Assessment of the Antagonistic Effects of bacteria isolated from Atrazine and Metribuzin–contaminated soil in sugarcane against Fungal Pathogens under Laboratory Conditions

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Abstract

Contaminated soil can be remediated by employing various types of microbes, such as bacteria, which have the ability to enhance plant growth and combat pathogenic microorganisms. Biological control is an environmentally adaptable method that utilizes living microorganisms like bacteria, fungi, and viruses to suppress plant pathogens through mechanisms such as competition, parasitism, antibiosis, and induced resistance. This study aims to assess the effectiveness of bioremediation bacteria as biological control agents against fungal pathogens affecting sugarcane. To achieve this objective, previously identified bacterial strains with potential in bioremediation of Atrazine and Metribuzin herbicides, including *Brucella* sp. (66A, 44A, and 65A), *Pseudomonas putida* 10A, *Ensifer adhaerens* 20m, *Pseudomonas aeruginosa* 15A, and *Stenotrophomonas* sp. 22A, were evaluated for their ability to inhibit the growth of *Thielaviopsis ethacetica*, *Cytospora sacchari*, *Fusarium proliferatum*, *Bipolaris drechslera*, *Curvularia* sp., *Alternaria* sp., and *Nigrospora* sp. The experiments were conducted using the dual culture method in a completely randomized design with three replications under laboratory conditions. The diameter of the bacterial inhibition zone and the percentage of fungal growth inhibition were calculated. The results indicated that *P. aeruginosa* 15A exhibited the highest growth inhibition against *T. ethacetica*, *Curvularia* sp., and *Alternaria* sp. On the other hand, *Stenotrophomonas* sp. 22A significantly inhibited *C. sacchari*, while both *Stenotrophomonas* sp. 22A and *Brucella* sp. 65A showed considerable inhibition against *F. proliferatum*. *Bipolaris drechslera* was most effectively inhibited by *Brucella* sp. 66A and *Brucella* sp. 44A, while *P. aeruginosa* 15A and *Brucella* sp. 66A exhibited prominent inhibition against *Nigrospora* sp. Notably, *P. putida* 10A did not display antagonistic properties against the tested fungi under laboratory conditions, whereas *P. aeruginosa* 15A significantly affected the growth of all examined fungi. Based on these findings, this study provides evidence that certain bioremediation isolates possess significant potential for biological control of specific fungal pathogens affecting sugarcane in laboratory conditions. Consequently, the antagonistic properties against important fungi in sugarcane can be utilized in agricultural activities. This strategy has the potential to decrease the reliance on fungicides in sugarcane farming, benefiting both the economy and the environment. Additionally, it can contribute to the maintenance and improvement of product quality and yield, while supporting sustainable cultivation.

Keywords: Antagonism, Bioremediation, Control, Sugarcane.

Introduction

Sugarcane is an important crop for the production of sugar and bioethanol. However, it is susceptible to various fungal diseases that significantly reduce its yield and quality. The most serious diseases affecting sugarcane include red rot, wilt, sett rot, and seedling rot, which are caused by *Colletotrichum falcatum*, *Ceratocystis paradoxa*, *Fusarium* spp., and *Pythium* spp., respectively (Viswanathan & Rao, 2011). These pathogens can infect different parts of the sugarcane plant, such as the stem, root, and leaf, leading to symptoms such as discoloration, wilting, rotting, and plant death (Del Gobbo *et al*., 2022). Conventional methods for disease control involve the use of resistant varieties, cultural practices, and chemical fungicides. However, these methods have limitations, including resistance breakdown, environmental impact, and the development of resistance by the pathogens

themselves (Viswanathan & Malathi, 2019). In contrast, biological control is an eco–friendly approach that utilizes living organisms, such as bacteria, fungi, and viruses, to suppress plant pathogens through mechanisms such as competition, parasitism, antibiosis, and induced resistance. These organisms can be applied to different parts of the plant system, including the soil, plant, or seed (Monjezi *et al*., 2023; Compant *et al*., 2005; Aeini *et al*., 2021). Bioremediation of contaminated soil can also be achieved by using microbial agents that not only enhance plant growth but also exhibit antagonistic activity against pathogens (Mirzavand *et al*., 2023). These microbial agents consist of various bacterial genera and species, such as *Bacillus*, *Streptomyces*, *Pseudomonas*, *Burkholderia*, and *Agrobacterium*, as well as fungal genera like *Trichoderma* and *Talaromyces* (Lombardi *et al*., 2018). Additionally, they can produce metabolites that have similar beneficial effects (d'Errico *et al*., 2020). The objective of this study was to assess the potential of bioremediation bacteria as biological control agents against fungal pathogens of sugarcane. To achieve this, previously identified bacterial strains with bioremediation potential for atrazine and metribuzin herbicides were evaluated against eight fungal pathogens of sugarcane using the dual culture method under laboratory conditions.

Materials and methods

Bacteria, including three strains of Brucella sp. (66A, 44A, and 65A), Pseudomonas putida 10A, Ensifer adhaerens 20m, Pseudomonas aeruginosa 15A, and Stenotrophomonas sp. 22A, which had been identified by Mirzvand *et al*., 2023, were obtained from the bacterial collection of the plant pathology department of Shahid Chamran University of Ahvaz. These strains have already been isolated from Atrazine– and Metribuzin–contaminated soils in sugarcane fields, and their bioremediation capabilities have been characterized (Mirzavand *et al*., 2023). Fungal isolates, including Thielaviopsis ethacetica, Cytospora sacchari, Fusarium proliferatum, Bipolaris drechslera, Curvularia sp., Alternaria sp., and Nigrospora sp., were received from the Iranian Sugarcane Research and Training Institute (ISCRTI). To evaluate the antagonistic ability of bacterial strains against fungi, dual–culture test was conducted following a modified Dinger and Sinclair method (1995). A 5–mm disc from the active margin of the fungal disease–causing strain was placed on a Petri dish containing Potato Dextrose Agar (PDA) medium, with another bacterial strain disc placed simultaneously 5.0 cm away from the fungal margin using a sterile loop. Similarly, fungal isolates alone, without bacterial presence, were cultured as control on PDA medium. Each Petri dish was labeled with the bacterial and fungal strain names, and the edges were completely sealed with parafilm. They were then maintained in an incubator at 28°C. The test continued until the fungal control's hyphal growth reached the Petri dish wall. Reduction in pathogenic growth compared to the bacteria–free control, the creation of an inhibitory zone by bacteria, and prevention of fungal growth were considered as criteria to evaluate the test. The assessment of bacterial antagonistic power against fungal growth was determined by measuring the mean diameter of the inhibition zone created between the bacterial strain margin and the fungus, according to Weller and Cook (1983). Additionally, the percentage of inhibition from fungal mycelium growth compared to the control was calculated using the following formula IR (%) post full growth of the control, as per Huang *et al*. (2017).

$$IR(\%) = \frac{(C - B)}{B} \times 100$$

In this equation, IR represents the percentage of inhibition from fungal growth, C is the radial fungal growth in Petri dishes for the control, and B is the fungal radial growth in the presence of the bacteria. All collected data throughout the experiment were organized using Excel. Statistical analysis was perfrmed using SPSS version 27, following a completely randomized design with three replications. Mean comparisons were conducted using Tukey's test at a significance level of alpha set at five percent.

Results

Results indicated that some bacterial strains reduced the growth of specific pathogenic fungi, considered as a positive, while their lack of effect was considered a negative result. Bacterial isolates significantly reduced the growth of pathogenic fungi in the dual culture test, although the degree of reduction varied among different strains (Figure 1). In the dual–culture test, all identified bacteria (except one strain, *P. putida*10A) exhibited significant inhibitory effects on the growth of the examined pathogenic fungi. Conversely, the strain *P. putida* 10A did not display any antagonistic properties against the mentioned fungi in laboratory conditions. *P. aeruginosa* 15A affected all examined fungi significantly (Table 1 and 2).

Table 1: Evaluation of Antagonistic Effects of Representative Bacterial Isolates against Fungi Isolated from Sugarcane in Laboratory Conditions.

Bacteria/Fungi	*Stenotrophomonas* sp. 22A	*P. aeruginos* 15A	*P.putida* 10A	*B.* sp. 65A	*B.* sp. 44A	*B.* sp. 66A	*E. adhaerens.* 20m
T. ethacetica	–	+	–	–	–	–	+
C. sacchari	+	+	–	–	+	+	–
F. proliferatum	+	+	–	+	–	+	+
Curvulari	+	+	–	–	+	–	+
Alternaria	–	+	–	+	–	–	–
B. drechslera	–	+	–	–	+	+	+
Nigrospora	–	+	–	–	+	+	+

A positive sign (+) indicates that bacteria have an inhibitory effect on fungal growth, whereas a negative sign (–) indicates that bacteria have no effect on fungal growth.

Table 2. Comparison of the mean diameter of the inhibition zone created by bacteria in millimeters (mm) under laboratory conditions in the dual–culture test.

Bacteria/Fungi	*Stenotrophomonas* sp. 22A	*P. aeruginos* 15A	*E. adhaerens* 20m	*B.* sp. 65A	*B.* sp. 44A	*B.* sp. 66A
T. ethacetica	*	17.16^{l}±0.120	7.13^{q}±0.088	*	*	*
C. sacchari	45.1^{a}±0.057	41.2^{b}±0.057	*	*	24.38^{i}±0.046	38.55^{c}±0.029
F. proliferatum	26.11^{g}±0.060	25.46^{h}±0.088	4.083^{r}±0.060	26.11^{g}±0.06	*	20.2^{j}±0.011
Curvulari	16.66^{m}±0.088	36.13^{e}±0.088	14.11^{p}±0/030	*	20.2^{j}±0.015	*
Alternaria	*	37.3^{d}±0.152	*	16.11^{n}±0.092	*	*
B. drechslera	–	15.6^{o}±0.057	14.04^{p}±0.092	*	19.1^{k}±0.057	19.1^{k}±0.057
Nigrospora	*	30.05^{f}±0.028	24.76^{i}±0.120	*	24.76^{i}±0.120	30.05^{f}±0.028

Table description: * Due to the no effect of bacteria in the dual–culture test (lack of inhibitory effect), the diameter of the inhibition zone created by the bacteria was not measured. The superscript letters on each number indicate in each column and row. The difference between the numbers that have at least one common letter is not significant based on the Tukey test at the 5% probability level. The numbers in front of the data show the standard deviation (±) of the mean in three replicates.

Table 3. Comparison of the fungal inhibition percentage mean in treatment with antagonistic bacteria under laboratory conditions in the dual–culture test

Bacteria/Fungi	*Stenotrophomonas* sp. 22A	*P. aeruginos* 15A	*E. adhaerens* 20m	*B.* sp. 65A	*B.* sp. 44A	*B.* sp. 66A
T. ethacetica	*	47.94^{l}±0.120	33.46^{q}±0.088	*	*	*
C. sacchari	85.8^{a}±0.057	81.52^{b}±0.057	*	*	64.27^{i}±0.046	78.48^{c}±0.029
F. proliferatum	66.78^{g}±0.060	65.52^{h}±0.088	16.74^{r}±0.060	66.77^{g}±0.060	*	55.75^{j}±0.011
Curvulari	44.77^{m}±0.088	70.14^{e}±0.088	38.5^{p}±0/030	*	55.75^{j}±0.015	*
Alternaria	*	72.53^{d}±0.152	*	43.74^{n}±0.092	*	*
B. drechslera	*	42.76^{o}±0.057	40.11^{i}±0.120	*	*	50.1^{k}±0.057
Nigrospora	*	68.93^{f}±0.028	64.60^{i}±0.120	*	64.60^{i}±0.120	30.05^{f}±0.028

Table description: * Due to the no effect in the dual–culture test (lack of inhibitory effect), the diameter of the inhibition zone created by the bacteria was not measured. The superscript letters on each number indicate the statistical difference at the 5% probability level in each column and row. The difference between the numbers that have at least one common letter is not significant based on the Tukey test. The numbers in front of the data show the standard deviation (±) of the mean in three replicates.

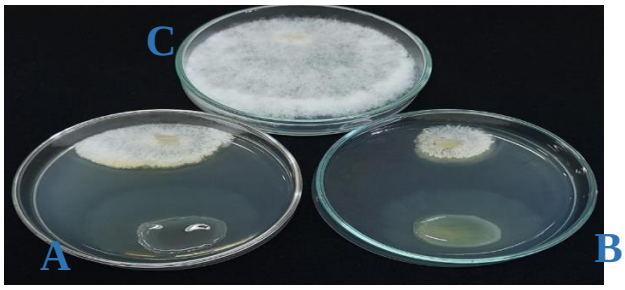

Fig. 1. Inhibition of the growth of *C. sacchari* by *Stenotrophomonas* sp., and *P. aeruginosa* (A, B) and *C. sacchari* without the presence of bacteria as a control (C).

Comparison of the inhibition mean diameter zone created by bacteria and the percentage of fungal inhibition in the presence of controls (Table 2 and 3), was calculated under a completely randomized design at a statistical level of five percent ($p \leq 0.05$) using the Tukey test in the dual–culture test. *P. aeruginosa* 15A showed the highest inhibition against *T. ethacetica*, *Curvulari* sp., and *Alternaria* sp. *Stenotrophomonas* sp. 22A and *P. aeruginosa* 15A closely followed, with sigificant inhibitory effects against these pathogens. For *C. sacchari*, *Stenotrophomonas* sp. 22A and *Brucella* sp. 65A exhibited the highest inhibition. Against *F. proliferatum*, *Stenotrophomonas* sp. 22A and *Brucella* sp. 65A demonstrated the highest inhibition. In *B. drechslera*, the most significant inhibition was caused by two strains, *Brucella* sp. 66A and *Brucella* sp. 44A, and in *Nigrospora* by *P. aeruginosa* 15A and *Brucella* sp. 66A.

Discussion

During the assessment of bacterial antagonistic properties, it was discovered that certain bacteria, in addition to their capability to degrade the herbicides atrazine and metribuzin, also exhibit the capacity to inhibit the growth of various sugarcane fungi. This dual functionality positions these bacteria as valuable agents not only for bioremediation purposes but also for effective biological control. This study revealed diverse antagonistic effects of bacterial isolates against fungi isolated from sugarcane, shedding light on their potential as biocontrol agents in agricultural environments. Some of these bacteria were able to significantly reduce the growth of the mentioned fungi, but the degree of reduction varied. Therefore, the antagonistic power of bacterial isolates against the fungi was evaluated by measuring the average diameter of the inhibition halo created by the bacteria and the percentage of inhibition of mycelial growth of the fungus. *P. aeruginosa* was able to significantly prevent the growth of all the studied fungi, making it a superior isolate in terms of antagonistic properties among the identified bacteria. *P. aeruginosa* is recognized as one of the most valuable commercial and biotechnological microorganisms. It secretes various redox–active phenazine compounds, with pyocyanin being the most prominent. Pyocyanin serves as a quorum sensing signaling molecule for *P. aeruginosa* and functions as both an electron shuttle for bacterial respiration and an antibacterial and antifungal agent (Jayaseelan *et al*., 2014). Previous studies have reported the antagonistic properties of *P. aeruginosa* against *Pythium aphanidermatum* in greenhouse soils and several fungal pathogens, such as *Macrophomina phaseolina*, *Fusarium solani*, and *Rhizoctonia solani*, which cause root–knot, root rot, and wilt diseases in mung bean plants (Al–Hinai *et al*., 2010), and these findings are supported by the present study. Isolates of *Stenotrophomonas* sp., as natural soil bacteria, have a wide range of applications in agriculture as potential biological control agents for fungal diseases and plant growth promotion (Mukherjee and Roy, 2016). This study also confirmed the bioremediation and biological control properties of this bacterium. *S. maltophilia*, an opportunistic pathogen with multidrug resistance, is commonly found in water, soil, plant rhizospheres, animals, and foods (Looney *et al*., 2009; Brooke, 2012). It has been introduced as a potential agent in biological control for *Ralstonia solanacearum*, which causes brown rot in potatoes (Messiha *et al*., 2007). *S. maltophilia* has applications in biotechnology, including agriculture, biological control, and bioremediation (Brooke, 2021). Considering that this study provides evidence of the high potential of certain isolates in the biological control of fungal pathogens in sugarcane under laboratory conditions, conducting field studies to confirm their effectiveness in the future would enable their integration into disease management strategies. Such an approach would not only reduce the use of fungicides in sugarcane cultivation but also have positive economic and environmental impacts by maintaining and improving the quality and yield of the product, as well as promoting sustainable cultivation practices.

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ارزیابی خاصیت مهار زیستی باکتری‌های جداشده از خاک‌های آلوده به آترازین و متریبوزین مزارع نیشکر علیه بیمارگرهای مهم قارچی نیشکر در شرایط آزمایشگاهی

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چکیده

خاک‌های آلوده می‌توانند به وسیله میکروارگانیسم‌های مختلفی مانند باکتری‌ها تیمار شوند. این میکروارگانیسم‌ها هم می‌توانند آلودگی‌ها را بر طرف کنند و هم در فرآیند مهار زیستی عمل نمایند. به طور کلی، مهار زیستی روش سازگار با محیط با استفاده از میکروارگانیسم‌هایی مانند قارچ‌ها، باکتری‌ها و ویروس‌هاست که می‌تواند از طریق مکانیسم‌های مختلفی از قبیل رقابت، پارازیتیسم، آنتی‌بیوزیست و القای مقاومت عمل نماید. این تحقیق با هدف بررسی توان باکتری‌های موثر در فرآیند زیست‌پالایی و قابلیت مهار زیستی آن‌ها در مقابل بیمارگرهای قارچی مهم نیشکر انجام شد. برای دستیابی به این هدف، سویه‌های باکتریایی شناسایی شده قبلی از جمله *Brucella* sp. (66A, 44A,65A) و *Pseudomonas putida* 10A، *Ensifer adhaerens* 20m، *Pseudomonas aeruginosa* 15A و *Stenotrophomonas* sp. 22A با توانایی زیست‌پالایی علف‌کش‌های آترازین و متریبوزین در برابر قارچ‌های *Thielaviopsis ethacetica*، *Cytospora sacchari*، *Fusarium proliferatum*، *Bipolaris drechslera*، *Curvularia* sp.، *Alternaria* sp.، و *Nigrospora* sp. ارزیابی شدند. آزمایش با استفاده از روش کشت دوگانه در طرح کاملا تصادفی با سه تکرار در شرایط آزمایشگاهی انجام شد. قطر بازداری از رشد باکتری‌ها و درصد مهار رشد قارچ‌ها به ترتیب محاسبه شدند. در بررسی خاصیت مهارزیستی جدایه‌های باکتریایی علیه برخی قارچ‌های نیشکر، بیشترین بازدارندگی رشد قارچ‌های *Thielaviopsis ethacetica*، *Curvularia* و *Alternaria* توسط جدایه *P. aeruginosa* 15A و قارچ *Cytospora sacchari* توسط جدایه *Stenotrophomonas* sp. 22A و در قارچ *Fusarium proliferatum* توسط جدایه‌های *Stenotrophomonas* sp. 22A و *Brucella* sp. 65A مشاهده شد. همچنین بیشترین بازدارندگی رشد *Bipolaris drechslera* توسط دو جدایه *Brucella* sp. 66A و *Brucella* sp. 44A و قارچ *Nigrospora* توسط جدایه‌های *P. aeruginosa* 15A و *Brucella* sp. 66A ایجاد شد. *P. putida* 10A هیچ خاصیت ضدقارچی در برابر قارچ‌های فوق در شرایط آزمایشگاهی نشان نداد، در حالی که *P. aeruginosa* 15A بر تمام قارچ‌های مورد بررسی تأثیر قابل توجهی داشت. بر اساس این یافته‌ها، این مطالعه شواهدی را ارائه می‌دهد که برخی از جدایه‌های زیست پالایی توانایی بالایی در مهار زیستی برخی از عوامل بیماری‌زای قارچی نیشکر در شرایط آزمایشگاهی دارند. در نتیجه، خاصیت ضدقارچی در برابر قارچ‌های مهم نیشکر می‌تواند در عملیات کشاورزی کاربرد داشته باشد. این استراتژی می‌تواند مصرف قارچ‌کش در کشت نیشکر را کاهش دهد و به اقتصاد و محیط زیست سود برساند. همچنین می‌تواند به حفظ و بهبود کیفیت و عملکرد محصول و در نتیجه کشاورزی پایدار کمک نماید.

واژه‌های کلیدی: آنتاگونیست، زیست‌پالایی، کنترل، نیشکر.