Rev Argent Microbiol. 2020;52(2):164–166

asociación argentina de microbiología

REVISTA ARGENTINA DE MICROBIOLOGÍA

www.elsevier.es/ram

LETTER TO THE EDITOR

Protective effect on wood by metabolic extracts from plant growth-promoting rhizobacteria against decay fungi

Efecto protector en madera de extractos metabólicos provenientes de rizobacterias promotoras del crecimiento vegetal contra hongos de pudrición

Dear Editor:

Plant Growth-Promoting Rhizobacteria (PGPR) of the genus *Bacillus* are useful as biological control agents by synthesizing and releasing different metabolic antimicrobial compounds such as surfactin, fengycin, hydrocyanic acid, pyrrolnitrin, dimethylhexadecylamine, phenazines and others; however, the antifungal mechanism and the application of these compounds to protect wood-based materials are still not known[4]. Wood is a lignocellulosic material that is an important source of carbon for insects, bacteria and fungi which have the potential to degrade it and generate significant economic losses[3]. In the agricultural system, the control mechanisms offered by the PGPR have been an environmentally sustainable alternative to the use of eco-toxic compounds; nonetheless, this strategy has not been adopted in the wood preservation industry. That is why bacterial metabolites can represent a biotechnological option to control the deterioration caused by decay fungi.

To test the above, we used isolates of xylophagous fungi identified using 18S rRNA gene sequences[2]. In an *in vitro* system, we confronted fungal isolates to already identified PGPR *Bacillus megaterium* UMCV1, *B. subtilis* BS-MIA02 and other *Bacillus* spp. (BA, SS and BM), we selected the strain with the highest antagonistic activity and performed a methanolic extraction of the bacterial metabolites[1]. The extracted compounds were incubated with wood pieces of *Pinus pseudostrobus* in Petri dishes containing Potato Dextrose Agar/Nutrient Agar medium ratio 1:1 at 28 °C in the dark for 10 days[5]. Using ImageJ software[6] we recorded the mycelial growth diameter and the percentage of the area colonized in the wood.

Table 1 shows the growth of fungal isolates confronted to PGPRs and we can observe a strong inhibition of mycelial growth by the five bacteria tested against the six identified fungi and the isolate FD. We also found that of the five bacteria tested, *Bacillus* sp. SS had the strongest mycelial growth inhibition. Using the metabolic extracts from the strain identified as *Bacillus* sp. SS, different pieces of wood were impregnated with the extracts and exposed to the fungal isolates. Results in this trial showed that the fungal isolates grew *ad libitum* on the lignocellulosic material (100% colonization) that did not receive treatment with the metabolic extracts. The pieces of wood that received the protective treatment showed a significant inhibition of fungal development ($p < 0.05$ regarding the untreated pieces), contrary to the percentages of colonization obtained, which were: *Trametes versicolor* (55.9%), *Fusarium verticillioides* (1.3%), *Mucor* sp. (0%), *Trichoderma* sp. (PP2, PP3 and PP5) (0%) and FD (0%). The metabolic extracts from the *Bacillus* strains tested in this work inhibited the growth of the xylophagous fungi. Although the metabolomic profile of the PGPRs tested is not known, the results were important and convincing. It has been proven that biological control strategies in field conditions are effective; on the other hand, in forest habitats the exploration is poor. With the information obtained in this work, it can be concluded that there is an interesting profile of metabolic compounds, still uncharacterized,

Table 1 Antagonistic activity of different PGPR strains against isolated xylophagous fungi.

Fungal isolate	*Bacillus* strain					
	Control	SS	BSMIA	BM	BA	UMCVI
Trametes versicolor	8.62 ± 0.66^{d}	2.50 ± 0.20^{a}	2.82 ± 0.49^{ab}	3.54 ± 0.20^{b}	6.20 ± 0.58^{c}	6.26 ± 0.23^{c}
Fusarium verticillioides	9.00 ± 0.20^{d}	2.54 ± 0.41^{a}	7.00 ± 0.50^{c}	7.10 ± 0.30^{c}	6.12 ± 0.23^{b}	8.42 ± 0.29^{d}
Mucor sp.	8.86 ± 0.21^{d}	2.70 ± 0.45^{a}	4.58 ± 0.42^{b}	7.18 ± 0.41^{c}	7.50 ± 0.50^{c}	7.94 ± 0.61^{cd}
Trichoderma koningii (PP2)	8.60 ± 0.22^{c}	4.22 ± 0.58^{a}	6.18 ± 0.24^{b}	8.02 ± 0.62^{c}	8.28 ± 0.33^{c}	8.72 ± 0.22^{c}
Trichoderma koningii (PP3)	9.16 ± 0.089^{d}	2.98 ± 0.34^{a}	4.86 ± 0.79^{b}	4.66 ± 0.27^{b}	7.24 ± 0.33^{c}	8.38 ± 0.23^{d}
Trichoderma koningii (PP5)	8.80 ± 0.2^{e}	2.62 ± 0.39^{a}	4.82 ± 0.47^{b}	6.18 ± 0.48^{cd}	6.84 ± 0.51^{d}	6.52 ± 0.48^{cd}
FD	8.40 ± 0.41^{e}	2.14 ± 0.13^{a}	4.14 ± 0.27^{b}	5.22 ± 0.44^{c}	6.58 ± 0.51^{d}	6.56 ± 0.26^{d}

Values correspond to the average mycelial growth diameter (cm), different letters show statistical differences according to the Tukey's test ($p < 0.05$, $n = 8$), $\pm$ shows the standard deviation.

produced by the mentioned tested bacterial isolates, indicating their broad-spectrum antagonism to wood-degrading fungi, as well as the potential or capacity for fungal repression by these metabolites does not show a homogeneous behavior among them. There is still work to do related to the mode of use, minimum repressing concentration, durability in woody tissue, application costs, among others; however, we believe that in the short term the compounds produced by the bacterial strains used here will be identified and used in wood protection susceptible to microbial attack and low durability.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

Thanks to CONACYT and UMSNH-CIC for its support in the work.

References

1. Dimkić I, Stanković S, Nišavić M, Petković M, Ristivojević P, Fira D, Berić T. The profile and antimicrobial activity of *Bacillus* lipopeptide extracts of five potential biocontrol strains. Front Microbiol. 2017;8:1–12.
2. Gontia-Mishra I, Tripathi N, Tiwari S. A simple and rapid DNA extraction protocol for filamentous fungi efficient for molecular studies. Indian J Biotechnol. 2014;13:536–9.
3. Guillén F, Martínez MJ, Gutiérrez A, Del Rio JC. Biodegradation of lignocellulosics: microbial, chemical, and enzymatic aspects of the fungal attack of lignin. Int Microbiol. 2005;8:195–204.
4. Liu C, Sheng J, Chen L, Zheng Y, Lee DYW, Yang Y, Xu M, Shen L. Biocontrol activity of *Bacillus subtilis* isolated from *Agaricus bisporus* mushroom compost against pathogenic fungi. J Agric Food Chem. 2015;63:6009–18.
5. Orozco-Mosqueda M, Valencia-Cantero E, López-Albarrán P, Martínez-Pacheco M, Velázquez-Becerra C. La bacteria *Arthrobacter agilis* UMCV2 y diversas aminas inhiben el crecimiento *in vitro* de hongos destructores de madera. Rev Argent Microbiol. 2015;47:219–28.
6. Schneider C, Rasband W, Eliceiri K. NIH Image to ImageJ: 25 years of image analysis. Nat Methods. 2012;9:671–5.

Vanessa García-Ortiz, Christian Hernández-Soberano, Mauro Martínez-Pacheco, Enrique Ambriz-Parra, Crisanto Velázquez Becerra*

Universidad Michoacana de San Nicolás de Hidalgo, Gral. Francisco J. Múgica S/N, Ciudad Universitaria, 58030 Morelia, Mich., Mexico

*Corresponding author.
E-mail address: cvelazquez@umich.mx
(C. Velázquez Becerra).

https://doi.org/10.1016/j.ram.2019.04.003

Alta seroprevalencia de *Neospora caninum* en perros con sospecha clínica de neosporosis en Montevideo, Uruguay

High seroprevalence of *Neospora caninum* in dogs with clinical suspicion of neosporosis in Montevideo, Uruguay

Sr. Editor:

La neosporosis causada por el parásito *Neospora caninum* está ampliamente distribuida en el mundo; es considerada una enfermedad emergente en Sudamérica[4], y produce alteraciones neurológicas en los perros. También origina trastornos reproductivos en los bovinos, por lo que ocasiona pérdidas millonarias para la ganadería[2]. En Uruguay, la mayoría de los estudios sobre neosporosis se enfocan en la ganadería, pero dada la importancia que tiene la producción de bovinos, es imperativo considerar también a los perros, sus principales hospederos definitivos[2]. A 21 años del último reporte del parásito en perros en Uruguay[1], su seroprevalencia actual se desconoce. El objetivo de esta investigación fue realizar un estudio retrospectivo para detectar la prevalencia de anticuerpos anti-*Neospora caninum* en perros domésticos clínicamente sospechosos de padecer neosporosis y su asociación con el sexo, la edad y la raza.

Se recopiló información de expedientes de 469 muestras de suero remitidas entre agosto de 2017 y marzo de 2018 al Laboratorio de Análisis Clínicos de la Facultad de Veterinaria de la Universidad de la República, Montevideo, Uruguay. Para la detección de anticuerpos IgG se empleó la técnica de inmunofluorescencia indirecta mediante el kit MegaFLUO® *Neospora caninum* (Diagnostik MEGACOR Gemeinde Hörbranz, Austria), utilizando distintas diluciones: 1:50, 1:100, 1:200, 1:400 y 1:800. Las muestras con manifestación total de fluorescencia en los taquizoítos desde la primera dilución fueron consideradas positivas.

El 47,3% de las muestras fueron positivas a *N. caninum*; no se observaron diferencias significativas ($p > 0,05$) de los perros positivos en relación con las variables sexo y edad, pero sí en cuanto a la raza (tabla 1). Las diluciones y las frecuencias de los casos positivos fueron, respectivamente 1:50, 43; 1:100, 6; 1:200, 42; 1:400, 30 y 1:800, 101.

Dado que en un artículo que resume 52 estudios de seroprevalencia del parásito en perros solo se hallaron 3 casos con seroprevalencias superiores a las del presente análisis[2], consideramos que la seroprevalencia hallada es alta. Ello puede obedecer a que, por tratarse de casos sospechosos a neosporosis, las muestras en nuestro estudio no fueron