BIOLOGIJA. 2009. Vol. 55. No. 1–2. P. 29–34
DOI: 10.2478/v10054-009-0006-3

Susceptibility of fungi to new bacterial isolates

Loreta Levinskaitė[1]*,

Vytautas Melvydas[2]

[1] *Laboratory of Bioderioration Research, Institute of Botany, Žaliųjų Ežerų 49, LT-8406 Vilnius, Lithuania*

[2] *Laboratory of Genetics, Institute of Botany, Žaliųjų Ežerų 49, LT-8406 Vilnius, Lithuania*

Susceptibility of 40 fungal strains from the genera *Aspergillus, Penicillium, Gliocladium, Paecilomyces, Scopulariopsis, Trichoderma, Ulocladium* and others to the bacterial isolates T1x, T2x, T3x, Ux and Ux308 was tested. The killer activity of the bacteria was evaluated as clear fungicidal zones formed by bacteria on fungal lawns. All the bacteria exerted killer activity against the fungi. Although the individual reaction of fungi varied, bacteria Ux and Ux308 showed a high fungicidal activity on most of *Penicillium, Aspergillus, Gliocladium* and other fungi. Some fungi (*Eupenicillium* sp., *Penicillium brevicompactum*, etc.) were more susceptible to Tx-type bacteria. A conspicuous individual susceptibility of some fungi to particular bacterial isolates was noted. The high and wide-range fungicidal activity of the bacteria tested can be promising in developing measures against harmful fungi.

Key words: fungi, killers, fungicidal effect, bacteria, secreted substances

INTRODUCTION

Many species of microscopic fungi such as *Penicillium, Aspergillus, Cladosporium, Stachybotrys, Alternaria, Paecilomyces*, etc. are found in living and working premises. These fungi damage fruit, vegetables, grains and processed food products. These micromycetes can produce harmful toxins, induce allergies or other diseases [1–3]. Therefore, their presence, especially in high amounts, in human environment is undesirable. For elimination of fungi and limitation of their distribution, various antifungal agents are applied. Nevertheless, often antiseptics are not of a wide coverage; moreover, in the long run, fungal resistance may develop. The capabilities and range of natural antimicrobial agents draw the attention of researchers [4]. It is known that yeasts can secrete proteins lethal to sensitive fungi [5]. Killer strains have been found among the genera *Saccharomyces, Candida, Debaromyces, Pichia, Torulopsiis, Cryptococcus, Rhodotorula, Hansenula*, etc. *Zygoccharomyces bailii* produces the protein toxin zygocin which is effective against some micromycetes [6]. Antimicrobial toxins are produced by *Ustilago mayadis, Hanseniaspora uvarum, Kluyveromyces phafii*, etc. [7]. Many bacteria secrete antibiotics affecting various microorganisms. Toxins with an antimicrobial effect are produced by *Pseudomonas* sp., *Lactobacillus* sp., etc [8–10]; Palumbo et al. [11] reported about bacteria fungicidally affecting *Aspergillus flavus*. Nevertheless, the knowledge of biological antifungal agents is still insufficient.

* Corresponding author. E-mail: loreta.levinskaite@botanika.lt

In our previous investigation, bacteria named Tx-type and Ux-type were isolated, which showed killing activity against some micromycetes. The aim of this work was to examine the impact of these bacteria on fungi (molds) distributed in the human environment (living or working premises, food, etc).

MATERIALS AND METHODS

Bacteria T1x, T2x, T3x and Ux were isolated by multiple cloning from spontaneous fruit and berry fermentations, and Ux308 was obtained from soil [12].

Fungi were isolated from living rooms, working offices, fruit, cereals, processed food products. To isolate and identify molds, MEA, Czapek, CYA, YES media were used [13–17]. Fungi were identified following the handbooks [10, 13–21].

Standard killer strains of *Saccharomyces cerevisiae* K7 (MAT*a arg9* [*kil-K1*]), DBY *4975* (MATα *ade2 his3-200 leu2-3-112 lys2-801 ura3-52 gal*[*kil-K1*]), Rom K-100 (*wt, HM / HM* [*kil-K2*]), M437 *wt, HM / HM* [*kil-K2*]), K28 (*wt, HM / HM* [*kil-K28*]) and MS 300 (MATα *leu2 ura3-52* [*kil-K28*]) were used as a control to compare activity of the bacterial strains, and the *S. cerevisiae* strain α′*1*(MATα *leu2-2* [*kil-0*]), a test-organism sensitive to all standard killer strains, was used to test the action of bacterial toxins [22].

To test killer activity, MB medium (without methylene blue) consisting of 0.5% peptone, 0.5% yeast extract, 2% glucose, 1.05% citric acid and 3.53% $Na_2HPO_4 \times 12H_2O$ was used. The medium pH was adjusted to 4.8, and 2% of agar was added.

The killer activity of the bacteria was evaluated by formation of lysis (fungicidal) zones on lawns of the test fungi. Fungal lawns were obtained by applying the deep plating method when the medium was poured onto the spore suspension (concentration $\approx 10^6$) in Petri dishes and thoroughly mixed. Then, bacterial isolates as well as standard killer strains were spotted onto these agar plates inoculated with fungi. The tests were run in triplicates. The plates were incubated for 3 days at 26 ± 2°C. Sterile lysis (fungicidal) zones formed around the bacterial (and standard yeast) colonies on fungal lawns were evaluated as a manifestation of the killer activity of the test microorganisms [12]. The radius of a sterile zone from the bacterium edge to the growing fungal mycelium was measured and the average was calculated.

RESULTS AND DISCUSSION

The reaction of 40 fungi was tested towards substances secreted by 5 bacteria isolated from berry spontaneous fermentations. Additionally, to evaluate the efficiency of the bacterial killer (fungicidal) impact, the effect of 6 standard killer strains of *Saccharomyces cerevisiae* was also tested. The standard strains were found to have no effect or in several cases showed a very weak impact (Figs. 1, 2). Fungal susceptibility towards bacteria was significantly higher. Examination of the sensitive strain *α′1* showed that standard yeast strains had a killer effect, whereas the majority of the bacterial strains were even more active.

The results showed that the growth of fungi from the genus *Aspergilllus* was inhibited by all the test bacteria (Fig. 3).

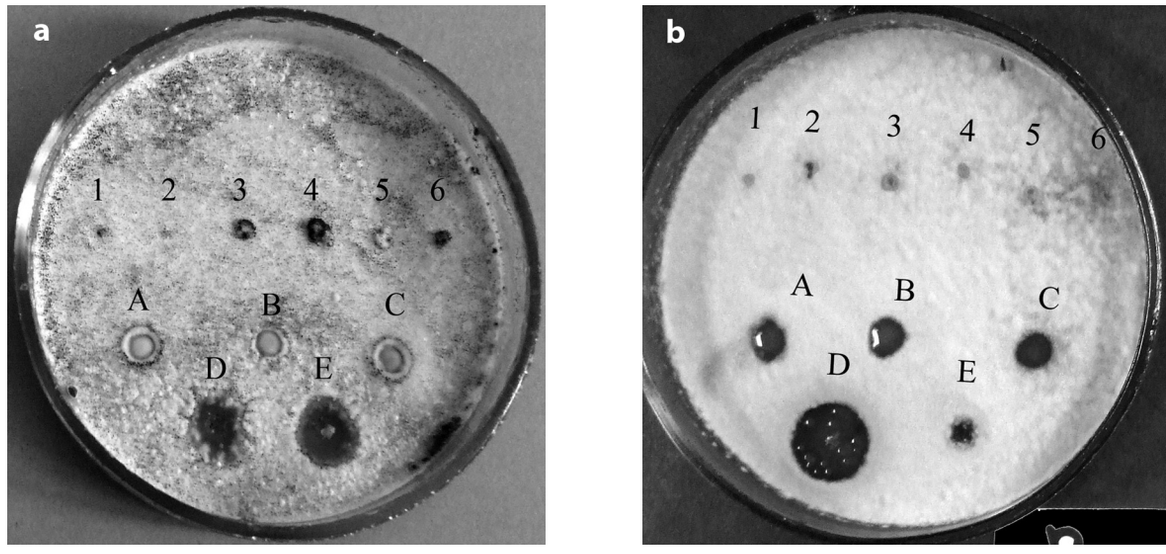

Fig. 1. Effect of bacterial isolates (A – T1x; B – T2x; C – T3x; D – Ux308; E – Ux) and standard *S. cerevisiae* killer strains (1 – DBY; 2 – MS300, 3 – Rom K100, 4 – K28, 5 – M437, 6 – K7) on *Aspergillus niger* 1AL (a) and *Penicillium expansum* 48 (b)

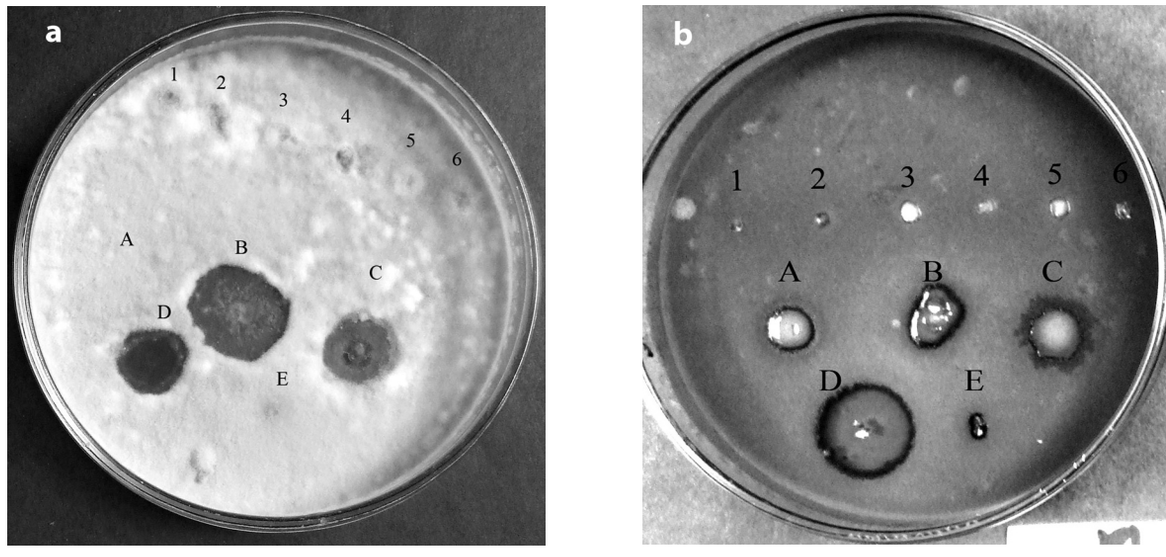

Fig. 2. Effect of bacterial isolates (A – T1x; B – T2x; C – T3x; D – Ux308; E – Ux) and standard *S. cerevisiae* killer strains (1 – DBY; 2 – MS300, 3 – Rom K100, 4 – K28, 5 – M437, 6 – K7) on *Gliocladium roseum* 24GL (a) and *Phoma* sp. (b)

The most significant effect on *Aspergillus* was excerted by bacteria Ux and Ux308 (Fig. 1). The bacterium Ux showed the highest effect on *A. glaucus* 16JL, *A. niger* 1AL: clear fungicidal zones on a fungal lawn were up to 5.3 mm in diameter (Fig. 1). Fungi *A. versicolor* 4SL, *A. nidulans* 5SL were the most sensitive toward the bacterium Ux308. The impact of bacteria T1x, T2x and T3x on the *Aspergillus* fungi was rather similar (fungicidal zones in the range of 1–3 mm), with the only exception of T2x which did not affect *A. versicolor* 4SL.

The 14 fungi from the genus *Penicillium* were susceptible to all bacterial strains, although the reaction varied among strains. High susceptibility towards all the bacteria (except Ux) was manifested by the fungus *P. janthinellum* 37L. The most significant killer effect on this fungus was exerted by T3x (fungicidal zone over 10 mm); a high inhibition was showed also by Tx2. Fungi *P. digitatum* 51L, *P. expansum* 48, *P. glabrum* 17G; *P. paxilli* 21L, *P. italicum* 1L and *P. oxalicum* 4DL, etc. were susceptible to most bacterial strains tested (Figs. 1, 3). There were only several cases when the bacteria had no effect. The fungus *P. brevicompactum* 11DL was not affected by Ux and Ux308, while all Tx-type bacteria exerted a clear fungicidal impact. Neither did

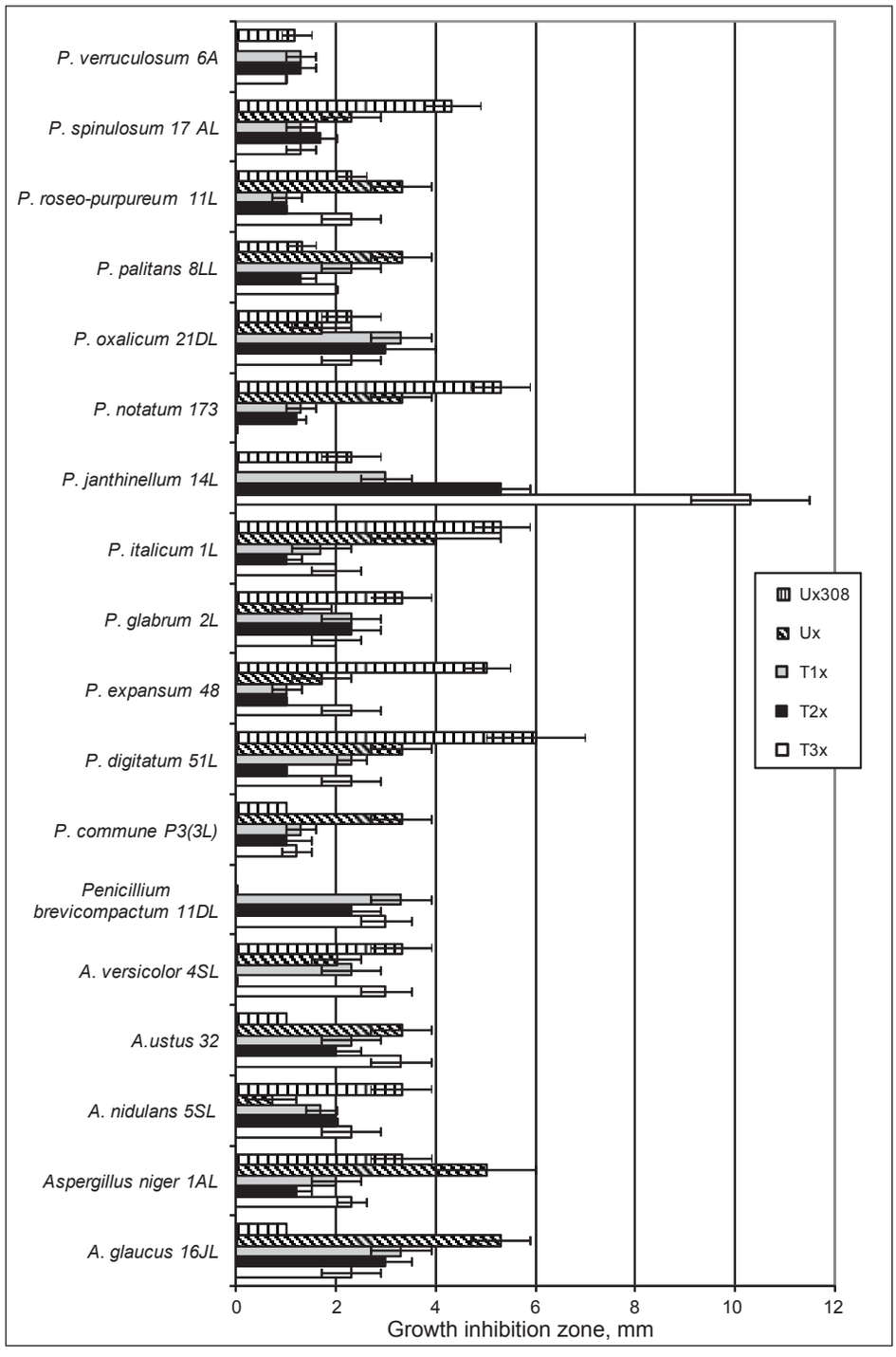

Fig. 3. Susceptibility of fungi *Aspergillus* and *Penicillium* to bacterial isolates T1x, T2x, T3x, Ux and Ux308. Bar length represents fungicidal effect (no fungal growth around the bacteria); the absence of bars indicates no fungicidal activity

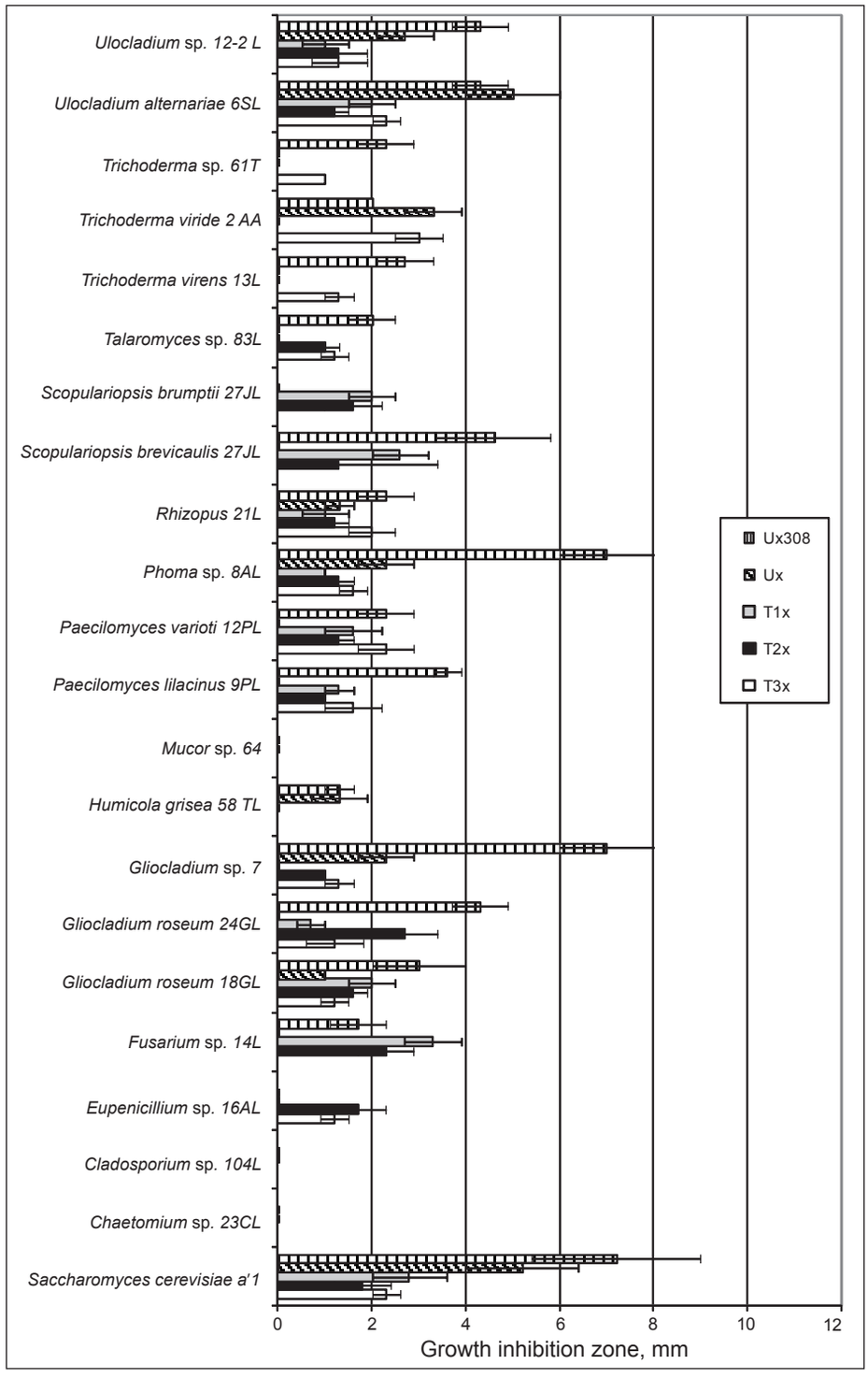

Fig. 4. Susceptibility of fungi from various genera to bacterial isolates T1x, T2x, T3x, Ux and Ux308. Bar length represents fungicidal effect (no fungal growth around the bacteria); the absence of bars indicates no fungicidal activity

the bacterium Ux fungicidally affect *P. janthinellum* 14L and *P. verruculosum* 6AL.

To find the most effective bacterium against *Penicillium* could be rather complicated; nevertheless, a tendency that the test *Penicillium* strains were more susceptible to Ux-type bacteria could be seen. On the other hand, some fungi such as *P. janthinellum* 37L and *P. brevicompactum* 14Dl, and partly *P. oxalicum* 4DL, were more inhibited by Tx-type bacteria. The differences in response among *Penicillium* fungi could have been caused by their individual peculiarities, firstly by their defense reaction and secreted metabolites.

None of the test *Trichoderma* strains was affected fungicidally by bacteria T1x and T2x (no clear lysis zones were formed); moreover, fungi *T. virens* 13L and *Trichoderma* sp. 61T were insensitive to Ux (Fig. 4). The fungus *T. viride* 2AA was most susceptible to Tx3 and Ux (zones about 3 mm), while *T. virens* 13L and *Trichoderma* sp. 61T were most significantly inhibited by Ux308 (zones over 2 mm). Comparison of

all three *Trichoderma* fungi has shown that the bacterial isolate Ux308 exerted an evident fungicidal effect. In cases of the other bacterial isolates, *Trichoderma* fungi showed a rather individual reaction. Fungi of the genus *Trichoderma* are known as intensive antagonists able to affect strongly fungicidally other microorganisms [23, 24]. This property of these fungi could have influenced their resistance to bacterial isolates. However, the fungicidal action of the bacterial isolates shows that these bacteria can be promising even against as strong antagonistic fungi as *Trichoderma*.

Fungi from the genus *Gliocladium* were more susceptible than *Trichoderma* to the influence of bacterial isolates. The most significant killer effect on all the *Gliocladium* strains was clearly exerted by Ux308 (fungicidal zones up to 7 mm) (Fig. 2). The other bacteria also influenced fungicidally the growth of these fungi, though weaker. As an exception, T1x had no effect on *Gliocladium* sp. Gl7 and Ux on *Gliocladium roseum* 24GL.

Paecilomyces lilacinus 9GL and *Paecilomyces variotii* 12PL reacted similarly towards bacterial toxic substances. The bacteria Ux had no effect on either of them. The most evident killer effect was exerted on *P. lilacinus* 9GL by Ux308. The fungicidal effect of Tx-type bacteria was slightly weaker and very simillar on both fungi.

Both fungi from the genus *Scopulariopsis*, *S. brumptii* 14JL and *S. brevicaulis* 27JL, were not affected by T3x and Ux, and *S. brumptii* 14Jl also by Ux308. The rest bacteria showed a fungicidal effect on the growth of these fungi. The highest effect on *S. brevicaulis* 27JL was exerted by Ux308 (zones over 4 mm), and rather effective killer influence was shown by T1x. The fungus *S. brumptii* 14JL was fungicidally affected by T1x and T2x.

Ulocladium alternariae 6Sl and *Ulocladium* sp. 12–2l were susceptible to all bacterial isolates, and the highest susceptibility was shown to bacteria Ux and Ux308 (zones up to 5 mm). A similar reaction was exerted by *Phoma* sp. 8AL.

There were several fungi that were not affected fungicidally by the test bacteria. The bacterial isolates had no killer effect on *Chaetomium* sp. 23CL, *Cladosporium* sp. 104L and *Mucor* sp. 64. However, *Rhizopus* (which as *Mucor* is representative of *Mucorales*) was fungicidally influenced by all the bacteria. The other fungi showed individual susceptibility to the bacteria. *Eupenicillium* sp. 16AL was susceptible to T2x and T3x, *Fusarium* sp. 14L to T1x, T2x and Ux308, *Talaromyces* sp. 83L to T2x, T3x and Ux308, and *Humicola grisea* 58TL was fungicidally affected by both Ux-type bacteria.

The effect of the bacterial isolates was often not only fungicidal, but also fungistatic (suppressed fungal growth). In many cases when fungicidal zones were not found, the fungistatic effect was present; e. g., *Penicillium notatum* 173 was not affected fungicidally by T3x, while suppressed growth was noted at the radius of 10 mm. There were rather many cases when the zones of fungistatic effect were larger than those of fungicidal impact (both *Scopulariopsis* strains, *Eupenicillium* sp. 16AL, etc.).

All the bacterial strains were fungicidally active against the fungi, whereas the killer effect of the bacteria on the test fungal strains differed. Bacterial isolates Ux and Ux308 could be indicated as more efficient towards the tested *Gliocladium,* most of *Penicillium* and some other fungi, while other micromycetes (e. g., *Eupenicillium* sp. 16AL, *Penicillium brevicompactum* 11DL) were more sensitive to Tx-type bacteria. The reaction of some fungi to particular bacterial isolates was very strong. *Scopulariopsis brevicaulis* 27JL was very sensitive to Tx2, *Penicillium janthinellum* 14L to Tx3, and *Gliocladium* sp. 7, *Phoma* sp. 8AL, *Ulocladium* sp. 12-2L to Ux308.

In our previous investigation, these bacterial isolates performed as killers against some plant pathogens. Bacteria T1x, T2x and Ux showed a killer effect on the plant pathogen *Verticillium albo-atrum*; T1x killed superficial hypha of the plant pathogen *Venturia ineaqualis*. These toxins were most effective at the initial stages of fungal growth [25]. The killer effect of Tx, Ux on plant pathogens *Fusarium* and *Alternaria* was also detected, especially at the early fungal growth [26]. Bacterial isolates T1x, T2x, T3x, Ux and Ux308 were also tested with some pathogenic yeasts, and all of them showed killer activity. Additionally, the killer effect of T1x was very stable against the test pathogens [27].

The investigation on killer substance origin and biochemical peculiarities is in progress. Previous experiments had shown that only Ux killer ability could be related to proteolytic activity. The primary analysis of the killer substances showed that the molecular mass of secreted substances of all the killer bacteria except Ux was less than 10 kDa [26]. Further studies are required to get more biochemical and genetic data for to characterize the origin of the toxin. The results obtained in this study and in previous ones allow to conclude that toxins produced by the study bacteria seem to affect a very wide range of fungi, and this feature can be promising for their future application.

ACKNOWLEDGEMENT

This work was supported by the Lithuanian State Science and Studies Foundation (grant No T-65/08).

Received 11 January 2009
Accepted 17 April 2009

References

1. Hyvärinen A, Meklin T, Vepsalainen A et al. Intern Biodet & Biodegr 2002; 49: 27–37.
2. Nielsen KF. Fungal Genetics and Biology 2003; 39: 103–17.
3. Lugauskas A, Paškevičius A, Repečkienė J. Patogeniški ir toksiški mikroorganizmai žmogaus supančioje aplinkoje. Vilnius, 2002.
4. Donini M, Lico C, Baschieri S et al. Appl Environment Microbiol 2005; 71: 6360–67.

5. Perez F, Ramirez M, Regodon A. Antonie Van Leewenhoek 2001; 76: 393–9.
6. Weiler F, Rehfeld K, Bautz F, Schmitt MJ. Molec Microbiol 2002; 46(4): 1095–105.
7. Selitrennikoff CP. Appl Environ Microbiol 2001; 67(7): 2883–94.
8. Anith KN, Tilak KV, Khanuja SPS et al. Microbiol Biotechnol 1998; 14(6): 939–41.
9. Lowe DP, Arendt EK. Journal of the Institute of Brewing 2004; 110(3): 163–80.
10. Kozakiewicz Z. Mycological Papers 1989; 161: 1–188.
11. Palumbo JD, Baker JL, Mahoney NE. Microb Ecol 2006; 52(1): 45–52.
12. Melvydas V, Černyšova O, Gedminienė G et al. Botanica Lithuanica 2005; 11(4): 241–6.
13. Domsch KH, Gams W, Anderson TH. Compendium of Soil Fungi. London: Academic Press, 1980.
14. Klich MA. Identification of Common *Aspergillus* Species. Utrecht, The Netherlands: Centraalbureau voor Schimmelcultures, 2002.
15. Samson RA, Hoekstra S. Introduction to food- and airborne fungi. Utrecht, The Netherlands: Centraalbureau voor Schimmelcultures, 2000.
16. Pitt JI. The genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces*. London: Academic Press, 1979.
17. Samson RA, Frisvad JC. Stud Mycol 2004; 49: 33–5.
18. Bridžiuvienė D, Levinskaitė L, Lugauskas A et al. Mikrobiologiniai medžiagų pažeidimai. Vilnius, 1997.
19. Ellis MB. Dematiaceous Hyphomycetes. Kew, 1976.
20. Lugauskas A, Mikulskienė A, Šliaužienė D. Katalog mikromitsetov – biodestruktorov polimernykh materialov. Moskva: Nauka, 1987.
21. Samson RA. Stud Mycol 1974; 6: 1–119.
22. Kondratienė L, Gulbinienė G, Servienė E et al. Sodininkystė ir daržininkystė (Horticulture and Vegetable Growing) 2003; 22 (3): 518–27.
23. Hjeljord LG, Tronsmo A. Phytopathology 2003; 93: 1593–98.
24. Eden MA, Hill RA, Stewart A. Plant Pathol 1996; 45(2): 276–84.
25. Melvydas V, Servienė E, Čapukoitiene B et al. Sodininkystė ir daržininkystė (Horticulture and Vegetable Growing) 2006; 25(2): 91–8.
26. Meškauskienė V, Melvydas V. Biologija 2007; 53(1): 50–3.
27. Melvydas V, Šakalytė J, Paškevičius A, Gedminienė G. Biologija 2007; 53(1): 45–9.

Loreta Levinskaitė, Vytautas Melvydas

GRYBŲ JAUTRUMAS NAUJIEMS BAKTERIJŲ IZOLIATAMS

Santrauka

Įvertintas 40-ies grybų padermių, priklausančių *Aspergillus, Penicillium, Gliocladium, Paecilomyces, Scopulariopsis, Trichoderma, Ulocladium* ir kt. gentims, jautrumas T1x, T2x, T3x, Ux ir Ux308 bakterijų izoliatams. Apie žudantį bakterijų poveikį buvo sprendžiama įvertinus bakterijų suformuotas skaidrias fungicidines zonas ant grybų pasėlio. Visos tiriamos bakterijos pasižymėjo grybus žudančiu aktyvumu. Nors individuali grybų reakcija buvo skirtinga, Ux ir Ux308 bakterijos ypač ryškiai fungicidiškai veikė daugumą tirtų grybų iš *Penicillium, Aspergillus, Gliocladium* ir kt. genčių. Kai kurie grybai (*Eupenicillium* sp., *Penicillium brevicompactum* ir kt.) buvo jautresni Tx bakterijoms. Pastebėtas kai kurių grybų ryškus individualus jautrumas atskiriems bakterijų izoliatams. Tirtų bakterijų plataus spektro ryškus fungicidinis aktyvumas gali būti pritaikomas kuriant antigrybines priemones prieš žalingus mikroskopinius grybus.