Relationship Between The Endophytic Bacteria Pseudomonas Aeruginosa Isolated From Medicinal Plants And The Production Of Bioactive Compounds

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ABSTRACT

Rice is one of the most important foods in the family basket. In recent years, rice has shown a decrease in its production due to diseases that have caused great economic losses. To control diseases, the application of agrochemicals is necessary. As an alternative to replace agrochemicals, we have opted for the application of beneficial microorganisms which confers protection against pathogens and favors plant growth.is necessary to search for beneficial microorganisms capable of controlling phytopathogens that cause diseases in crops, in order to replace the application of agrochemicals which cause environmental problems. Evaluate the antagonistic activity in vitro of Pseudomonas aeruginosa against Burkholderia glumae. The morphotypes isolated from Lippia origanoides were used, which were activated and purified in R2A medium. Each morphotypes was inoculated in 3s medium for 7 days for the production of secondary metabolites. After the time, we proceeded to concentrate the bacterial extract and evaluate it on filter paper discs in culture medium King B previously plated with pathogens. Likewise, the morphotypes were molecularly

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identified from the 16S rRNA gene. An analysis of variance and the Duncan multiple range test were applied for the inhibitory activity of morphotypes against pathogens. To phylogenetic analyses, the morphotypes evaluated showed 100% homology with Pseudomonas aeruginosa. In addition, they had the ability to control the growth of pathogens by releasing secondary metabolites. This species is characterized by producing secondary metabolites that control the growth of pathogens. The application of beneficial microorganisms with antagonistic potential may become in the future a great alternative to replace the application of agrochemicals.

Keywords: Pseudomonas aeruginosa, Burkholderia glumae, inhibition.

1. INTRODUCTION

Endophytic bacteria are microorganisms that can colonize approximately 90 % of living plant tissues of vascular plants without damaging them or gaining any advantage other than finding protection and habitat (Anantha et al., 2017; Thanh-Dung et al., 2023). Endophytic bacteria have been isolated from a high diversity of host plants (Rosenblueth and Martinez-Romero, 2006). Endophytic bacterial communities established on plants show a capacity for the formation of various bioactive metabolites in plants (Tiwari et al., 2013; Santos et al., 2018; Thanh-Dung et al., 2023).

As raised by Abla et al., (2015), there is a question whether only plants or microorganisms produce phytochemicals or do they interact for their production, are there interactions between plants and endophytic bacteria in the production of bioactive compounds, and can endophytic bacteria be considered as triggers? Several studies link the roles of endophytic bacteria and plants in the production of bioactive compounds, many studies have reported the ability of endogenous bacteria to produce bioactive compounds in medicinal plants. In addition, the studies also provided new and useful insights needed for pathogens for human health and other potential medical applications.

Plant-associated endophytic bacteria serve as novel bioactive agents (Nongkhlaw and Joshi, 2015), producing various compounds that promote plant growth and protection (Basit et al., 2021).

Endophytic bacteria can produce multiple bioactive metabolites in a single plant or microorganism, making them an excellent source of drugs for the treatment of various diseases and are alternative products for wide use in agricultural, pharmaceutical, food and cosmetic industries (Jalgaonwala et al., 2011; Omojate Godstime et al., 2014; Shukla et al., 2014; Strobel and Daisy, 2003).

Secondary metabolites are classified into different functional groups, such as alkaloids, benzopyranones, quinones, flavonoids, phenolic acids, quinones, steroids, saponins, tannins, terpenoids, tetralones and xanthones (Ek-Ramos et al., 2019; Jalgaonwala et al., 2011; Joseph and Priya, 2011; Omojate Godstime et al., 2014; Pimentel et al., 2011; Schulz et al., 2002; Strobel and Daisy, 2003).

Secondary metabolites produced by endophytic bacteria are considered as antimicrobial agents, which are a natural way to effectively combat pathogens in the context of multidrug resistance of pathogenic microorganisms (Singh et al., 2017). In addition, a wide range of bioactive compounds produced by endophytic bacteria are beneficial not only for plants, but also for humans (Basit et al., 2021). These compounds play important roles in therapeutic applications as antibacterial, anticancer, antioxidant, antidiabetic and antibiotic agents (Korkina, 2007; Rustamova et al., 2020).

Given the current problems faced by agricultural growers in Colombia, due to the presence of phytopathogens in agricultural crops of commercial interest and the productive losses that these microorganisms produce in productivity and crop profitability, there is a need to find new bio-actives of microbial origin associated with medicinal plant species, as an alternative for the management of phytosanitary problems in the field, as a measure to mitigate the indiscriminate use of drugs, avoid phytopathogens resistance and protect crop safety, it was proposed to isolate endophytic bacteria from Lippia origanoides and evaluate the inhibitory activity through secondary metabolites produced by these bacteria against the bacteria causing the bacterial blast of panicle blast in rice crops.

2. MATERIALS AND METHODS

Sampling. Samples of Lippia origanoides were collected in the Corregimiento de Segovia, municipality of Sincelejo, Sucre, Colombia, at 9°13'53.13''N and 75°22'41.59''W at 114 masl. The material collected in the field was labelled, stored and refrigerated

for processing in the microbiological research laboratory of the University of Sucre.

Isolation of endophytic bacteria. The root, stem and leaves of each Lippia origanoides plant were washed with sterile distilled water and cut into segments of approximately 1 cm, then subjected to surface disinfection as described by Perez et al.

Method to determine the antimicrobial activity of metabolic extracts. The method to evaluate the inhibitory activity of microbial metabolic extracts was performed following the protocol proposed by Barraza et al. (2017), as follows: A pure colony of the phytobacterium B. glumae was transferred to 20 ml of King B broth in incubated at 30 °C at 150 rpm for 24 h, after this time the bacterial suspension was adjusted to a concentration of 0.5 Macfarland. On the other hand, samples of the metabolic suspensions taken at different times (24, 48, 72 and 120 h), dilutions were prepared at 3, 6, 12, 25 to 50 % concentrations. The bactericidal activity of each extract was evaluated separately using the microdilution technique in 96-well plates**.**

From the stock solution, 5 serial dilutions were made in triplicate in King B medium, in a 1:1 ratio, for a final volume of 90 µL/well of each dilution, to obtain the aforementioned concentrations, 10µL of the bacterial suspension was added to each well to complete a final volume of 100 mL per well. Each plate included medium sterility controls (100 µL King B broth), growth control (90 µL King B medium + 10 µL bacterial suspension) and metabolite control (100 µL), the plates were capped and sealed with crista Flex and incubated at 150 rpm at 30°C for 24 hours in a Heidolph 1000 incubator (model D-91126).

After incubation time, 15 µL of an aqueous solution (0.5 mg/ml) of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) was added to the wells containing the treatments and the controls. After incubation for 45 minutes, the contents of the wells were discarded and 100 µL/well of concentrated DMSO was added and the optical density was determined in a Chromate Awareness reader, model 4300 using a wavelength of 492 nm. With the results of the readings, the percentage inhibition was determined using the following formula:

%IB= 1-(Aai/Acc)*100

Where: Aai: average growth rate of each treatment Acc: average growth rate control test (Barraza et al., (2017).

Molecular identification of endophytic bacterial strains. Genomic DNA of endophytic bacterial strains was performed according to the protocol proposed by Oliveira et al. (2013). Universal primers that amplify the small subunit of the 16S rRNA were used. The amplified products were purified and sent for sequencing to Macrogen. The sequences obtained were compared with those stored in Genbank. Base alignment was performed in the Clustal W program; phylogenetic inferences were obtained by the Neighbor Joining method based on the kimura-2-parameter model with bootstrap 1,000 test in the MEGA X program.

3. RESULTADOS AND DISCUSSION

Figure 1 shows the inhibitory activity of the metabolic extracts of P. aeruginosa and the positive control (oxolinic acid) against Burkholderia glumae. The in vitro inhibitory activity of P. aeruginosa was similar to that of the positive control, indicating the power of the bacterial bioactive for the management of bacterial blast of rice panicle in the field.

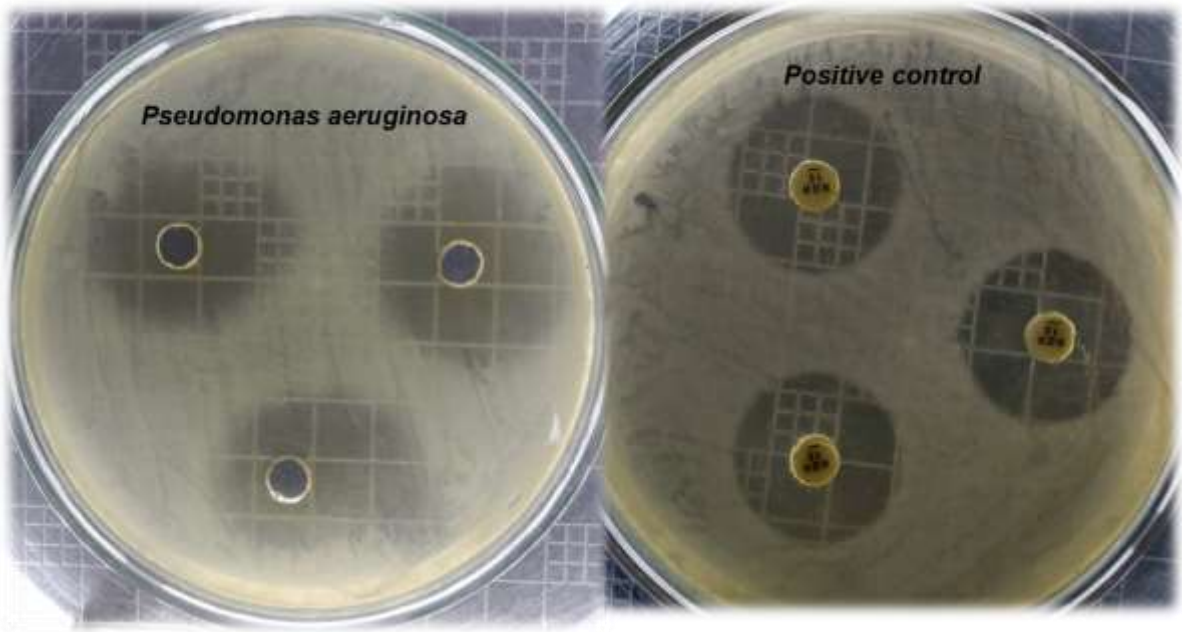

Figure 1. In vitro inhibitory activity of metabolic extracts of Pseudomonas aeruginosa against B. glumae.

Endophytic bacteria colonize different compartments such as apoplast, including intercellular and vascular spaces in plant tissues without expressing pathogenicity (Pérez et al., 2013) creating a symbiotic type association between them (Nair and Padmavathy, 2014). This type of colonization is a complex process that involves the immune response of the plant to the microorganisms and the ability of the latter to "invade" and colonise the interior of the host without causing any deleterious effect on it (Hardoim, et al., 2008, Compant, et al., 2010), forming a system in which the microorganisms transmit information from the host plant to them and vice versa (Pérez et al., 2010).

Figure 2 shows the results of the percentage inhibition of P. aeruginosa extracts against B. glumae compared to the positive control. The figure shows that from the first hour after the start of the experiment, P. aeruginosa showed inhibitory activity of 80% compared to the biological control with oxolinic acid, which by the same time had an inhibition percentage of 59%. Likewise, it is observed that the inhibitory activity of the bacterial extract reached 100 % of activity from the 7th hour onwards, while with the positive control it was reached from 11 hours after the start of the experiment.

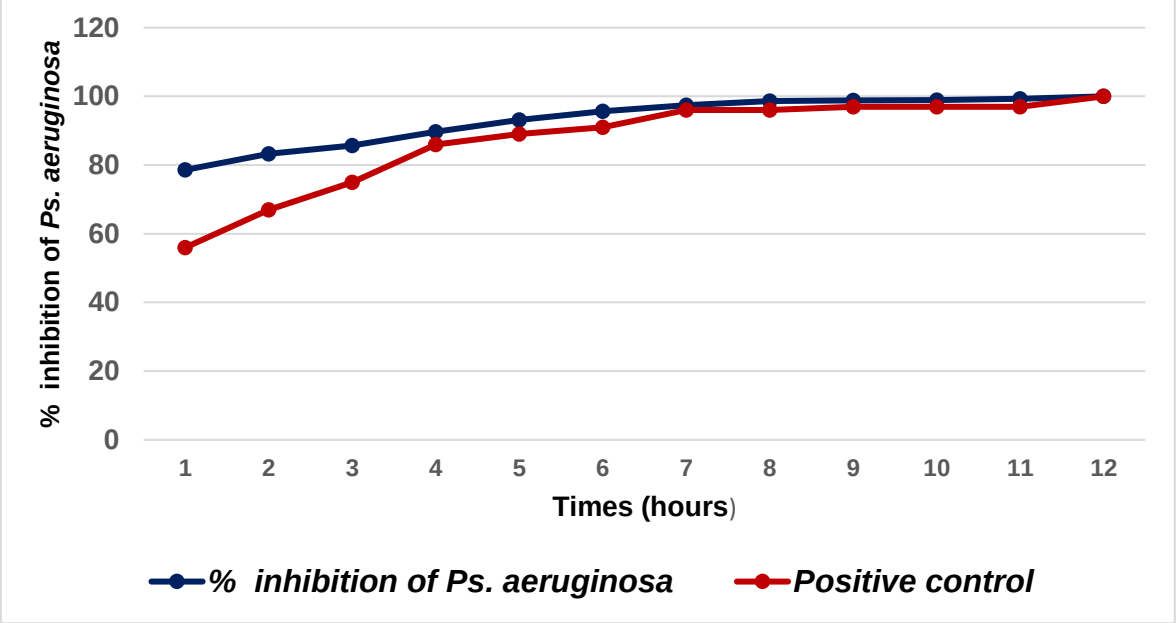

Figure 2. Percentage inhibition of P. aeruginosa and the positive control against Burkholderia glumae.

According to the results of the phylogenetic analysis of the isolates under study, they showed similarity with the sequences stored in GenBank which presented 100% homology with the species Pseudomonas aeruginosa FJ972534.1 (figure 3), which corresponded to the strain studied to evaluate the capacity to produce metabolic extracts and antibacterial activity against Burkholderia glumae.

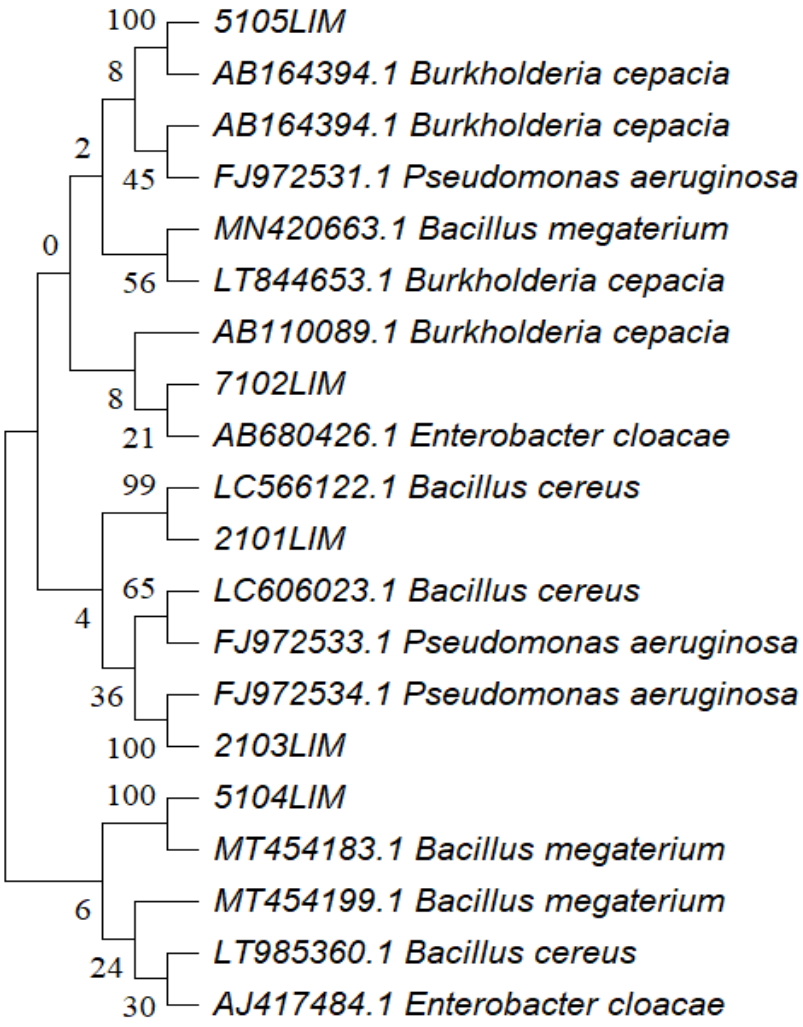

Figure 3. Sequence dendogram from the 16S rRNA gene of the morphotypes under study.

Among the bacteria used for biological control, species of the genus Pseudomonas spp. stand out for their versatility in producing antimicrobial secondary metabolites, such as siderophores and antibiotics, which belong to groups of chemically defined compounds with specific biological functions against phytopathogenic fungi and pathogenic bacteria. Inhibition of the

pathogen by the production of antimicrobial metabolites or iron chelator (siderophores) are considered to be the main biocontrol mechanisms. The production of secondary metabolites allows plants to protect themselves from attack by various pathogenic microorganisms (Rojas et al., 2012).

According to the results of research carried out by (Hui et al., 2013), there are about 244 strains of bacteria isolated and identified from Polygonum cuspidatum, which correspond to the groups and/or genera Lysinibacillus, Paenibacillus, Pseudomonas, Bacillus, Kocuria, Streptomyces, Providence, Rhizobium, Leucobacter, Brachybacterium and Mycobacterium. A diversity of these endophytic bacteria showed antibacterial activity against Klebsiella pneumoniae, 12 isolates showed antibacterial activity against Staphylococcus aureus and 6 isolates inhibited Bacillus subtilis. Likewise, secondary metabolites produced from Streptomyces SUK 06 isolated from Thottea grandiflora (Joseph and Priya, 2011) and Streptomyces sp. from Greviilea pteridifolia (Castillo et al., 2003) showed antibacterial activity Guzman-Trampe ' et al. (2015) reported antibacterial activity of 8 strains of Pseudomonas isolated from the medicinal plant Magnolia dealbata Zucc (Guzman-Trampe et al., 2015).

Most endophytic bacteria produce multiple antibiotics. Ecomycin, pseudomycin and kacadumycin are some of the new antibiotics produced by endophytic bacteria (Christina et al., 2013). The endophytic bacterium Streptomyces sp. LJK109 isolated from the root of Alpinia galangal produces 3-methylcarbazole which is the main anti-inflammatory component and also suppresses macrophage production of inflammatory mediators NO,PGE2, TNF-α, IL-1β, IL-6 and IL-10 in a dose-dependent manner (Taechowisan et al., 2012). Pseudomonas viridiflava, an epiphytic or endophytic microorganism of plants, particularly the leaves of many grass species, produces ecomycin, which is a molecule found in grass plant tissues.

The diversity and characteristics of medicinal plants determine the diversity and characteristics of endophytic bacteria. The physiological diversities, metabolites and growth habits of different plant species influence their ability to recruit diverse endophytic bacteria (Campisano et al., 2014; Kawaguchi and Minamisawa, 2010) (Figure 4).

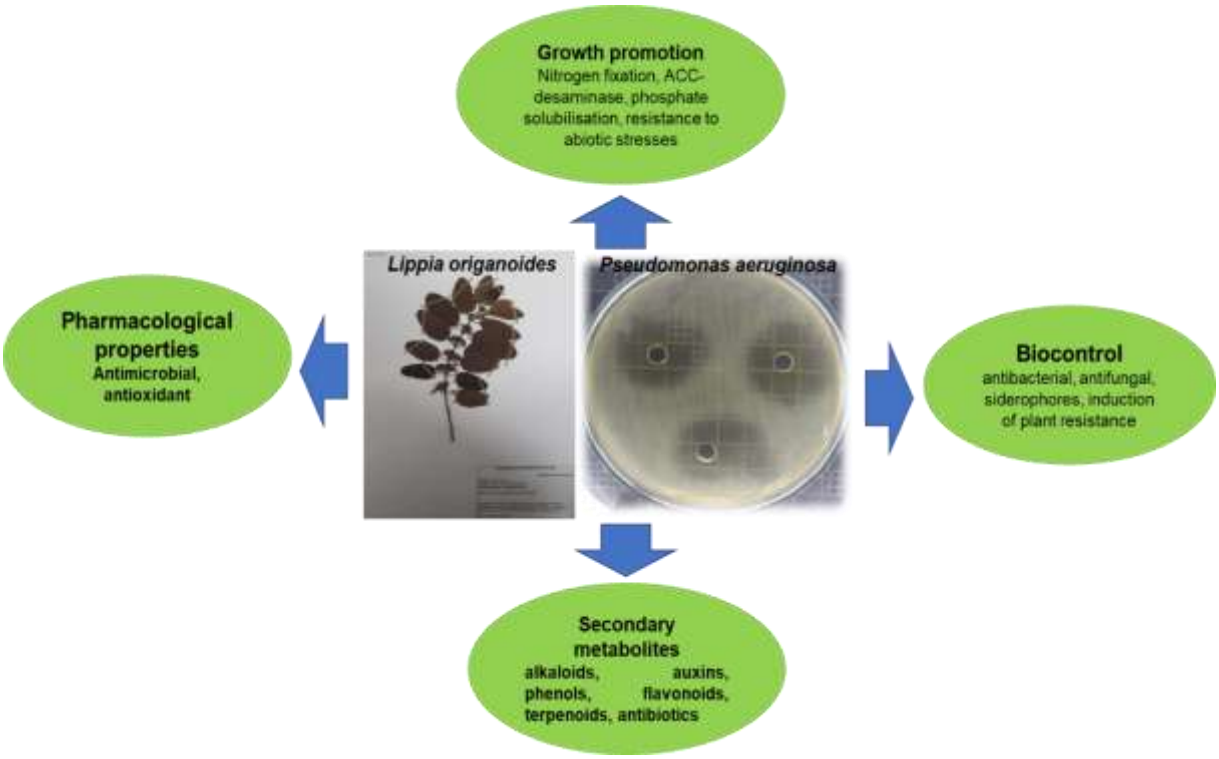

Figure 4. Possible interaction between Pseudomonas aeruginosa and Lippia origanoides.

Medicinal plants of Lippia origanoides harbour endophytic bacteria such as Pseudomonas aeruginase, an endophytic bacterium with potential for distinct biosynthetic characteristics derived from the enormous and diverse range of metabolite richness. There are differences between endophytic bacteria associated with medicinal plants and those related to cross-modulation of phytohormone production or enhancement of plant tolerance to stress. Under conditions of nutrient deficiency or stress, the host plant increases the production of metabolites for the recruitment of nutrient-contributing endophytic bacteria (López-Ráez' et al., 2011).

One of the aromatic plants producing essential oils is Lippia origanoides H.B.K., an aromatic plant commonly known as "Orégano de monte", which grows wild in Central America, northern South America and the Antilles (Hennebelle, et al., 2008), 2008), in Colombia this plant is found at altitudes between 500 and 800 masl (Stashenko, et al., 2008) in the departments of Guajira, Magdalena, Cauca, Cundinamarca, Cauca, Norte de Santander, Santander and Nariño (Albesiano et al., 2003; Ruiz et al., 2007). Studies have shown that the essential oils of Lippia origanoides,

have inhibitory activity against plant pathogenic fungi B. maydis, R. solani, Fusarium oxysporum, antimicrobial activity against human pathogens causing respiratory and gastrointestinal diseases (Dos Santo et al., 2004; Oliveira et al., 2007), anti-protozoal activity against Leishmania chagasi, causative agent of Leishmaniasis and anti-repellent activity against pests (Caballero-Gallardo et al., 2012).

4. CONCLUSION

The endophytic bacterium Peudomonas aeruginosa isolated from Lippia origanoides, becomes a biological potential for the management of the bacterium Burkholderia glumae causing the bacterial blast of the rice panicle in the department of Sucre. In addition, it could be an alternative to reduce the continuous use of fungicides and in the future it could become a natural resource to reduce environmental pollution due to the indiscriminate use of drugs for the management of the disease in rice fields in Colombia and worldwide.

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6. AUTHORSHIP CONTRIBUTIONS

All authors have jointly and equally contributed to the argumentation and writing of the manuscript.

7. FUNDING.

None.

8. CONFLICT OF INTEREST

None.

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