academicJournals

Vol. 9(5), pp. 30-36, August 2017
DOI: 10.5897/JBR2017.0244
Article Number: 045B41365844
ISSN 2006-9871
Copyright © 2017
Author(s) retain the copyright of this article
http://www.academicjournals.org/JBR

African Journal of Bacteriology Research

Full Length Research Paper

Antimicrobial activity of secondary metabolites obtained with different carbon sources at different stages of the *Pseudomonas* growth curve isolated from Fresnillo, Zacatecas, México mineral soils

Mayela Robles-Huízar[1], Susana De La Torre-Zavala[1], Myriam A. De La Garza-Ramos[2*] and Luis J. Galan-Wong[1]

[1]Universidad Autonoma de Nuevo Leon, Centro de Innovacion Biotecnologica (CINNOB), Instituto de Biotecnologia, Ave. Pedro de Alba s/n cruz con Ave. Manuel L. Barragan s/n, Cd. Universitaria, San Nicolás de los Garza, Nuevo Leon, Mexico.

[2]Centro de Investigación y Desarrollo en Ciencias de la Salud (CIDICS), Avenida Gonzalitos s/n, Colonia Mitras Centro, Monterrey, Nuevo Leon, Mexico.

Received 15 June, 2017; Accepted 27 July, 2017

The obtention of secondary metabolites from bacterial filtrates has permitted the identification of new compounds with diverse biotechnological applications. These metabolites were generated under different conditions. Eighty-six strains of *Pseudomonas* were isolated from mineral soils of the central region of Zacatecas to determine if these generated secondary metabolites possess antimicrobial activity against phytopathogenic microorganisms. Afterwards, parameters, such as nutrients, were identified using seven different mediums, time of production of bioactive metabolites using growth curves, the determination of antimicrobial activity during fermentation, and their minimum inhibitory concentration (MIC). One strain of *Pseudomonas* was able to generate specific secondary metabolites for one or all microorganisms during different stages of the growth curve. The best phase for development of these metabolites was the stationary phase; however, in enriched media supplemented with glycerol and mannitol, less antimicrobial activity was observed than that with minimal salts medium supplemented with glucose, since in the latter, five strains were susceptible. Additionally, as the growth curve advanced, the generated metabolites were specific for one microorganism and lost activity against others. It was also determined that the MIC of the secondary metabolites generated in minimal salts medium was much lower than that obtained in enriched media supplemented with glycerol and mannitol. The *Pseudomonas* strain obtained from mining soils is capable of generating specific bioactive metabolites of one microorganism at different stages of growth.

Key words: Phytopathogens, minimal inhibitory concentration, metabolism, biochemical applications, strains.

INTRODUCTION

The genus Pseudomonas has become one of the most studied bacterial groups that are widely distributed in nature. It is estimated that half of the natural isolates in mineral water and soil consist of Pseudomonas (Guillot and Leclerc, 1993). They belong to the family of gamma proteobacteria with more than 100 different species whose lifestyle is widely varied (Bennasar et al., 2010; Yadav et al., 2014). In addition, they have been selected

due to their metabolic versatility and their biotechnological value. Since they adapt to different stress environments and produce a wide range of bioactive metabolites with antimicrobial activity, such as phenazine 1-carboxylic acid (PCA), pyoechelin, and salicylic acid (Duffy and Defago, 1999; Ramos and Filloux, 2010; Reder-Christ et al., 2012; Siddiqui and Shaukat, 2004; Trippe et al., 2013). These metabolites, produced by *Pseudomonas,* have been widely studied; however, to date, various *Pseudomonas* species are able to generate compounds with a broad biological activity as in the production of antimicrobial compounds and plant growth promotors (Glick, 1995). It has even been found that *Pseudomonas aeruginosa* produces various compounds with potential activity against tumor cells (Yamada et al., 2002). However, this activity has been observed in compounds that have been purified without taking into account the probability that the metabolites generated in a culture medium can act synergistically allowing this effect to potentialize. It has been observed that the crude extract derived from *Pseudomonas* sp., In5, has suppressive activity against *Rhizotocnia solani* and *Pynthium aphanidermatum* by induction of a mitochondrial stress response. Other compounds such as nunapeptin and nunamicin show different antimicrobial activity by themselves, suggesting that the bioactivity of crude extract derived from *Pseudomonas* sp. In5 involves the presence of both compounds working in synergy (Michelsen et al., 2015). This is of great importance because the search for new antimicrobial compounds should not only be based on the use of an active compound, but on the possible combination of several. Furthermore, the generation of secondary metabolites is influenced by conditions of fermentation (Higgs et al., 2001). Because of this, the search for new bioactive compounds depends not only on testing microorganisms in places that have not been studied, but also in fermentation conditions, taking into account the possibility that these metabolites act synergistically. In this study, different strains of *Pseudomonas* were isolated from mining soils in order to determine their capability to generate secondary metabolites with antimicrobial activity. Also, this work is aimed at determining the stage of the tested metabolites with high activity under different culture media conditions. It is important to mention that this is the first study of *Pseudomonas* isolated from mine soils of this region.

MATERIALS AND METHODS

Biological materials

The phytopathogens used in this study were donated by the Center for Research and Advanced Studies (CINVESTAV, Irapuato). All strains were maintained at -80°C in Luria-Bertani liquid medium (LB medium) containing a final concentration of 20% (v/v) glycerol (Sambrook and Russell, 2001).

Sample collection and bacterial culture

Soil samples were randomly collected between August 2013 and August 2014 with the purpose of obtaining a wide collection of *Pseudomonas* isolated from mineral soils in different locations of Fresnillo, Zacatecas, Mexico. Primary isolation of *Pseudomonas* was made according to the protocol described by Li et al. (2013). One gram of soil was weighed and mixed with 9 ml of 0.5% sterile saline, serial dilutions were prepared and 1 ml of the sample was placed in Petri dishes with King's B medium (KB) supplemented with cyclohexamide (100 µg/L), nystatin (100 µg/L), penicillin G (75 U/L) and chloramphenicol (50 µg/L). The sample was spread onto the surface of medium and the plates were incubated for 48 h at 28°C. Gram staining was performed to select the presumptive *Pseudomonas* strains. Isolates were maintained at -20°C in King´s B liquid medium containing a final concentration of 20% (v/v) glycerol.

Production and extraction of secondary metabolites

Isolated *Pseudomonas* were inoculated into modified *Pseudomonas* minimal salts medium (PMS medium) according to the methodology described by Trippe et al. (2013), with some modifications. The secondary metabolites from the *Pseudomonas* isolate were inoculated into PMS medium, with constant mixing at 200 rpm (Thermo Scientific Max Q8000, Thermo Fisher Scientific Inc., Waltham, MA) for 7 days at 27°C. To prepare culture filtrates, 50 ml of the 7-day cultures were centrifuged (3000 rpm for 20 min) and the supernatant was passed through a bacteriological filter (Corning Sterile Syringe Filter, pore 0.20 µM, Corning Inc., New York, NY). The resulting sterile culture filtrate was stored at 4°C for microbial susceptibility testing.

Determination of antimicrobial activity

Antimicrobial activity was determined according to the method described by Halgren et al. (2011) with some modifications. These consisted of placing 250 µL of culture of the problem strains previously adjusted at 10^8 CFU/ml in 25 ml of minimum salts agar (MSA) Wells were made with an inverted sterile Durham tube and 150 µl of *Pseudomonas* bacterial filtrates were added and incubated at 27°C for 48 h. Afterwards, inhibition halos present in the medium were measured. Three replicate plates were prepared for each culture filtrate and the experiment was repeated twice.

Culture medium optimization and growth curves

Based on background for the generation of metabolites, it was determined that the carbon and nitrogen sources influence the metabolism of *Pseudomonas* for their production; therefore, 7 culture media were designed with the following formulations: (1) 0.2 g/L KCl, 1 g/L KNO_3, 1 g/L K_2HPO_4, 1.5 g/L KH_2PO_4, 20 g/L peptone, 20 ml/L 20% glycerol and 2 ml/L 1 M $MgSO_4$; (2) 0.2 g/L KCl, 1 g/L KNO_3, 1 g/L K_2HPO_4, 1.5 g/L KH_2PO_4, 20 g/L peptone,

*Corresponding author. E-mail: myriam.garzarm@uanl.edu.mx.

Author(s) agree that this article remains permanently open access under the terms of the Creative Commons Attribution License 4.0 International License

20 ml/L 20% mannitol and 2 ml/L of 1 M $MgSO_4$; (3) 20 ml/L of 10% casamino acids, 100 µl of 1 M $CaCl_2$, 20 ml/L of 20% glycerol, 2 ml/L of 1 M $MgSO_4$ and 957.9 ml/L of M9 salts; (4) 20 ml/L of 10% casamino acids, 100 µl of 1 M $CaCl_2$, 20 ml/L of 10% mannitol, 2 ml/L of 1M $MgSO_4$ and 957.9 ml/L of M9 salts, (5) 0.2 g/L KCl, 1 g/L KNO_3, 1 g/L K_2HPO_4, 1.5 g/L KH_2PO_4, 20 g/L peptone, 20 ml/L 20% glucose and 2 ml/L 1 M $MgSO_4$, (6) 20 ml/L of 10% casamino acids, 100 µl of 1M $CaCl_2$, 20 ml/L of 20% glucose, 2 ml/L of 1M $MgSO_4$ and 957.9 ml/L of M9 salts, (7) MM9 700 ml of sterile distilled water, 200 ml/L of sterile M9 minimum salts medium, 2 ml/L of 1 M $MgSO_4$, 20 ml/L of 20% glucose, 100 µL/L of 1M $CaCl_2$ and adjusted to 1 L with sterile distilled water. Later, 50 ml of each medium were placed in a 250 ml Erlenmeyer flask and a *Pseudomonas* preculture 1% (v/v) (adjusted to 0.5 McFarland at an OD of 620 nm) was added and incubated at 27°C for seven days mixing at 200 rpm (Thermo Scientific Max Q8000). Aliquots of 500 µL were drawn every 24 h and centrifuged (3000 g, 5 min). The supernatant was filtered with filter paper (Corning Sterile Syringe Filter, pore 0.20 µM, Corning Inc.), and the antimicrobial susceptibility assays carried out.

Extraction of secondary metabolites and determination of the minimum inhibitory concentration (MIC)

The culture filtrates of *Pseudomonas* were centrifuged (3500 rpm, 25 min) and the supernatant was passed through a bacteriological filter (Corning Sterile Syringe Filter, pore 0.20 µM, Corning Inc.). The previously acidified (pH 2, 2N HCl) (Jousset et al., 2006) cell-free medium was mixed with 2 volumes of ethylacetate. After incubation overnight at -20°C, the unfrozen (ethylacetate) fraction that contained the active compounds was evaporated in a rotavapor (BM500, Yamato Scientific Co.,Ltd., Tokyo, Japan) at 225 hPa at 35 to 40°C (Garbeva et al., 2011). The dried extracts were dissolved in 3% DMSO (Wadhwani et al., 2009). The minimum inhibitory concentration (MIC) was determined using the plate microdilution method. This consisted of placing 150 µL of the corresponding culture medium (2X) for each problem strain in each well and then adding 150 µL of the secondary metabolite extract with 3% DMSO to the first well and performing serial dilutions. The problem strain 1% (v/v) was added to each well and incubated (28°C, 24 h). After incubation, the microplate was read at 620 nm (Synergy 2 Multi-Mode Microplate reader, BioTek Instruments, Inc., Winooski, VT) and the MICs were determined.

RESULTS

Isolation and antimicrobial activity of *Pseudomonas* filtrates

A total of 86 bacterial filtrates of *Pseudomonas* were obtained. Thirteen presented antimicrobial activity showing inhibition halos of up to 0.9 cm (Table 1). The results show that the filtrates MR-IB23, MR-IB29, MR-IB35, MR-IB36, MR-IB43, MR-IB66 and MR-IB67 have antimicrobial activity against at least 5 of the panel microorganisms. However, filtrate MR-IB66 was the only one that showed activity against *P. syr* pv *glycinea* with inhibition halos of 0.47 mm with no significant difference with regard to the control. On the other hand, the most susceptible strain was *Pseudomonas syringae* sr61 when challenged against the MR-IB23 filtrate showing an inhibition halo of 0.94 cm; the least susceptible strain was *Erwinia* sp. which presented inhibition halos of 0.4 cm being similar to the control. Based on these results, the filtrate MR-IB66 was chosen to carry out culture medium optimization (Table 2).

The extracts were generated in minimal salt medium suplemented with glucose (20%) incubated at 27°C for 7 days. The well test was performed in M9 medium with 100 µL of Phytopathogen adjusted to 0.5 by McFarland and incubated for 24 h at 27°C. Assays were performed in duplicate with 3 replicates.

Culture medium optimization and antimicrobial activity at different stages of the growth curve

The results of this study showed that the generation of secondary metabolites with antimicrobial activity occurs at different stages of the growth curve. In addition, their specificity varies depending on the strain (Figure 1).

As shown in Figure 1, the secondary metabolites present in the *Pseudomonas* MR-IB66 filtrate are generated at the middle of the stationary phase (3rd day of fermentation) in which it was observed that the specificity of the metabolites depends on the formulation of the medium. According to these results, medium 1 is specific for *P. syringae* sr61 and *P. syringae* pv *tomato* DC3000 with inhibition halos of 0.39 and 0.2 cm, respectively; the metabolites present in the culture filtrate of medium 3 only had activity against *P. syringae* pv *tomato* DC3000 with inhibition halos of 0.18 cm. On the other hand, the metabolites present in filtrates of medium 6 and 7 showed antimicrobial activity against *P. syringae* sr61, *P. syringae* pv *tomato* DC3000, *E. coli* JM103 and *Erwinia* sp. However, the most active filtrate was media 7 showing higher inhibition compared to those obtained in medium 6. On day 4 of fermentation it was seen that there was a loss of antimicrobial activity of filtrate 1 against *P. syringae* sr61 and *P. syringae* pv *tomato* DC3000. Nevertheless, these were susceptible to media 3, 4 and 5. In contrast, the metabolites present in medium 7 lost antimicrobial activity against *Erwinia* sp. and *E. coli* JM103. On the 5th day of fermentation, it was observed that the metabolites generated in this stage by media 1, 2 and 5 were specific against *E. coli* JM103 while in medium 7, activity was maintained and reappeared against *Erwinia* sp. These results suggest that once secondary metabolites are generated, some appear momentarily and can have biological activity against different bacterial cells.

Extraction of secondary metabolites and determination of the minimum inhibitory concentration (MIC)

The determination of the minimum inhibitory concentration was done by obtaining the secondary metabolites present in media 1, 5 and 7 in two phases of the growth curve. The first at the beginning of the stationary phase (SP1)

Table 1. Bacteria that are sensitive to *Pseudomonas* culture filtrate.

Test species	***Culture filtrate***						
	MR-IB3	**MR-IB14**	**MR-IB16**	**MR-IB22**	**MR-IB23**	**MR-IB29**	**MR-IB35**
	Zone size (cm)						
P. syringae sr61	0.80±0.23	0.64±0.39	0.52±0.13	0.52±0.08	0.940±0.134	0.80±0.20	0.72±0.17
P. syringae pv. Tomato DC3000	0.64±0.07	0.62±0.34	0.60±0.37	0.62±0.00	0.74±0.18	0.82±0.14	0.60±0.07
P. syringae pv. Glycinea	-	-	-	-	-	-	-
E. coli JM103	0.48±0.17	0.60±0.12	0.44±0.27	0.56	0.56±0.054	0.56±0.05	0.40±0.10
Erwinia sp.	-	-	0.42±0.11	0.40±0.10	-	0.42±0.08	0.42±0.10
Agrobacterium sp.	0.44±0.05	-	-	0.42±0.00	0.66±0.26	0.58±0.08	0.58±0.13
Test species	**MR-IB36**	**MR-IB43**	**MR-IB66**	**MR-IB67**	**MR-IB68**	**MR-IB73**	**Control**
P. syringae sr61	0.84±0.08	0.60±0.07	0.74±0.16	0.52±0.04	0.78±0.13	0.76±0.19	0.62±0.08
P. syringae pv. Tomato DC3000	0.60±0.00	0.48±0.27	-	0.54±0.05	0.66±0.15	0.70±0.18	1.00±0.07
P. syringae pv. Glycinea	-	-	0.47±0.05	-	-	-	0.48±0.04
E. coli JM103	-	0.50±0.07	0.46±0.08	0.44±0.89	0.46±0.26	0.48±0.08	1.06±0.08
Erwinia sp.	0.40±0.10	0.48±0.04	0.28±0.08	0.44±0.55	-	-	0.46±0.05
Agrobacterium sp.	0.50±0.00	0.58±0.04	0.36±0.23	0.50±0.10	0.58±0.04	0.56±0.11	1.00±0.00

Table 2. Antimicrobial minimal inhibition concentrations (MICs) of the filtrate culture media obtained by *Pseudomonas* MR-IB66.

Test species	**Culture media**		
	M1 SP2	**M5 SP2**	**M7 SP2**
E. coli JM103	4.5±0.01	3.5±0.01	2.0±0.01
Erwinia sp.	4.5±0.01	3.5±0.00	2.5±0.01
Agrobacterium sp.	2.5±0.07	1.5±0.08	2.5±0.04
P. syringae pv. tomato DC3000	9.0±0.02	7.5±0.02	3.5±0.01
P. syringae sr61	8.0±0.01	8.5±0.01	3.7±0.04
P. syringae pv glycinea	12.0±0.02	10.0±0.01	5.0±0.01

M1, Medium 1; M5, Medium 5; M7, Medium 7; SP, Stationary phase.

and the second in the constant stationary phase (SP2). As shown in Figure 2, medium 1 stationary phase (SP2).

As shown in Figure 2, medium 1 in SP1 and medium 5 in SP2 have a MIC ranging from7.5 to 15 mg/ml for *E. coli* JM103, Erwinia sp. and Agrobacterium sp., while medium 1 in SP2 and medium 5 in SP2 have a MIC that ranges from 3.75 to 7.5 mg/ml for the same phytopathogens.

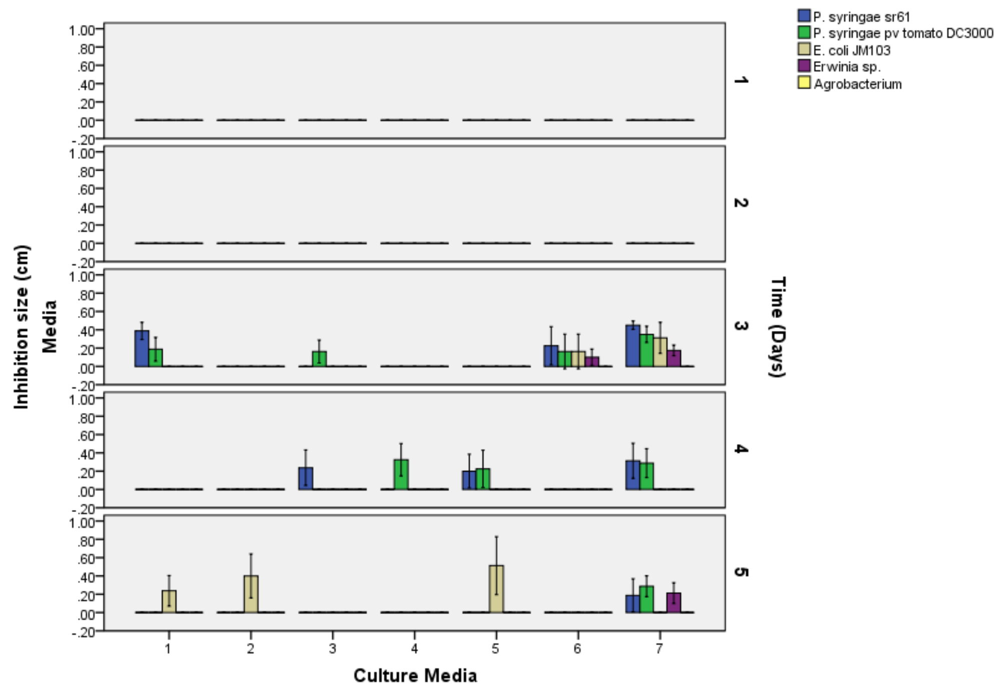

Figure 1. Antimicrobial activity of Pseudomonas MR-IB66 culture filters in different culture media. The tests were performed in duplicates and a comparison of means (ANOVA) with a value of $p<0.05$.

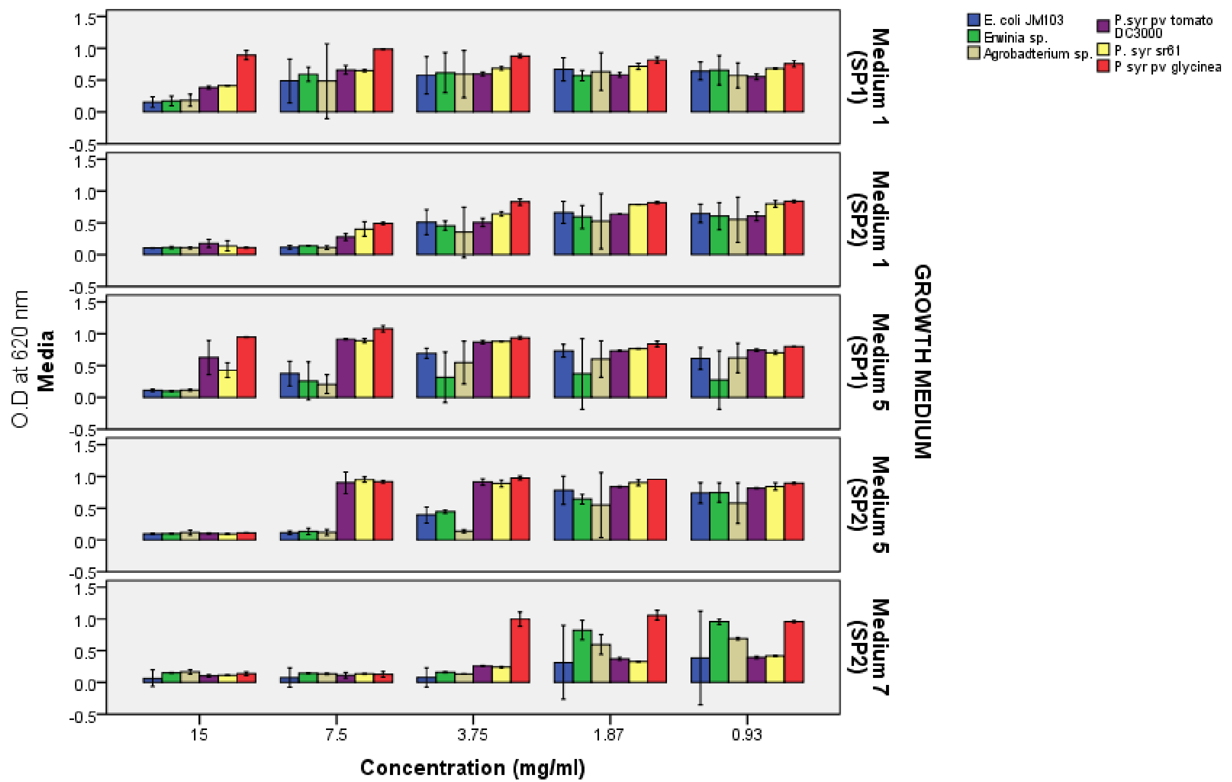

Figure 2. Minimal inhibitory concentration (MIC) of different culture filtrate produced by MRIB-66 at two stages of growth curve.

In addition, medium 7 showed smaller MIC ranges not only for *E. coli* JM103, *Erwinia* sp. and *Agrobacterium* sp. but also for *P. syringae* pv. *tomato* DC3000 and *P. syringae* sr61, with MIC ranges of 1.87 to 3.75 mg/ml; while the MIC of *P. syringae* pv *glycinea* is between 3.75 and 7.5 mg/ml.

Based on previous results, it was found that *Agrobacterium* sp. has a MIC of 1.5 mg/ml for medium 5 SP2, 2.5 mg/ml for medium 1 SP2 and medium 7 SP2. The MIC for *E. coli* JM103 and *Erwinia* sp. is very similar in the three media with a CMI of 4.5 mg/ml for medium 1 SP2, 3.5 mg/ml for medium 5 SP2 and 2 to 2.5 mg/ml for medium 7 SP2.

It is also observed that nutrient-rich media (medium 1 and 5) present MICs higher than 9 mg/ml against the 3 different strains of *P. syringae* tested here. While in the minimal medium (medium 7) MICs of 3.5 mg/ml for *P. syringae* pv. *tomato* DC3000 and *P. syringae sr61*, and 5 mg/ml for *P. syringae* pv. *glycinea* were found (Table 1).

DISCUSSION

Antimicrobial activity of *Pseudomonas* isolated from mining soils

Many strains of *Pseudomonas* show potential for biological control of phytopathogens especially root pathogens and have become prominent models for analysis of bacterial secondary metabolism (Couillerot et al., 2009). They are capable of suppresing a wide range of plant diseases due to their ability to biosynthesize antimicrobial metabolites like cyclic lipopeptides (CLPs), siderophores, hydrogen cyanide (Gross and Loper, 2009) and non-proteinogenic aminoacids like FVG (L-2-amino acid 4-formylaminooxy-*trans*-3-butenoic acid) and MVG (L-2-amino-4-methoxy-*trans*-3-butenoic acid). This selectively inhibit the growth of *Erwinia amylovora* (Halgren et al., 2011; Lee et al., 2013) and 3-methylarginine which has previously been known only in nature as a constituent of the peptide lavendomycin from *Streptomyces lavendulae* that suppresses the growth of *P. syringae* pv. *glycinea* (Braun et al., 2008). It has been observed that the production of 3-methylarginine can be overcome by supplementing the growth medium with the essential amino acid, l-arginine suggesting that the toxin acts as an inhibitor of arginine biosynthesis. These data are of great importance because only one of the 86 filtrates tested in this study (MR-IB66) did not only showed activity against *Erwinia* sp. but also against *P. syringae* pv *glycinea* suggesting that the *Pseudomonas* MR-IBB66 could produce non-proteinogenic aminoacids like FVG or 3-methylarginine. In a previous study, it was found that the antimicrobial activity against *P. syringae* pv. *glycinea* is only seen when the strain is grown in a minimal salt medium. Therefore, it would be interesting to determine if the bacteria used in nutritional stress is affected in some way that it can generate some derivative allowing it to act as a microbial antagonist.

The production of secondary metabolites depends mostly on the nutrients available in the medium (Higgs et al., 2001). This makes it possible for different species of *Pseudomonas* to generate secondary metabolites of different composition during their growth. Polanski-Cordovano et al. (2013) found that the production of extracellular antibiotics produced by *P. fluorescens* CL0145A against *B. subtilis* depends on the content of carbon sources as well as the presence of amino acids and vitamins, since when glycerol, hydrolyzed soy, tryptophan, glutamine, biotin and riboflavin were added to the medium, these antibiotics were generated. Siddiqui and Shaukat (2004) determined that the nematicide activity of *P. aeruginosa* IE-6S and *P. fluorescens* CHA0 was influenced by the carbon source, fatty acid precursors, and nitrogen sources, finding that glycerol, propionate and L-lysine increase this activity, while glucose, L-valine and Pi suppress it. In this case, it was found that the antimicrobial activity was also affected by the main carbon source (Figure 1) such as glycerol, mannitol, and glucose (medium 1, 2 and 5, respectively). On the other hand, the addition of casamino acids with glycerol and mannitol (medium 3 and 4, respectively) showed antimicrobial activity against *P. syringae* pv *tomato* DC3000 and *P. syringae* sr61, while medium supplemented with casamino acids in the presence of glucose had antimicrobial activity against *P. syringae* sr61, *P. syringae* pv *tomato* DC3000, *Agrobacterium* sp., and *Erwinia* sp. suggesting that the presence of casamino acids stimulates the generation of secondary metabolites in media supplemented with glucose. On the other hand, as shown in Figure 2, the fermentation time of the strain used influences the type of antimicrobial activity observed. Most of the bioactive compounds have been identified at different incubation times such as pyoluteorin, which was obtained at 24 h in a glycerol-enriched medium, pyrrolnitrins which were obtained after 5 days of incubation in a medium supplemented with glycerol, and furanomycin, which was obtained in a minimal salt medium at 7 days of incubation (Kidarsa et al., 2011; Trippe et al., 2013, Upadhyay and Srivastava, 2008). Thus, it can be assumed that the microorganism generates this type of compounds; however, it is still necessary to determine if a single type of compound provides the antimicrobial effect or if there is synergy between them. It can also be determined that the minimal medium, which is a nutrient-poor medium compared to medium 1 and 5, in some way, causes *Pseudomonas* MR-IB66 to produce metabolites that confer antimicrobial activity against other *Pseudomonas* species.

With these results, it is also observed that in addition to the components of the culture medium, the time in which

these metabolites are obtained in the MR-IB66 bacterium is very important because the MIC of each phytopathogen varies depending on these factors. Besides, only in the minimal salts medium (medium 7), it was observed that the required MIC is lower for other *Pseudomonas* species compared to those obtained in medium 1 with a MIC range from 3.5 to 5 mg/ml.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors extend their thanks to the Center for Research and Advanced Studies (CINVESTAV) in Irapuato for providing some strains. They also extend their thanks to the National Council of Science and Technology (CONACyT) for their support in this research and Sergio Lozano-Rodriguez, M.D. for his help in translating the manuscript.

REFERENCES

Bennasar A, Mulet M, Lalucat J, Garcia-Valdes E (2010). PseudoMLSA: a database for multigenic sequence analysis of Pseudomonas species. BMC Microbiol. 10(118).

Braun SD, Volksch B, Nuske J, Spiteller / D (2008). 3-Methylarginine from Pseudomonas syringae pv. syringae 22d/93 suppresses the bacterial blight caused by its close relative Pseudomonas syringae pv. glycinea. Chembiochem: a Euro. J. Chem. Biol. 9(12):1913-1920.

Couillerot O, Prigent-Combaret C, Caballero-Mellado J, Moenne-Loccoz Y (2009). Pseudomonas fluorescens and closely-related fluorescent pseudomonads as biocontrol agents of soil-borne phytopathogens. Lett. Appl. Microbiol. 48(5):505-512.

Duffy BK, Defago G (1999). Environmental factors modulating antibiotic and siderophore biosynthesis by Pseudomonas fluorescens biocontrol strains. Appl. Environ. Microbiol. 65(6):2429-2438.

Garbeva P, Silby MW, Raaijmakers JM, Levy SB, Boer W (2011). Transcriptional and antagonistic responses of Pseudomonas fluorescens Pf0-1 to phylogenetically different bacterial competitors. ISME J. 5(6):973-985.

Glick BR (1995). The enhancement of plant growth by free-living bacteria. Can. J. Microbiol. 41(2):109-117.

Gross H, Loper JE (2009). Genomics of secondary metabolite production by Pseudomonas spp. Natural product reports 26(11):1408-1446.

Guillot E, Leclerc H (1993). Bacterial flora in natural mineral waters: characterization by ribosomal ribonucleic acid gene restriction patterns. Systematic Appl. Microbiol. 16(3):483-493.

Halgren A, Azevedo M, Mills D, Armstrong D, Thimmaiah M, McPhail K, Banowetz G (2011). Selective inhibition of Erwinia amylovora by the herbicidally active germination-arrest factor (GAF) produced by Pseudomonas bacteria. J. Appl. Microbiol. 111(4):949-959.

Higgs RE, Zahn JA, Gygi JD, Hilton MD (2001). Rapid method to estimate the presence of secondary metabolites in microbial extracts. Appl. Environ. Microbiol. 67(1):371-376.

Jousset A, Lara E, Wall LG, Valverde C (2006). Secondary metabolites help biocontrol strain Pseudomonas fluorescens CHA0 to escape protozoan grazing. Appl. Environ. Microbiol. 72(11): 7083-7090.

Kidarsa TA, Goebel NC, Zabriskie TM, Loper JE (2011). Phloroglucinol mediates cross-talk between the pyoluteorin and 2,4-diacetylphloroglucinol biosynthetic pathways in Pseudomonas fluorescens Pf-5. Mol. Microbiol. 81(2):395-414.

Lee X, Azevedo MD, Armstrong DJ, Banowetz GM, Reimmann C (2013). The Pseudomonas aeruginosa antimetabolite L-2-amino-4-methoxy-trans-3-butenoic acid inhibits growth of Erwinia amylovora and acts as a seed germination-arrest factor. Environ. Microbiol. Rep. 5(1):83-89.

Li L, Abu Al-Soud W, Bergmark L, Riber L, Hansen LH, Magid J, Sorensen SJ (2013). Investigating the diversity of pseudomonas spp. in soil using culture dependent and independent techniques. Curr. Microbiol. 67(4):423-430.

Michelsen CF, Jensen H, Venditto VJ, Hennessy RC, Stougaard P (2015). Bioactivities by a crude extract from the Greenlandic Pseudomonas sp. In5 involves the nonribosomal peptides, nunamycin and nunapeptin. Peer J. (3):1476.

Polanski-Cordovano G, Romano L, Marotta LL, Jacob S, Soo Hoo J, Tartaglia E, Asokan D, Kar S, Demain AL (2013). Nutritional studies on production of antibacterial activity by the zebra mussel antagonist, Pseudomonas fluorescens CL0145A. J. Microbiol. Biotechnol. 23(5): 656-660.

Ramos JL, Filloux A (2010). Pseudomonas: Volume 6: Molecular Microbiology, Infection and Biodiversity, Springer Science & Business Media.

Reder-Christ K, Schmidt Y, Dorr M, Sahl HG, Josten M, Raaijmakers JM, Gross H, Bendas G (2012). Model membrane studies for characterization of different antibiotic activities of lipopeptides from Pseudomonas. Biochim Biophys Acta 1818(3):566-573.

Sambrook J, Russell, DW (2001). Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press.

Siddiqui IA, Shaukat SS (2004). Liquid culture carbon, nitrogen and inorganic phosphate source regulate nematicidal activity by fluorescent pseudomonads in vitro. Lett. Appl. Microbiol. 38(3):185-190.

Trippe K, McPhail K, Armstrong D, Azevedo M, Banowetz G (2013). Pseudomonas fluorescens SBW25 produces furanomycin, a non-proteinogenic amino acid with selective antimicrobial properties. BMC Microbiol. 13(111).

Upadhyay A, Srivastava S (2008). Characterization of a new isolate of Pseudomonas fluorescens strain Psd as a potential biocontrol agent. Lett. Appl. Microbiol. 47(2):98-105.

Wadhwani T, Desai K, Patel D, Lawani D, Bahaley P, Joshi P, Kothari V (2009). Effect of various solvents on bacterial growth in context of determining MIC of various antimicrobials. Internet J. Microbiol. 7(1).

Yadav S, Yadav S, Kaushik R, Saxena AK, Arora DK (2014). Genetic and functional diversity of fluorescent Pseudomonas from rhizospheric soils of wheat crop. J. Basic Microbiol. 54(5):425-437.

Yamada T, Goto M, Punj V, Zaborina O, Chen ML, Kimbara K, Majumdar D, Cunningham E, Das Gupta TK, Chakrabarty AM (2002). Bacterial redox protein azurin, tumor suppressor protein p53, and regression of cancer. Proc. Natl. Acad. Sci. U.S.A. 99(22):14098-14103.