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ISOLATION AND EVALUATION OF ACTINOMYCETES AGAINST PHYTOPATHOGENIC AND BACTERIA

Ma. Guadalupe Maldonado Blanco
Universidad Autónoma de Nuevo León, Facultad de Ciencias Biológicas, Instituto de Biotecnología, San Nicolás de los Garza, Nuevo Léon, Mexico

Orquídea Pérez González
Universidad Autónoma de Nuevo León, Facultad de Ciencias Biológicas, Instituto de Biotecnología, San Nicolás de los Garza, Nuevo Léon, Mexico

Eduardo Isaí Sauceda Tovar
Universidad Autónoma de Nuevo León, Facultad de Ciencias Biológicas, Instituto de Biotecnología, San Nicolás de los Garza, Nuevo Léon, Mexico

Ma. Guadalupe Rojas Verde
Universidad Autónoma de Nuevo León, Facultad de Ciencias Biológicas, Instituto de Biotecnología, San Nicolás de los Garza, Nuevo Léon, Mexico

Ma. del Socorro Flores González
Universidad Autónoma de Nuevo León, Facultad de Ciencias Biológicas, Instituto de Biotecnología, San Nicolás de los Garza, Nuevo Léon, Mexico

Abstract: Microorganisms belonging to the genus *Streptomyces* are among the major producers of antibiotics, representing more than 50% of the total antimicrobial compounds produced. In this work, the antibacterial activity of 57 species of actinomycetes and 19 bacillary bacteria from different soil samples obtained from different regions of our country was evaluated, where isolation was carried out in potato dextrose medium added with malt and yeast extracts, and then in Petri dish experiments each of the isolated microorganisms were confronted against 2 species of phytopathogenic bacteria and 8 species of bacteria of medical interest. The results obtained indicated that 53% (41) of all isolates showed activity against at least one of the bacteria tested. *B. subtilis, B. cereus* and *S. aureus* species were inhibited by a greater number of isolates, while few species showed inhibitory activity against *Pseudomonas* sp., *K. pneumoniae*, *L. monocytogenes*, *Salmonella* sp. and *E. coli* (1-4 isolates). The best bacterial inhibition results were obtained on Gram (+) strains; against *K. pneumoniae* one strain showed 100% inhibition.
Keywords: Actinomycetes, pathogenic bacteria, inhibition, antibacterial activity

INTRODUCTION

Of the microbial antagonists evaluated worldwide, microorganisms belonging to the genus *Streptomyces* are of particular importance and interest (Hassan *et al.,* 2011). Among the 10,000 antimicrobial compounds produced by microorganisms, more than 50% were isolated from actinomycetes (Anderson and Wellington, 2001). Throughout the twentieth century, antibiotics have been a primary defense against bacterial diseases. Unfortunately, inappropriate and excessive use of antibiotics in animal husbandry is threatening their efficacy (Jassim and Limoges, 2014). The emergence of infectious diseases caused by antibiotic-resistant bacteria requires alternatives to already known drugs (Barrow and Soothill, 1997; Alisky *et al*., 1998). The search for new drugs is becoming critical, due to the growing concern of the lack of new drugs. There is great interest in investigating alternative and natural antimicrobial agents, which has also increased due to the consumption of chemical preservatives in food and on food processing surfaces. Even now, *Salmonella* remains the major causative bacterium of foodborne diseases worldwide, where poultry are known to be the main reservoirs of this zoonotic pathogen (FSA, 2011; Bardina *et al*., 2012). This bacterium is the second leading cause of foodborne bacterial disease in the United States and the vast majority of these infections are associated with the consumption of products such as chicken and eggs contaminated with *Salmonella* (Foley *et al*., 2008). This microorganism has evolved in its virulence and antimicrobial resistance mechanisms leading to continuous challenges to our public health. In addition to this microorganism, there are others that also cause serious diseases such as *Listeria monocytogenes* which is also transmitted by the consumption of contaminated food, *Bacillus cereus* and *Escherichia coli, Pseudomonas sp, Staphylococcus aureus, Listeria monocytogenes, Klebsiella pneumoniae and within* the plant pathogens*, Xanthomonas campestris*.

Among these pathogens*, Klebsiella pneumoniae* stands out for its ability to form biofilm (Vuotto *et al*., 2014; Chung, 2016) that protects the pathogen from the host immune response as well as antibiotics (Jagnow and Clegg, 2003; Bandeira *al.,* 2014).

On the other hand, some members of the genus *Streptomyces* have been reported to significantly reduce the growth of fungal pathogens (Taechowisan *et al*., 2005; Errakhi *et al*., 2007). S. *aureofaciens* improves protection against postharvest anthracnose in mango caused by *Colletotrichum gloesporioides* (Haggag and Abdall, 2011). Talc-based formulations of *S. griseus* were used to control *Fusarium* wilt of tomato (Anitha and Rabeeth, 2009). *S. rochei* in combination with *Trichoderma harzianum*

was used to control root rot of pepper caused by *Phytophthora capsici* (Ezziyyani *et al*., 2007).

Thus, in this work, different actinomycetes were isolated from soil, tested against various food-borne pathogenic bacteria, as well as other plant pathogenic microorganisms, and the percentages of inhibition were determined in Petri dish cultures. The results showed that 57 species of actinomycetes and 19 of bacillary bacteria were isolated, of which 53% (41) of all the isolates showed activity against at least one of the bacteria. Fewer isolates showed inhibition against *Pseudomonas* sp., *K. pneumoniae*, *L. monocytogenes*, *Salmonella* sp. and *E. coli* (1 to 4 isolates). Inhibition was higher against Gram (+) bacteria.

OBJECTIVES

To detect the antibacterial activity presented by microorganisms isolated from soil against phytopathogenic bacteria and bacteria of importance in food.

MATERIALS AND METHODS

Soil samples were collected from different environments in Mexico, including forested, desert, and cultivated areas. One gram of each was taken and added to 10 ml of sterile distilled water, then subjected to temperatures of 50°C for 10 minutes and then serial dilutions were made up to 10^{-4}, then a volume of 0.1 ml was taken and spread with a glass spatula on Petri dishes containing potato dextrose agar added with 0.3% yeast extract and 0.3% malt extract. The boxes were incubated at 25°C for 10 days. Subsequently, a sample was taken to observe under the microscope and look for the characteristic morphology of actinomycetes (Gram + and filamentous form). Each colony was then isolated and transferred to slant agar tubes containing the same medium. On the other hand, strains of the different bacteria used, including *B. cereus, B. subtilis, Salmonella sp, Salmonella choleraesuis, Escherichia coli, Pseudomonas sp., Staphylococcus aureus, Listeria monocytogenes, Klebsiella pneumoniae and Xanthomonas campestris*, from the collection of the Instituto de Biotecnologia FCB-UANL, were activated on nutrient agar for 24 hours at 30-37°C. For the inhibition tests, plates were prepared with nutrient agar, and lines were marked on the bottom of the plate separated by approximately 2 cm, where the pathogens would be seeded; a line was also drawn through the center of the plate, on which the actinomycete strain was seeded 48 hours before seeding the bacteria and incubated at 25°C. Then, in the same plate, 5 species of bacteria were seeded in the lines previously drawn at the bottom of the box, which were incubated for 24 hours at 30-37°C and it was subsequently observed if it showed inhibition of bacterial growth. All experiments were performed in triplicate and at the end the inhibition percentages were determined based on the growth obtained in the line for each pathogen with respect to the distance in cm from the line drawn in the center of the box, where the actinomycete was sown.

RESULTS AND DISCUSSION

The continuing ability of bacteria to develop antibacterial resistance (Kim and Chung, 2004; Narayanan, 2004), has motivated research into new and more potent antibiotics and it is here, where countries such as Mexico, which have a great biodiversity, offer great potential in the search for new metabolites with inhibitory action against bacteria of interest in food and also against plant pathogens. Of the 76 species of actinomycetes isolated and tested against pathogenic bacteria, shown in Table 1, 41 (53.9%) of them were able to inhibit at least one bacterial species. The highest inhibition capacity was against the Gram-positive bacteria *B. subtilis*, *B. cereus* and *S. aureus*; while *L. monocytogenes* which is a bacterium that presents high resistance to antibiotics, could only be inhibited by strain M5 R2 by more than 50%. Of the Gram-negative bacteria,

shown in Table 2, *X. campestris* was inhibited by more than 50% by 12 of the isolated microorganisms. Against *Salmonella* sp. four isolates showed inhibition between 42.8-70%, while against *S. cholerasuis* there were 7 isolates that inhibited it, in similar proportions to the previous one; while against *E. coli*, three actinomycetes showed inhibition and only one isolate showed activity against *K. pneumonie* and another against *Pseudomonas* sp. A total of 56.7% of the isolated microorganisms had antibacterial activity only against Gram (+) bacteria, 29.7% against Gram (-) bacteria and 13.5% against both bacteria. These results differ from those reported by Jeffrey (2008) who reported actinomycete species that inhibited Gram (-) bacteria in a higher percentage. Our results coincide with those reported by Sierra-García *et al*. (2012) who mentioned that *S. aureus* and *B. subtilis* were the most sensitive bacteria in their evaluation.

The search for new products used against pathogenic species, especially actinomycetes, has been focused on unexplored niches to access new species, which may provide substances with potential for the development of new antibiotics.

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S. aureus	%	*B. cereus*	%	*B. subtilis*	%	*L. monocytogenes*	%
M4 R 5	51.7	D 17	57.1	D 17	68.9	M 5 R 2	55.1
M4 R 2	86.2	M1 R 6	42.8	D5	86.2		
D5	51.7	M1 R 8	85.7	M1 R 6	68.9		
M1 R 3	86.2	Ma 2	68.6	M1 R 3	68.9		
M R 3	100	M3 Col.	85.7	M1 R 8	100		
M1 R 8	68.9	A7	65.7	Ma 2	76.8		
M1 Col 2	71.4	A8	54.3	M3 Col.	100		
Ma 2	84.2	M4 R 4	54.3	A7	71.4		
M3 Col.	100	M4 R 1	42.9	M4 R 6	67.9		
M4 R 1	69.7	M1 R 13	57.1	A8	71.4		
A20	60.4	M1 R 10	42.9	M1 R 9	83.9		
M1 R 5	49.1	M R 10	71.4	M4 R 4	53.6		
M2 R	53.6	Narr1	40	M4 R 1	62.5		
M3 R 3	71.4	M4 R 3	42.8	M1 R 5	42.9		
M2 R 16	71.4	M1 R 2	52.9	M2 Col.	53.6		
M2 Col.	53.6	M5 R 1	51.4	M R 10	71.4		
M1 Col.	71.4			Narr1	44.8		
				Narr 2	65.5		

Tabla 1. Actividad Antibacteriana (% de inhibición) presentada por microorganismos aislados de muestras de suelo contra diversas bacterias patógenas Gram (+).
Fuente: Elaborado por los autores

S. *cholerae suis*	%	*X. campestris*	%	*E. coli*	%	*Salmonella* sp.	%	Pseudomonas sp	%	*K. pneumoniae*	%
M4 R 5	45.7	M1 R 3	62.5	D5	42.8	D5	42.8	M1 R 8	37.5	M2 Col.	100
D5	57.1	M R 3	70	M1 R 13	85.7	M5 R 5	42.8				
M R 3	42.8	M1 R 8	62.5	MA 23	57.1	M1 Col. 2	85.7				
Ma 2	60	Ma 2	61			Ma 2	70				
M4 R 6	40	A8	62.5								
M4 R 1	42.9	M1 R 13	62.5								
Narr 2	71.4	M R 10	61								
		M4 R 3	67.5								

		M 1 R 7	50
		M 4 R1	62.5
		A 20	50
		M 1 R5	65.7

Tabla 2. Actividad Antibacteriana (% de inhibición) presentada por microorganismos aislados de muestras de suelo contra diversas bacterias patógenas Gram (-)

Fuente: Elaborado por los autores

S. aureus	%	*B. cereus*	%	*B. subtilis*	%	*L. monocytogenes*	%
M4 R 5	51.7	D 17	57.1	D 17	68.9	M 5 R 2	55.1
M4 R 2	86.2	M1 R 6	42.8	D5	86.2		
D5	51.7	M1 R 8	85.7	M1 R 6	68.9		
M1 R 3	86.2	Ma 2	68.6	M1 R 3	68.9		
M R 3	100	M3 Col.	85.7	M1 R 8	100		
M1 R 8	68.9	A7	65.7	Ma 2	76.8		
M1 Col 2	71.4	A8	54.3	M3 Col.	100		
Ma 2	84.2	M4 R 4	54.3	A7	71.4		
M3 Col.	100	M4 R 1	42.9	M4 R 6	67.9		
M4 R 1	69.7	M1 R 13	57.1	A8	71.4		
A20	60.4	M1 R 10	42.9	M1 R 9	83.9		
M1 R 5	49.1	M R 10	71.4	M4 R 4	53.6		
M2 R	53.6	Narr1	40	M4 R 1	62.5		
M3 R 3	71.4	M4 R 3	42.8	M1 R 5	42.9		
M2 R 16	71.4	M1 R 2	52.9	M2 Col.	53.6		
M2 Col.	53.6	M5 R 1	51.4	M R 10	71.4		
M1 Col.	71.4			Narr1	44.8		
				Narr 2	65.5		

Table 1. Antibacterial activity (% inhibition) presented by microorganisms isolated from soil samples against various Gram pathogenic bacteria ( ).+

Source: Prepared by the authors

S. *cholerae suis*	%	*X. campestris*	%	*E. coli*	%	*Salmonella* sp.	%	Pseudomonas sp	%	*K. pneumoniae*	%
M4 R 5	45.7	M1 R 3	62.5	D5	42.8	D5	42.8	M1 R 8	37.5	M2 Col.	100
D5	57.1	M R 3	70	M1 R 13	85.7	M5 R 5	42.8				
M R 3	42.8	M1 R 8	62.5	MA 23	57.1	M1 Col. 2	85.7				
Ma 2	60	Ma 2	61			Ma 2	70				
M4 R 6	40	A8	62.5								
M4 R 1	42.9	M1 R 13	62.5								
Narr 2	71.4	M R 10	61								
		M4 R 3	67.5								
		M 1 R 7	50								
		M 4 R1	62.5								
		A 20	50								
		M 1 R5	65.7								

Table 2. Antibacterial activity (% inhibition) presented by microorganisms isolated from soil samples against several Gram (-) pathogenic bacteria.

Source: Prepared by the authors