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Antagonistic Properties of some Bacterial Strains Isolated from Sources of Drinking Water against Fungal Pathogen *Rhizoctonia solani*

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ABSTRACT

Control of phytopathogens by microorganisms means biological control. In this study, three bacterial strains *Pantoea agglomerans* B1, *Serratia plymuthica* B2 and *Proteus mirabilis* B3 are the strains of bacteria originally isolated from source of drinking water a temperate site at El-Gharbia Governorate, Egypt. The origin of isolated bacterial strains contains three sources of drinking water: Nile, tap and ground. The commercialization of this aquaculture has of bacteria generated economic profits while the water contain bacteria produced the antagonistic materials have an adverse effect on the soil-borne fungi. These sources of water are very important in agriculture especially when using for irrigation of crops. *In vitro*, *Pantoea agglomerans* B1, *Serratia plymuthica* B2 and *Proteus mirabilis* B3 are examined for its antagonistic activities against soil-borne fungi *Rhizoctonia solani*, the seedlings damping-off fungus for several plants. *Serratia plymuthica* B2 showed high level of antagonism against fungal growth. Also, production of lytic enzymes (chitinase, β -1,3 – glucanase), siderophores, salicylic acid (SA) and hydrogen cyanide (HCN) by the three strains of bacteria were evaluated. All antagonistic materials production recorded high-level values with strains B2 and B1 respectively. A most high relationship between the antagonistic potential of three bacterial strains against *R. solani* and its level of β -1,3 – glucanase, SA and HCN were observed. The antifungal metabolites by the bacterial strains mentioned were considered contributing to the antagonistic activities of these bacteria.

Keywords: Antagonistic materials, Bacteria in drinking water, *Rhizoctonia solani*



INTRODUCTION

The pathogen of *Rhizoctonia solani* kuhn is main reason to infect the plant at seedling stage causing great economic losses under different conditions of several crops worldwide (Nyvall, 1981 & Wolf and Verreet 1999). Pathogen that most commonly cause seedling disease problems in many plants is *Rhizoctonia solani* kuhn (Robert, 2008). Under different conditions, a fungal pathogen of *Rhizoctonia* able to survive for many years in organic matter and soil (as mycelium or sclerotia) *Rhizoctonia* pathogen has a wide host scale (Ogoshi 1987), furthermore *R. solani* is saprophytic life supported by organic matter, these factors increase problems to control *Rhizoctonia* disease and increase problems in crop production. Biological control as biocontrol agents (BCAs) are environmentally friendly to protect plants against soil-borne pathogens (Weller *et al.* 2002). Researchers have developed microflora to decrease diseases caused by *R. solani* (Ross *et al.* 1998). Today, biological control by strains of bacteria successfully used to suppress several fungal pathogens (Vidhyasekaran and Muthamilan, 1999 & Whipps, 2001). *In vitro* and *in vivo* assays, the antifungal activity of 434 bacterial strains was evaluated against *R. solani* based on hierarchical mixtures (Faltin *et al.* 2004). *In vitro*, isolates of bacteria were tested against the phytopathogenic fungi showed antagonism between various species and pathogenic fungi (Sheikh *et al.* 2022). Screening strategy development, three bacterial strains from *Serratia plymuthica* with antagonistic effects (ranked in the order) showed activity of antagonism *in vivo* by a leaf of plant and with plant-growth on lettuce seedlings (Grosch *et al.* 2005) and on cotton

seedlings (Affify and Ashour 2024). Also, strains of *Pantoea* spp. as bioagents are able to produce antimicrobial metabolites to suppress several soil-borne fungi (Smits *et al.* 2010). Biological control of phytopathogens by bacteria generally involve the following mechanisms: induced systemic resistance, plant growth promoting effects in assays on plant seedlings, production of siderophores, hydrogen cyanide (HCN), antibiotics, and lytic enzymes (Van Loon *et al.* 1998). The best bacterial antagonists are combination of mechanisms for a successful antifungal interaction (O'Sullivan and O'Gara 1992). And cell walls of phytopathogens consist of chitin and laminarin (Bartnicki-Garcla 1973). The current work was to evaluate the suppressing *R. solani* by bacterial strains isolated from three sources of drinking water (*Pantoea agglomerans* B1, *Serratia plymuthica* B2 and *Proteus mirabilis* B3) *in vitro*, through the production of chitinase, β -1,3 – glucanase, siderophores, salicylic acid (SA) and hydrogen cyanide (HCN).

MATERIALS AND METHODS

Strains of bacteria

This study used three strains of bacteria as biocontrol agents (BCAs). The strains of bacteria were isolated and identified during the following study "Bacterial and physico-chemical evaluation of drinking water quality at El-Gharbia Governorate, Egypt" (Affify and AbdAllah 2023). The strains *Pantoea agglomerans* B1, *Serratia plymuthica* B2, and *Proteus mirabilis* B3 were derived especially from different sources of water like the Nile, tap and ground. These sources of water are very important for agriculture. All bacterial strains were tested for their antagonism against phytopathogen as *R. solani*.

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Pathogen

R. solani Kuhn as soil-borne fungus was isolated from soil and maintained on potato dextrose agar (PDA) medium by Plant Pathology Research Institute, Agricultural Research Center (ARC), Giza, Egypt.

In vitro interaction between bacterial strains and fungal pathogen

The interaction between bacterial strains: *Pantoea agglomerans* B1, *Serratia plymuthica* B2, *Proteus mirabilis* B3 and fungal pathogen: *R. solani* were studied using plate culture technique according to Vidhyasekaran *et al.* (1997) zone of inhibition (mm) as antagonism was recorded. Five replications were kept for each strain.

Chitinases assay

Bacterial strains were cultured for chitinase production (Lim *et al.* 1991). Chitinase was determined by using bovine serum albumin as the standard as described by Bradford (1976).

β -1,3 – glucanase assay

Bacterial strains were cultured for β -1,3 – glucanase production (Lim *et al.* 1991). Enzyme was determined by using bovine serum albumin as the standard as described by Bradford (1976).

Salicylic acid (SA) production

Bacterial strains were cultured on succinate medium containing succinic acid (Meyer and Abdallah, 1978). SA (μ g/ml) was recorded at 527 nm in a spectrophotometer as described by Meyer *et al.* (1992).

Siderophore production

For siderophore production as (μ mol benzoic acid/ml), this method determined by a spectrophotometer at 700nm according to Reeves, *et al.* (1983).

HCN production

Bacterial strains were cultured for HCN production, the HCN was measured when the color in filter paper reddish at 625nm (Meena *et al.* 2001).

Mean of data obtained from five replications were kept for each strain.

RESULTS AND DISCUSSION

Inhibition of *R. solani* by *Pantoea agglomerans* B1, *Serratia plymuthica* B2, and *Proteus mirabilis* B3 strains in vitro

Three bacterial strains of *Pantoea agglomerans* B1, *Serratia plymuthica* B2, and *Proteus mirabilis* B3 were isolated from three sources of drinking water. The strains of bacteria showed high level of antagonism against the tested fungus *in vitro*. Among them, B2 was the most effective one as value of zone inhibition (13.8 mm), after that the B1 and B3 strains recorded inhibition zones (10.6 and 9.8 mm), respectively (Fig. 1). Fifty years ago, Howell and Stipanovic (1979) reported that the antagonism exhibited by the bacterium is possibly the result of the production of the antifungal, which is itself an effective protectant against damping-off. These results are in agreement with the reports indicated that three bioagents are bacteria-associated-plant with high inhibition pathogens *in vitro* (Faltin *et al.* 2004). *Serratia plymuthica* and other several bacteria showed antagonistic activity *in vitro* for *R. solani* and reduced disease effect (Grosch *et al.* 2005). Other bacteria distributed among the Gram-negative bacteria of several families can be used for development microbial biopesticides. They reduce plant pathogenic fungi infections; these bacteria include species such as *Pantoea* spp. (Bonaterra *et al.* 2022).

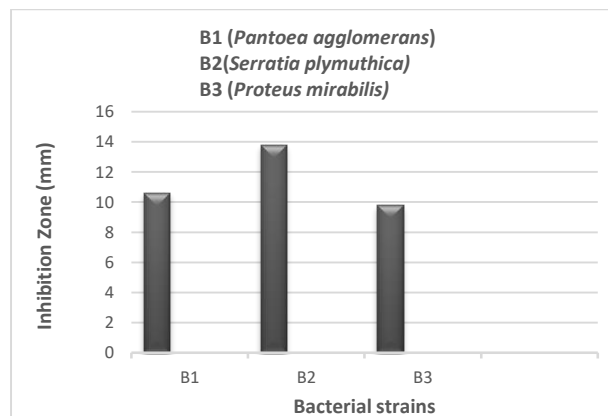


Fig. 1. Inhibition growth of *R. solani* by three bacterial strains isolated from sources of drinking water, Data are mean of five replications.

Production of lytic enzymes by three bacterial strains isolated from sources of drinking water

Among the three strains of *Pantoea agglomerans* B1, *Serratia plymuthica* B2 and *Proteus mirabilis* B3 tested for production of chitinase, *Pantoea agglomerans* B1 recorded the highest chitinase activity followed by *Serratia plymuthica* B2 and *Proteus mirabilis* B3 (Fig. 2). No relationship was observed between the antagonistic potential of three bacterial strains and their values of chitinase production. The strain *Pantoea agglomerans* B1 recorded inhibition zone of 10.6 mm less chitinase than the strains *Serratia plymuthica* B2 and *Proteus mirabilis* B3. *Serratia plymuthica* B2 introduced the high values β -1,3 – glucanase activity followed by *Pantoea agglomerans* B1 (Fig. 3). Their relationship between inhibition zone recorded and the antagonistic activity of three bacterial strains, exhibited the lowest β -1,3 – glucanase activity. Some biocontrol agents such as *Serratia marcescens* were able to degrade fungal cell wall by enzymes (chitinase and β -1,3 – glucanase) (Lee *et al.* 1992). Sadeghi *et al.* (2006) showed that bacterial isolates had antifungal materials such as siderophore and chitinase. Also, the chitinase production from *Serratia* has shown high activity against *R. solani* (Jaganmohan *et al.* 2010). The same authors reported that anti-fungal materials from chitinolytic bacteria have been reported positive action by production other lytic enzymes. Several studies have reported that bacterial strains reduced the growth of many plant pathogens by their antagonistic activity, with different modes of action such as production of enzymes (chitinase and 1,3-glucanase) (Sheikh *et al.* 2022).

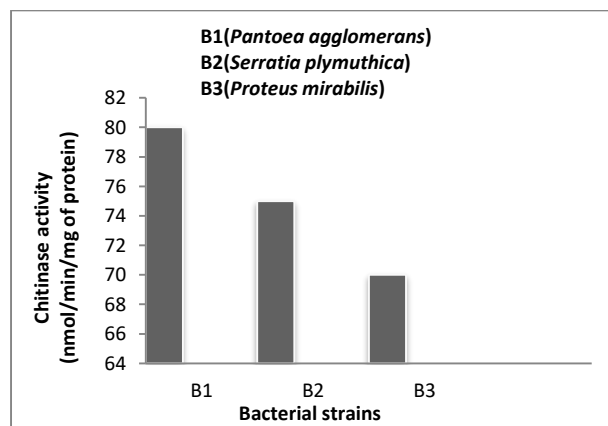


Fig. 2. Production of chitinase by three bacterial strains isolated from sources of drinking water, Data are mean values calculated from replicates.

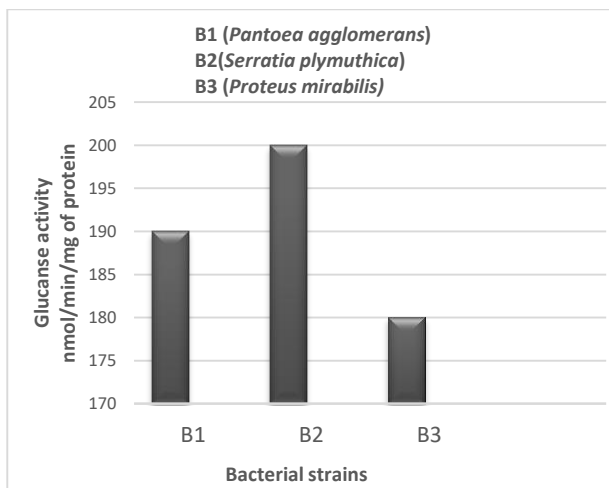


Fig. 3. Production of β -1,3 – glucanase by three bacterial strains isolated from sources of drinking water, Data are mean values calculated from replicates.

Production siderophore and SA by three bacterial strains isolated from sources of drinking water

The results recorded maximum siderophore production with strain *Serratia plymuthica* B2 followed by *Pantoea agglomerans* B1 and *Proteus mirabilis* B3 (Fig. 4). There was no relationship between siderophore production and the antagonistic effect of three bacterial strains. Of the three strains recorded, the maximum SA production was found with *Serratia plymuthica* B2 followed by *Pantoea agglomerans* B1 (Fig. 5). The strains *Proteus mirabilis* B3 showed low SA production. Observations of results showed that there is relationship between values from SA production and the antagonistic effect of three bacterial strains. The isolates of bacteria were found produced SA *in vitro*. Systemic acquired resistance (SAR) known by siderophores and SA (Leeman *et al.* 1996). Similarity, SA- mutants of *Serratia marcescens* strain 90-166 retained the same ISR activity against the pathogen in cucumber plant (Press *et al.* 1997). In alkaline media bacterial strain of *Pantoea eucalypti* M91 is able to produce siderophores (pyoverdine and pyochelin) (Campestre 2016). Most studies showed that inhibition of fungal pathogens as *Fusarium* as well as increase improvement plants growth by produced bacterial metabolites such as siderophores (Sheng *et al.* 2020).

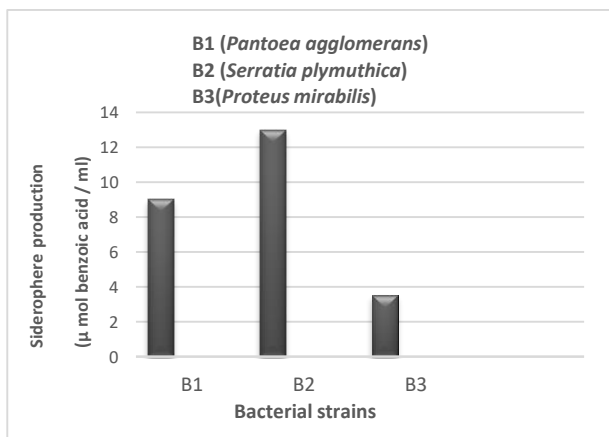


Fig. 4. Production of siderophore by three bacterial strains isolated from sources of drinking water, Data are mean values calculated from replicates.

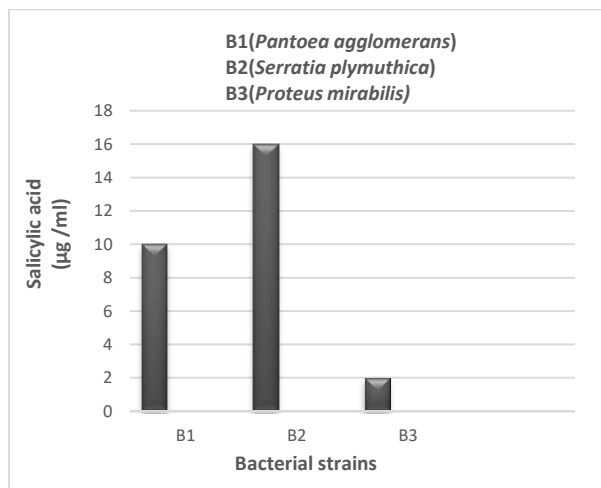


Fig. 5. Production of SA by three bacterial strains isolated from sources of drinking water, Data are mean values calculated from replicates.

Production of HCN

From the three strains of bacteria tested for the HCN determination, the strains *Pantoea agglomerans* B1 and *Serratia plymuthica* B2 showed higher production of HCN. The third strain recorded negligible amount of HCN (Fig. 6). Bacteria are found to play an important role in biological control by the production volatile compounds (ammonia and hydrogen cyanide) (Brimecombe *et al.* 2001). The microorganisms can be found decrease the deleterious effects of pathogens on crop yield through the production of hydrogen cyanide and siderophore (Larkin 2020; Mohammed *et al.* 2020).

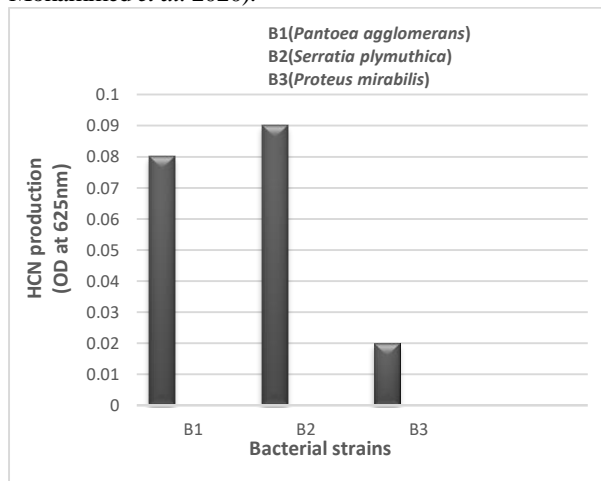


Fig. 6. Production HCN by three bacterial strains isolated from sources of drinking water, Data are mean values calculated from replicates.

CONCLUSION

The current research is important as the several possible mechanisms with the especially antagonistic bacterial species. Research should focus on pathogenic fungi as cause damping-off disease and biocontrol agents, it is a prior knowledge of the interaction recorded, as even a producer of antagonistic materials can be reduced and suppress growth of soil-borne pathogens. Important aim of this work was to know high potential of bacteria which isolated from three sources of drinking water as a biological

control agent against *Rhizoctonia* pathogen *in vitro*. To understand role of these bacteria as biological control agents must be underlying field studies are required.

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خصائص التضاد لبعض السلالات البكتيرية المعزولة من مصادر لمياه الشرب ضد الفطر الممرض ريزوكتونيا سولاني

عايدة حافظ عفيفي

قسم الميكروبيولوجيا الزراعية – كلية الزراعة – جامعة المنصورة – المنصورة – مصر

الملخص

هذه الدراسة تهدف إلى المقاومة الحيوية والتي تعرف بأنها مقاومة الفطريات الممرضة للنباتات بواسطة الكائنات الحية الدقيقة. وقد تناولت هذه الدراسة ثلاث سلالات من البكتيريا تم عزلها من مصادر مختلفة لمياه الشرب تحت الظروف العادية للمياه. هذه المصادر لمياه الشرب عبارة عن مياه النيل، مياه الصنبور (الحفنية)، ومياه جوفية (ماء أرضي) وكانت مصادر المياه من مواقع مختلفة بمحافظة الغربية بجمهورية مصر العربية حيث تم الحصول على هذه السلالات البكتيرية في تجارب سابقة لإجراء الحصر الميكروبي لمياه الشرب في هذه المحافظة. وكانت السلالات البكتيرية الثلاثة على الترتيب: بنتويا أجلوميرانس و سيرانيا بلميكتيا و بروتيوس ميرابلس. ووجود هذه السلالات البكتيرية بمياه الشرب كان له أهمية وتأثير خاص وأعطى لهذه المصادر من مياه الشرب صفة الأهمية وخصوصا وأن مثل هذه المصادر من المياه تستخدم في ري المحاصيل الزراعية. وقد أختبرت هذه السلالات من البكتيريا المائية في تجربة معملية بتنميتها مع أحد فطريات التربة التي تسبب موت البادرات لكثير من المحاصيل الإقتصادية وهو فطر الريزوكتونيا سولاني. فقد وجدنا في إختبارات التضاد في المعمل أن الثلاث سلالات البكتيرية سجلت مناطق تثبيط لنمو الفطر في أطباق بتري وكانت السلالة البكتيرية الثانية أكثرهم تثبيطا لنمو الفطر يليها السلالة الأولى ثم الثالثة على الترتيب. وبتقدير قدرة السلالات البكتيرية على إنتاج مواد التضاد الفطرية والتي شملت تقدير إنزيمي الكيتينيز و 1, 3 بيتا جلوكونيز و تقدير إنتاج السايديروفورز و حامض السلسيليك وكذلك إنتاج المواد السامة للفطريات مثل سيانيد الهيدروجين. سجلت السلالتين من البكتيريا الثانية والأولى أعلى القيم يلي ذلك السلالة الثالثة على الترتيب ولوحظ أن هذه النتائج للمواد المضادة متوافقة مع نتائج إختبار التضاد للسلالات البكتيرية الثلاثة والفطر الممرض مع وجود إختلاف واحد في أحد التقديرات. من هذه النتائج نستطيع أن نستنتج أنه يمكن للبكتيريا الموجودة في مياه الشرب أن تكون ذات فائدة كبيرة من خلال إنتاجها للمواد المضادة لفطريات التربة الممرضة لكثير من النباتات، وبهذا تعتبر مثل هذه السلالات من البكتيريا مهمة في المقاومة الحيوية.