- Pimentel, Silvano Montes-Villafan, Marcial García-Pineda, Leonor Angélica Guerrero-Zúñiga and Angélica Rodríguez-Dorantes. 2018. Morphological Changes in *Fouquieria splendens* Callus Cocultivated with Endophyte Bacteria. *Int.J.Curr.Microbiol.App.Sci.* 7(02): 1577-1586.
doi: https://doi.org/10.20546/ijcmas.2018.702.190