Webology (ISSN: 1735-188X)
Volume 19, Number 5, 2022

Control Of Anthracnose In Yam Crop Through The Antifungal Activity Of Burkholderia Cepacia

Alexander Perez-Cordero[1*], Donicer Montes –Vergara[2] and Yelitza Aguas –Mendoza[3]

[1]Grupo Bioprospección Agropecuaria, Laboratorio de investigaciones microbiológicas, Facultad de Ciencias Agropecuarias, Universidad de Sucre, Sincelejo, Sucre Colombia.

[2]Departamento de Zootecnia, Facultad de Ciencias Agropecuarias, Universidad de Sucre, Sincelejo, Sucre-Colombia.

[3]Grupo de Investigación Gestión Integral de Procesos, Medio Ambiente y Calidad, Facultad de Ingeniería, Universidad de Sucre, Sincelejo, Sucre- Colombia.

* Correspondence: Author: Alexander Perez-Cordero

ABSTRACT

The aim of this work was to evaluate in vitro the production of microbial secondary metabolite-like compounds in carbon sources against Colletotrichum gloeosporioides. For this purpose, the antagonist strain Burkholderia cepacea was grown on different carbon sources (Glycerol, Honey, Homemade) in order to optimize the production of secondary metabolites and to evaluate in vitro its activity against C. gloeosporioides. Five replicates per treatment were performed, the assays were incubated for 15 days to determine the inhibition activity. The presence of inhibition zones showed positive antifungal activity of B. cepacia against C. gloeosporioides. The highest percentages of antifungal activity against was obtained in the secondary metabolites optimized in the medium with honey (36.32%±6.65). The analysis of the chemical composition of the metabolites was carried out by gas chromatography coupled to mass, finding 45 compounds in the medium with glycerol, 50 compounds for the medium with honey and 17 compounds for the homemade medium, where in the Glycerol and honey media the pyrrolo [1,2-a] pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl) compound predominated. Further studies are recommended to continue evaluating the antifungal activity of these bacteria against C. gloeosporioides and in the future be a biological alternative for the in situ management of this disease.

Keywords: Inhibition, Secondary metabolites, Endophytic bacteria, phytopathogens.

INTRODUCTION

In world agriculture, phytopathogenic fungi cause pre- and post-harvest diseases in vegetable, cereal and fruit crops, being responsible for substantial economic losses, the damage they cause not only refers not only to losses in economic production, but also to losses in biological production, i.e. the alteration in the growth and development of host plants attacked by these microorganisms (Agrios, 2005).

The most important pathogens causing high losses of fruits and vegetables are usually bacteria and fungi, however, more often it is fungal species that cause pathological deterioration of fruits, leaves, stems and underground products (roots, tubers, corms, etc.). Some sources estimate such losses to be in the order of 5-25% in developed countries and 20-50% in developing countries and 20-50% in developing countries (FHIA, 2007).

A wide range of fungi have been characterized as causing pathological deterioration in a variety of commodities, the most common being Alternaria, Botrytis, Diplodia, Monilinia, Penicillium, Colletotrichum, Phomopsis, Fusarium, Rhizopus and Mucor species (FHIA, 2007).

The genus Colletotrichum sp causes diseases on virtually all agricultural crops worldwide (WHITELAW et al., 2007). Typical symptoms of Colletotrichum sp infection are called 'anthracnose' which are characterized by necrotic collapse of tissue where masses of conidia are produced within an acervulus (FREEMAN et al., 2000).

Anthracnose occurs on developing and mature plant tissues, affecting fruit during development in the field as well as mature fruit during storage (PRUSKY et al., 2000). In the Colombian Atlantic Coast, it is known as iron spot or scorch and affects yields up to 50% (Bustamante, 2006). The severity of this disease leads to the excessive use of fungicides, which is costly, ineffective and harmful to the environment (Pérez et al., 2003).

Fungicides are the main solution for fungal disease control, but their use is controversial due to the undesirable environmental effects they cause. In recent decades. Recent research has shown new alternatives to replace the use of agrochemicals. Endophytic bacteria are used in order to substitute agrochemicals that are used for the control of plant pathogens causing plant diseases.

Endophytic bacteria reside in plant tissues, mainly in the intercellular space and within vascular tissues without harming the plant and provide benefit to other microbial residents (Hallmann et al., 1997; Kobayashi and Palumbo, 2000; Seghers et al., 2004; Zinniel et al., 2002). They are considered a biotechnological tool due to the production of secondary metabolites, which have activity against different plant pathogens.

The phylotypes within the genus of Burkholderia bacteria encompass a biotechnological potential of importance ranging from plant growth and development to protection against various biotic and abiotic stresses. Burkholderia spp. are known to promote plant growth by direct and indirect mechanisms. Direct growth promotion takes place through plant phytohormones synthesis by the bacterium (Singh et al., 2013), ACC deaminase activity (Sun et al., 2009), phosphate solubilization (Pal et al., 2022), nutrient delivery through rhizophagy cycle activity (White et al., 2019), nitrogen fixation (Pandey et al., 2005), etc. while indirect growth promotion involves the inhibition of bacterial or fungal pathogens through the synthesis of antagonistic substances such as siderophores (Pandey et al., 2005), volatile organic compounds (VOCs) (Liu et al., 2020), antimicrobial compounds (Mendes et al., 2007), among others.

Burkholderia cepacia is an endophytic bacterium that has shown antagonistic activity against a wide range of plant pathogens (Jayaswal et al., 1990; Cartwright & Benson, 1995). The bacterium is known to produce a wide range of secondary metabolites such as pyrrolnitrin, phenazine, cepabactin and other unidentified volatile or non-volatile compounds (Roitman et al., 1990; Cartwright et al., 1995). The aim of this study was to evaluate in vitro the production of secondary metabolites of B. cepacea on different carbon sources and their antagonistic activity against C. gloeosporioides.

MATERIALS AND METHODS

Endophytic bacteria. Burkholderia cepacia was isolated from varieties of hawthorn yam (D. rotundata), and identified at the molecular level by the Agricultural Bioprospecting group of the University of Sucre.

Phytopathogens. The phytopathogenic fungus used and identified was Colletotrichum gloesporioides obtained from leaves of yam variety Espino (D. rotundata), in the department of Sucre.

Antagonistic activity of Burkholderia cepacia against Colletotrichum gloeosporioides. For the antagonistic confrontation test, the dual culture medium PDA-R2A was used. B. cepacia was surface seeded using the single streak technique at one end of the Petri dish with dual medium. Petri dishes were incubated at 28°C for 2 days. After this time, the medium was seeded with mycelium of the fungus C. gloeosporioides in exponential growth phase. The boxes were incubated at 28°C for 15 days to determine the zone of inhibition between bacteria and fungus. The fungus in box without bacteria was used as a negative control (Palaniyandi et al., 2011; Doncel and Pérez, 2018).

Obtaining metabolite-like compounds from Burkholderia cepacia. To optimize the production of secondary metabolites from B. cepacia, 3 media with different carbon sources

were evaluated. The media used were: M1 (8 g Nutrient broth; 1.20 g Na2HPO4; 0.25 g KH2PO4; 1g/L Glycerol), M2 (8 g Nutrient broth; 1.20 g Na2HPO4; 0.25 g KH2PO4; 1g/L honey), M3 (8 g Nutrient broth; 1. 20 g Bicarbonate; 0.25 g Epson's salt; 1g/L Whey), in 1000 mL of distilled water, the initial pH of the homemade medium was adjusted to 6.8 with 0.1 N sulphuric acid. The experiment was carried out in 500 mL Erlenmeyer flasks, to which 200 mL of medium was added and inoculated with 2 mL of pre-culture of each bacterium. It was incubated at 28ºc ± 2ºc, with 160 RPM shaking. The cultures were fermented for 7 days (Cho et al., 2007).

Extraction and identification of microbial metabolites. The following steps were used for the extraction process: 100mL of each fermented medium were taken and centrifuged at 7000 rpm for 45 minutes. To each filtrate 80mL of ethyl acetate was added, then the organic layer was collected and concentrated using a rotary evaporator. The chemical composition of the secondary metabolite-type compounds was carried out as follows: The concentrate was analyzed using gas coupled mass chromatography (GC-MS) using an Agilent Technologies 7820a gc gas chromatograph with an agilent technologies 5977e mass spectrometer, using a HP5-MS working column, internal diameter: 250mm, stationary phase thickness 0.25 μm, stationary phase: 5% Phenyl, Methylpolyxiloxane and the following conditions: Initial oven temperature 60°C for 5 min rising to 160° for 7 min at a rate of 4°C/min and finally was raised to 240° for 7min at a rate of 15°C/min. Transfer line: 250°C. Injection port: 250°C. Ionization source 230°C. Helium mobile phase flow rate grade 5.0: 1.0 mL/min. Working solvent: ethyl acetate. Sample volume: 1μL. Time for separation of compounds: 42,41 min. Library: NIST (National Institute of Standards and Technology).

Antifungal activity of microbial secondary metabolites. The evaluation of the antimicrobial activity of the secondary metabolite-type compounds was carried out as follows: A hole was made in the surface of the PDA medium with the aid of a sterile punch previously inoculated with the fungus. 25 uL of the concentrate of each medium was added to the well. The boxes were incubated at 28°C for 15 days and the inhibitory activity was expressed as the percentage inhibition of fungal growth compared to the negative control (ethyl acetate in the well) (Islam et al., 2012). The percentage growth inhibition was determined by the following equation:

Inhibition (%)= [(Rc - Rb)/Rc x 100]

Where Rc is the radius of the control and Rb is the radius of the fungal colony interacting with the antagonistic bacteria (Rahman, 2007). Five replicates per treatment were used. The presence of inhibition zones indicated positive antifungal activity of the endophytic bacteria.

Statistical analysis. Results were expressed as the mean ± S.D. To establish differences, an ANOVA was performed using a completely randomized design for the inhibitory activity of

microbial metabolites, having previously determined the criterion of normality of the data. Significant statistical differences for the results were evaluated using the Tukey test (p-value ≤0.05). Data were analyzed in the free version of InfoStat software.

RESULTS AND DISCUSSION

Figure 1 shows the confrontation test between B. cepacea and C. gloeosporioides. against

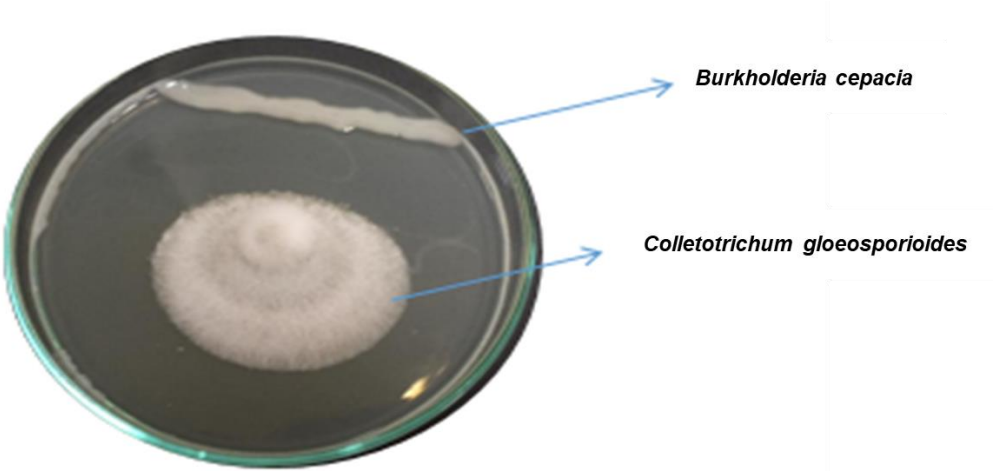

Figure 1. In vitro inhibition activity of B. cepacea against C. gloeosporioides in dual medium (R2A-PDA).

On analyzing the results and comparing the means obtained in the different tests in terms of the production of antifungal agents of strain AT31 against the fungus Colletotrichum gloeosporioides, significant differences were found between the homemade culture medium and the medium with glycerol and honey, However, no significant differences were found between the two latter media (Figure 2)

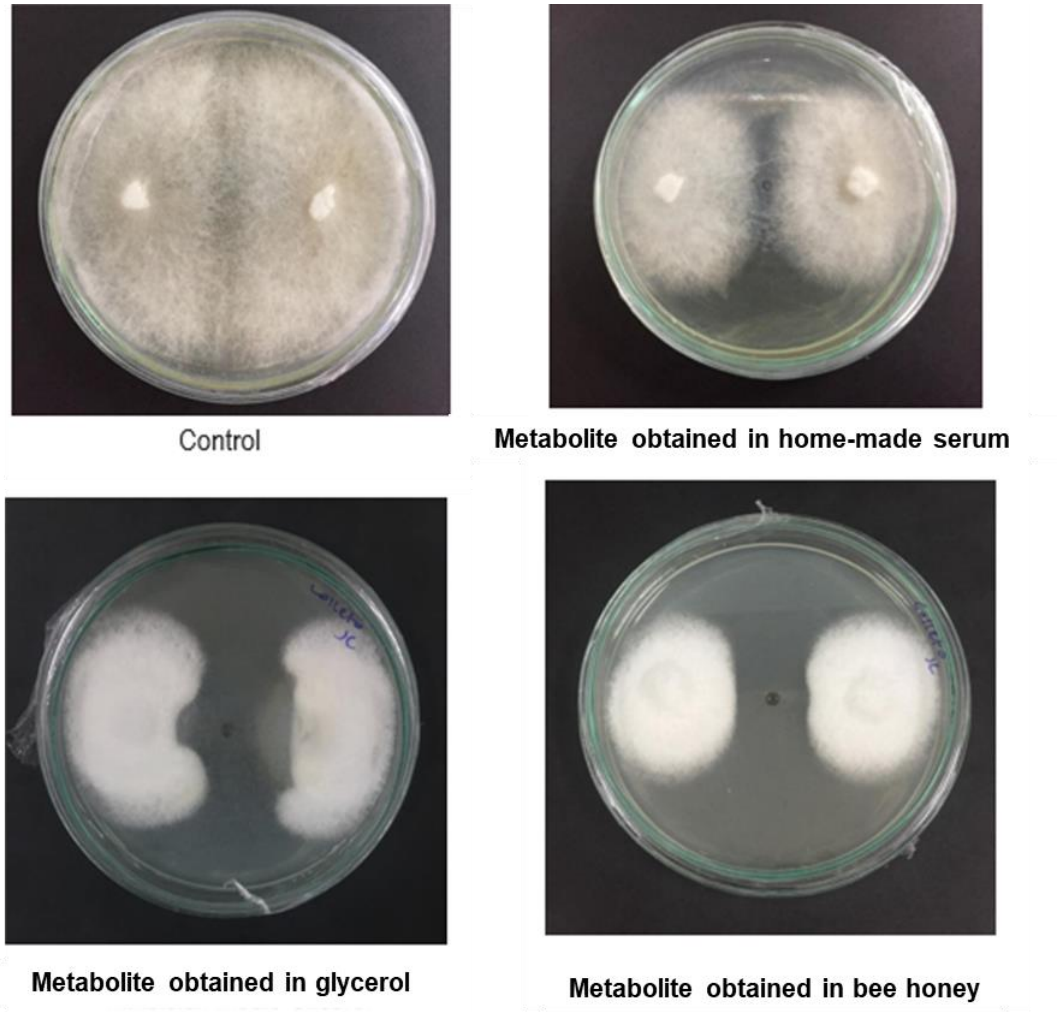

Figure 2. Inhibitory activity in culture media of B. cepacea metabolite-like compound against C. gloeosporioides**.**

The medium with honey showed the highest inhibition index with a total of 36.32%±6.65, followed by the medium with glycerol which showed an inhibition percentage of 27.61%±8.32 and finally the homemade medium with 5.50% ±1.60 (Figure 3).

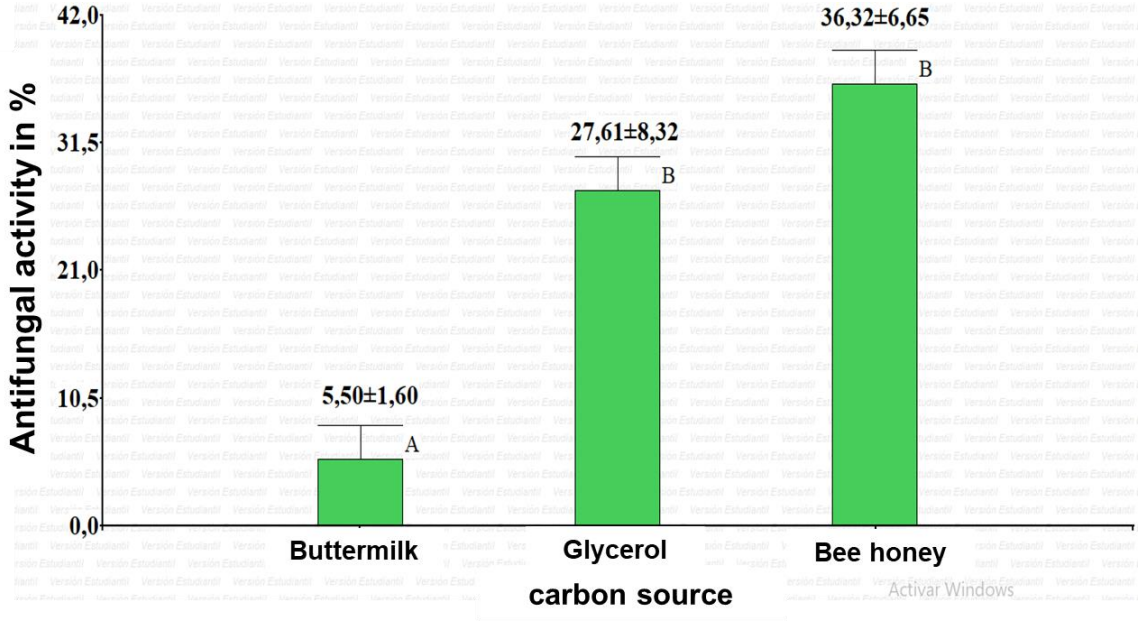

Figure 3. Antifungal activity of metabolite-like compound obtained from B. cepacea in different carbohydrate sources against C. gloeosporioides.

The chemical composition of the bacterial concentrate indicates that the most abundant compound was pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-, the most abundant of all the compounds tested, found in Honey and Glycerol media with a combined peak area of %25.704.

Previous research has demonstrated the antifungal activity of B. cepacia against plant pathogenic fungi (Parra et al, 2009). This property has led researchers to be interested in this bacterium as a potential biological control agent, as well as for inhibiting the growth of human pathogenic fungi (Li X et al, 2008).

Another study by Devi and Wahab (2012) illustrated that pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)- in endophytic fungi isolated from Camellia sinensis possesses strong antimicrobial activity. Furthermore, this compound was detected as the fraction of the extracts responsible for the high antibacterial capacity against Proteus vulgaris, Salmonella paratyphi, Staphylococcus aureus and Bacillus cereus in Streptomyces sp (Devi N et al, 2007).

The production of antimicrobial agents, which are often secondary metabolites, depends on primary metabolites that are produced by the catabolism of carbon, nitrogen and other important nutrients used by microorganisms, these primary metabolites are considered precursors and providers of building blocks for secondary metabolism through different pathways (Boaisha, 2012). Multiple reports have shown that antibiotic production occurs

late in the stationary phase, as a result of exposure to extreme conditions, nutrient deficiency or feedback phenomena, for this reason, metabolite extraction was performed on the seventh day of culture (Martin and Demain, 1980, Roitman, et al., 1990, El-Banna & Winkelmann, 1998).

The results obtained in this study show that B. cepacia inhibits the growth of the phytopathogenic fungus Colletotrichum gloeosporioides by the production of antifungal compounds, with higher antifungal activity observed when the medium with honey and glycerol was used. Variations in the nutritional and environmental factors of the bacteria often result in altered antibiotic production. The alteration involves changes in both the production and composition of the compound (Parra et al, 2009).

Media with Honey and Glycerol can contain precursors for antifungal metabolic activity, as (Ismet et al. 2004) demonstrated an increase in antifungal production when peptone is used as a nitrogen source. This factor is of utmost importance as it can change the ionising state of important essential molecules such as proteins and enzymes (Doncel 2018), according to Parra et al, 2009 demonstrated that temperature is also a fundamental factor in the antifungal activity of B. cepacia strains isolated from maize against phytopathogenic fungi.

According to (Parra et al, 2009), they reported changes in the composition and production of secondary metabolites by varying the growth conditions of B. cepacia. The results obtained in this study agree with those reported by these authors, where the production of the antifungal compound was influenced by variation in the growth factors of B. cepacia strains such as carbon source factor and pH.

B. cepacia is a nutritionally versatile bacterium due to the ability to utilize a large number of carbon sources as well as various amino acids as nitrogen sources (Li X et al, 2008). Nutritional factors had a great impact on the antifungal activity of B. cepacia, demonstrating, in the present study, the ability of the bacterium to inhibit fungal growth in the presence of a variety of carbon sources, making it a good prospect in the field of biotechnology for the control of fungal plant diseases of considerable economic importance.

CONCLUSION

In this study, the antifungal activity of the identified strain AT31 Burkholderia cepacia isolated from yam varieties against the fungus Colletotrichum gloeosporioides was proved, observing a higher antagonistic activity in the culture media with honey and glycerol as carbon sources, It is evident with these results and those based on the different literatures, that the bacterium presents a high degree of adaptability to different conditions to produce compounds that can be of great biotechnological interest, benefiting agriculture in the control of fungal diseases.

ACKNOWLEDGEMENTS

The authors would like to thanks the agricultural bioprospecting group of the University of Sucre for providing me with all the conditions to carry out this work.

REFERENCES

Agrios, G. N. 2005, Plant Pathology. Quinta edición. Academic Press. Nueva York. 803 p.

Berg, G., & Hallmann, J. (2006). Control of plant pathogenic fungi with bacterial endophytes. En Schulz B. J. E., Boyle C. J. C. and Sieber T.N. (Eds.), Microbial root endophytes (p. 53-69). Berlin: Springer- Verlag.

Boaisha, O. (2012). Burkholderia cepacia complex bacteria and their antimicrobial activity. Tesis Doctoral, Cardiff University.

Bustamante, S., Guzmán, M., & Buitrago, G. (2003). Caracterización molecular del germoplasma de ñame colombiano utilizando "DNA Amplification Fingerprinting (DAF)" en condiciones radiactivas. Revista Colombiana de Biotecnología, 5(2), 57-63.

Cho, K. M., Hong, S. Y., Lee, S. M., Kim, Y. H., Kahng, G. G., Lim, Y. P., … Yun, H. D. (2007). Endophytic bacterial communities in ginseng and their antifungal activity against pathogens. Microbial Ecology, 54(2), 341–351. https://doi.org/10.1007/s00248-007-9208-3

Cho, K. M., Hong, S. Y., Lee, S. M., Kim, Y. H., Kahng, G. G., Lim, Y. P., … Yun, H. D. (2007). Endophytic bacterial communities in ginseng and their antifungal activity against pathogens. Microbial Ecology, 54(2), 341–351. https://doi.org/10.1007/s00248-007-9208-3

Devi, N. N., & Wahab, F. (2012). Antimicrobial properties of endophytic fungi isolated from medicinal plant Camellia sinesis. Int J Pharm Bio Sci, 3(3), 420-427.

Doncel. 2017 bacterias endófitas productoras de metabolitos aisladas del cultivo de ñame (Dioscorea spp.) con actividad antifúngica contra Colletotrichum gloeosporioides penz. en el departamento de sucre. (Trabajo de grado de maestria). Maestria en Biologia, Facultad de Educaciíon y Ciencias, Universidad de Sucre.

Doncel, P., & Pérez-Cordero, A. (2017). Burkholderia cepacia aisladas de variedades de ñame con actividad antimicrobiana contra Colletotrichum gloeosporioides. Revista Colombiana de Ciencia Animal-RECIA, 9(S), 31-38.

FHIA, 2007. Deterioro poscosecha de las frutas y hortalizas frescas por hongos y bacterias. 4:2- 5.http://fhia.org. hn/dowloads/fhiainfdic2007 pdf, Accesada 02/11/10.

Fishal EM, Meon S, Yun WM. (2010). Induction of tolerance to fusarium wilt and defense-related mechanisms in the plantlets of susceptible Berangan Banana preinoculated with Pseudomonas sp. (UPMP3) and Burkholderia sp. (UPMB3). Agricul. Sci. China 9: 1140-114}

Freeman, S.; Katan, T.; Shabi, E. Characterization of Colletotricum gloeosporioides isolates from avocado and almond fruits with molecular and pathogenicity test. Applied and Enviromental Microbiology, Washington, v.62, n.3, p.1.014-1.020, 1996.

Hwang J, Chilton WS & Bensona DM (2002) Pyrrolnitrin production by Burkholderia cepacia and biocontrol of Rhizoctonia stem rot of poinsettia. Biological Control. 25: 56- 63.

Islam, R., Jeong, Y. T., Lee, Y. S., & Song, C. H. (2012). Isolation and identification of Antifungal compounds from bacillus subtilis C9 Inhibiting the growth of plant pathogenic fungi. Mycobiology, 40(1), 59–66. https://doi.org/10.5941/MYCO.2012.40.1.059

Ismet A, Vikineswary S, Paramaswari S, et al. (2004) Production and Chemical Characterization of Antifungal Metabolites From Micromonospora sp M39 Isolated From Mangrove Rhizosphere Soil. World Journal of Microbiology and Biotechnology. 20: 523-528.

Kadir, J., M.A. Rahman, T.M.M. Mahmud, R. Abdul Rahman and M.M. Begum, 2008. Extraction of antifungal substances from Burkholderia cepacia with antibiotic activity against Colletotrichum gloeosporioides on papaya (Carica papaya L.). Int. J. Agri. Biol., 10: 15-20

Kobayashi, DY, Palumbo, JD (2000) Bacterial endophytes and their effects on plants and uses in agriculture. In: Bacon, CW, White, JF, Jr (Eds.) Microbial Endophytes, Marcel Dekker, New York

Li X, Quan CS, Yu HY, Fan SD. Multiple effects of a novel compound from Burkholderia cepacia against Candida albicans. FEMS Microbiol Lett.2008; 285: 250-6.

Liu B, Qiao H, Huang L, Buchenauer H, Han Q, Kang Z, Gong Y. 2009. Biological control of take-all in wheat by endophytic Bacillus subtilis E1R-j and potential mode of action. Biol. Control 49: 277-285

Liu, A., Zhang, P., Bai, B., Bai, F., Jin, T., Ren, J., 2020. Volatile Organic Compounds of Endophytic Burkholderia pyrrocinia Strain JK-SH007 Promote Disease Resistance in Poplar. Plant Dis. 104, 1610–1620.

Machavariani N.G., Ivankova T.D., Sineva O.N., Terek hova L.P. (2014). Isolation of endophytic actinomycetes from medicinal plants of the Moscow region, Russia, World Appl. Sci. J. 30 (11) 1599–1604

Martin Jf & Demain AL (1980) Control of antibiotic biosynthesis. Microbioloy Reviews. 44: 230-251

Mendes, R., Pizzirani-Kleiner, A.A., Araujo, W.L., Raaijmakers, J.M., 2007. Diversity of cultivated endophytic bacteria from sugarcane: genetic and biochemical characterization of Burkholderia cepacia complex isolates. Appl. Environ. Microbiol. 73, 7259–7267.

Palaniyandi, S. A., Yang, S. H., Cheng, J. H., Meng, L., & Suh, J. W. (2011). Biological control of anthracnose (Colletotrichum gloeosporioides) in yam by Streptomyces sp. MJM5763. Journal of applied microbiology, 111(2), 443-455.

Parra, E. Centeno, S., Araque Y., (2009) Actividad antifúngica de Burkholderia cepacia aislada de maíz amarillo (Zea mays L.) bajo diferentes condiciones de cultivo. Revista de la Sociedad Venezolana de Microbiología 2009; 29:103-109.

Pal, G., Kumar, K., Verma, A., Verma, S.K., 2022. Seed inhabiting bacterial endophytes of maize promote seedling establishment and provide protection against fungal disease. Microbiol. Res. 255, 126926.

Pandey, P., Kang, S.C., Maheshwari, D.K., 2005. Isolation of endophytic plant growth promoting Burkholderia sp. MSSP from root nodules of Mimosa pudica. Curr. Sci. 177–180.

Prusky, D.; Freeman, S.; Dickman, M. Colletotrichum. Host specificity, pathology, and host-pathogen interaction. . St. Paul: American Phytopathological Society Press, 2000. 393 p

Pérez Lm, Baquero Mj, Beltran Jd. Caracterización morfológica y patogénica de Colletotrichum spp. como agente causal de la antracnosis en ñame Dioscorea sp. Rev Col Biotecnol. 2003;V:24-35.

Rahman, M. A., Kadir, J., Mahmud, T. M. M., Rahman, R. A., & Begum, M. M. (2007). Screening of antagonistic bacteria for biocontrol activities on Colletotrichum gloeosporioides in papaya. Asian Journal of Plant Sciences

Singh, R.K., Malik, N., Singh, S., 2013. Improved nutrient use efficiency increases plant growth of rice with the use of IAA-overproducing strains of endophytic Burkholderia cepacia strain RRE25. Microb. Ecol. 66, 375–384.

Sun, Y., Cheng, Z., Glick, B.R., 2009. The presence of a 1-aminocyclopropane-1-carboxylate (ACC) deaminase deletion mutation alters the physiology of the endophytic plant growth-promoting bacterium Burkholderia phytofirmans PsJN. FEMS Microbiol. Lett. 296, 131–136.

Whitelaw, W.M.A.; Curtis, S.J.; Huang, R.; Steel, C.C.; Blandchard, C.L.; Roffey, P.E. Phylogenetic relationships and pathogenicity of Colletotrichum acutatum isolates from grape in subtropical Australia. Plant Pathology, Chichester, v.56, n.3, p.448-463, 2007.

White, J.F., Kingsley, K.L., Zhang, Q., Verma, R., Obi, N., Dvinskikh, S., Elmore, M.T., Verma, S.K., Gond, S.K., Kowalski, K.P., 2019. Review: endophytic microbes and their potential applications in crop management. Pest Manag. Sci. 75, 2558–2565.