Antifungal Activity of Sodium Acetate and Lactobacillus rhamnosus†

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ABSTRACT

The inhibition of molds by sodium acetate in deMan Rogosa Sharpe (MRS) medium, along with the antifungal activity of Lactobacillus rhamnosus VT1, was studied by the slope agar plate method. MRS agar prepared with and without sodium acetate was used as the agar substrate. A total of 42 strains of Aspergillus, Penicillium, Fusarium, Alternaria, Cladosporium, and Rhizopus were used to compare sensitivities to the inhibitory activity of sodium acetate and L. rhamnosus VT1. It was found that sodium acetate in MRS medium affected the growth of 33 of the 42 mold strains tested to various degrees. The highest sensitivity to sodium acetate was shown by strains of Fusarium, followed by strains of Penicillium, Aspergillus, and Rhizopus. L. rhamnosus VT1 also inhibited mold growth. A significant finding was that sodium acetate and L. rhamnosus VT1 in combination exhibited a possible synergistic action. Thirty-nine of the 42 mold strains tested were completely inhibited by the presence of both antifungal agents. This finding confirms that sodium acetate, a basic component of commercial MRS medium, has strong antifungal properties, and this must be taken into consideration when evaluating the antifungal activity of Lactobacillus cultures grown in MRS broth.

Molds cause quality problems as contaminants of various foods, including dairy products. Molds also pose food safety and potential health risks for humans by producing mycotoxins in foods (4, 9–11, 16). Chemical antifungal agents, including acetates, have long been used to control fungi in foods (3–6). In some situations, however, the prolonged use or overuse of these chemicals has led to the development of fungal resistance (17, 18). The levels of chemicals used in the food industry are fungistatic and do not kill or completely inhibit growth for an indefinite time (4). Increasing consumer concerns about chemicals in foods has led to interest in novel preservation methods. Antagonistic microorganisms or their antifungal metabolites have been shown to have potential as natural biopreservatives to control undesirable fungi (17, 18). There have been numerous reports of the inhibition of mold growth and mycotoxin production by antifungal metabolites produced by lactic acid bacteria (1, 10, 12–15, 19, 20). Lactobacillus rhamnosus VT1 isolated from salad dressing has been found to exhibit strong antifungal properties against species of Aspergillus, Penicillium, Fusarium, Alternaria, Cladosporium, and Rhizopus (19, 20). In these studies, the inhibitory effect of L. rhamnosus VT1 was observed in cell-free supernatants after growth in lactobacilli deMan Rogosa Sharpe (MRS) broth (8). However, clear zones of inhibition were also observed on control plates on which sterile lactobacilli MRS agar was tested in agar wells, suggesting that a component in the lactobacilli MRS medium might have antifungal activity as well and hence lead to false conclusions regarding the actual antifungal properties of bacterial strains tested. The only component of MRS medium with known antifungal properties is sodium acetate. Hence, the objective of this study was to evaluate the inhibitory effect of sodium acetate alone and in combination with L. rhamnosus VT1 to confirm this activity.

MATERIALS AND METHODS

Bacterial strain. The antifungal strain L. rhamnosus VT1 was isolated and identified at the Department of Dairy and Fat Technology (DMF), Institute of Chemical Technology, Prague, Czech Republic. L. rhamnosus VT1 was cultivated at 37°C for 18 h in commercial MRS broth (Difco Laboratories, Sparks, Md.) and in freshly prepared MRS broth without sodium acetate for a microbial concentration of approximately 10⁷ CFU/ml.

Fungal strains. The Fusarium strains DMF 0101 and DMF 0102 were isolated and identified at the DMF: Fusarium culmorum DMF 0103 was isolated from a soil sample and identified at the Department of Botany, Charles University of Prague, Czech Republic. The remaining Fusarium species were obtained from the Fusarium Research Center at Pennsylvania State University, University Park, Pa. Penicillium, Cladosporium, Alternaria, and Rhizopus species were obtained from the NRRL Culture Collection of the U.S. Department of Agriculture’s National Center for Agricultural Utilization Research in Peoria, Ill. Aspergillus species

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TABLE 1. Composition of commercial MRS medium (Difco)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amt of ingredient (g/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteose peptone</td>
<td>10.00</td>
</tr>
<tr>
<td>Beef extract</td>
<td>10.00</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>5.00</td>
</tr>
<tr>
<td>Dextrose</td>
<td>20.00</td>
</tr>
<tr>
<td>Tween 80</td>
<td>1.00</td>
</tr>
<tr>
<td>Ammonium citrate</td>
<td>2.00</td>
</tr>
<tr>
<td>Magnesium sulfate</td>
<td>0.10</td>
</tr>
<tr>
<td>Manganese sulfate</td>
<td>0.05</td>
</tr>
<tr>
<td>Dipotassium phosphate</td>
<td>2.00</td>
</tr>
<tr>
<td>Sodium acetate</td>
<td>5.00</td>
</tr>
<tr>
<td>Bacteriological agar</td>
<td>7.00</td>
</tr>
</tbody>
</table>

Preparation of MRS medium. The commercial MRS medium used contained 5 g of sodium acetate per liter of medium. In this study, modified MRS broth or agar without sodium acetate (mMRS) was freshly prepared according to the composition of the commercial product (Table 1) but omitting sodium acetate. The beef extract (10 g) was dissolved in 200 ml of distilled water and filtered through Whatman no. 41 filter paper (Whatman Ltd., Maidstone, UK). The remaining ingredients were weighed separately, mixed together thoroughly, dissolved in 800 ml of distilled water, combined with the 200 ml of beef extract solution, and then autoclaved at 121°C for 20 min.

Slope agar plate method. The inhibitory effect of sodium acetate, along with the antagonistic activity of L. rhamnosus VT1 grown in MRS broths with and without sodium acetate, was studied using a slope agar plate method. Twenty milliliters of each type of MRS agar inoculated with 1% (vol/vol) L. rhamnosus VT1 was poured onto petri plates. Before the solidification of the agar, the plates were slanted to a slope of about 30° with a slanting device in order to obtain a control within the test plate. After solidification, all plates were overlaid in a level horizontal position with 20 ml of one MRS agar or the other. The points of high and low concentration of L. rhamnosus VT1 on MRS agar without sodium acetate.

RESULTS AND DISCUSSION

Inhibitory effect on Aspergillus species. Most of the Aspergillus species showed comparable growth on control plates of both types of MRS agar, suggesting that sodium acetate did not significantly affect the growth of Aspergillus species at the concentration used. Ten of 12 strains were completely inhibited by the presence of both L. rhamnosus VT1 and sodium acetate, suggesting possible synergistic activity. Aspergillus clavatus QM 8360 was not inhibited by either of the two antifungal agents. While there was almost no difference in the growth of control Aspergillus species on both types of MRS agar, 3 of 12 Aspergillus strains tested, namely, Aspergillus repens NRRL 13, Aspergillus versicolor M 1069, and Aspergillus candidus C 25, were completely inhibited by L. rhamnosus VT1 on MRS agar plates without sodium acetate.

Inhibitory effect on Penicillium species. Eight of 13 Penicillium strains tested were affected by the presence of sodium acetate in commercial MRS agar. All 13 Penicillium strains were completely inhibited by the presence of both L. rhamnosus VT1 and sodium acetate, suggesting possible synergistic activity. Only two of 13 strains, Penicillium italicum NRRL 983 and Penicillium puberulum NRRL 1899, were completely inhibited by L. rhamnosus VT1 on MRS agar plates without sodium acetate.

Inhibitory effect on Fusarium species. Thirteen of 14 Fusarium strains tested were affected by the presence of sodium acetate in commercial MRS agar. Six Fusarium strains were completely inhibited by its presence, suggesting that Fusarium species are quite sensitive to sodium acetate. Thirteen of 14 Fusarium strains were completely inhibited by the presence of L. rhamnosus VT1 and sodium acetate together. Only three out of 14 strains, Fusarium sp. DMF 0101, Fusarium poae T 0919, and Fusarium sporotrichioides T 42, were completely inhibited by L. rhamnosus VT1 on MRS agar plates without sodium acetate.

Inhibitory effect on Rhizopus, Cladosporium, and Alternaria species. Rhizopus stolonifer NRRL 1519 showed very slight sensitivity to the presence of sodium acetate in commercial MRS agar. Cladosporium cladosporioides NRRL 6421 and Alternaria alternata NRRL 5255 showed no sensitivity at all. However, all three strains were completely inhibited by the presence of both sodium acetate and L. rhamnosus VT1 in the medium together. C. cladosporioides NRRL 6421 and A. alternata NRRL 5255 were also completely inhibited by L. rhamnosus VT1 on MRS agar plates without sodium acetate.

Results from this study showed that sodium acetate, a component of MRS medium, has strong antifungal activity. This fact must be taken into consideration when evaluating the antifungal activity of Lactobacillus cultures that have been grown in MRS broth, especially if the cell-free supernatant (MRS broth) of the cultures is used for testing. The cell-free MRS broth may contain enough residual acetate to be inhibitory to certain molds by itself, leading to erroneous observations. In this study, sodium acetate affected the growth of 33 of the 42 mold...
TABLE 2. Growth of molds on MRS agar with and without sodium acetate and with and without L. rhamnosus VT1 after 30 days of incubation*

<table>
<thead>
<tr>
<th>Mold strain</th>
<th>MRS agar</th>
<th>L. rhamnosus VT1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With sodium acetate</td>
<td>Without sodium acetate</td>
</tr>
<tr>
<td>Aspergillus candidus C 25</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>A. clavatus QM 8360</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>A. flavus NRRL 1290</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>A. nidulans NRRL 157</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>A. niger NRRL 326</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>A. ochraceus NRRL 3174</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>A. parasiticus NRRL 2999</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>A. petrakii WDC 23</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>A. repens NRRL 13</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>A. sydowii NE M1</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>A. terreus WDC 6</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>A. versicolor M 1069</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Penicillium aurantiogriseum NRRL 6093</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>P. citrinum NRRL 1841</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>P. crustosum NRRL 969</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>P. digitatum NRRL 786</td>
<td>–</td>
<td>+++</td>
</tr>
<tr>
<td>P. expansum NRRL 2304</td>
<td>++</td>
<td>+++</td>
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<tr>
<td>P. glabrum NRRL 766</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>P. griseofulvum NRRL 2300</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>P. chrysogenum NRRL 807</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>P. italicum NRRL 983</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>P. puberulum NRRL 1899</td>
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<td>++</td>
</tr>
<tr>
<td>P. roqueforti NRRL 849</td>
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<tr>
<td>P. verrucosum NRRL 846</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>P. viridicatum NRRL 958</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Fusarium sp. DMF 0101</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Fusarium sp. DMF 0102</td>
<td>–</td>
<td>–</td>
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<tr>
<td>F. acuminatum R 666</td>
<td>–</td>
<td>–</td>
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<tr>
<td>F. crookwellense R 3090</td>
<td>–</td>
<td>–</td>
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<tr>
<td>F. culmorum DMF 0103</td>
<td>–</td>
<td>–</td>
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<tr>
<td>F. equiseti R 8466</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>F. graminearum R 4053</td>
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<td>++</td>
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<tr>
<td>F. chlamydosporum T 925</td>
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<td>++</td>
</tr>
<tr>
<td>F. verticillioides M 1325</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>F. oxyssporum O 1077</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>F. poae T 919</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>F. proliferatum M 3659</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>F. sporotrichioides T 42</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>F. subglutinans M 5640</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Alternaria alternata NRRL 5255</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Cladosporium cladosporioides NRRL 6421</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Rhizopus stolonifer NRRL 1519</td>
<td>++</td>
<td>+++</td>
</tr>
</tbody>
</table>

*+++*, extensive normal growth with typical sporulation characteristic of the species; +++, typical, but less mycelial growth, with reduced amounts of sporulation; +, little mycelial growth with no sporulation; –, no mold growth.

strains tested to various degrees. *Fusarium* species were especially sensitive to sodium acetate, since many *Fusarium* species were completely inhibited by its presence in sterile MRS agar. *L. rhamnosus* VT1 exhibited antifungal properties when used as an inhibitory agent alone; however, in the presence of sodium acetate, greater inhibitory activity was observed (Table 2). This finding also suggests that the use of biological control agents in combination with a suitable chemical could be advantageous and could extend the duration of control beyond that given by chemicals or biological agents alone (2, 7, 14, 17). Thus, acetate may also be of use in combination with *L. rhamnosus* VT1 and other lactobacilli in the food industry to prevent mold growth in products with shelf lives of about 21 days.

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REFERENCES