

Fungicide Effect on Nitrogenase Activity in Methylo-trophic Bacteria

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Abstract

The study comprised tests on the effect of fungicides: Oxafun T, Funaben T and Baytan Universal on the nitrogenase activity of methylo-trophic bacteria, selected from the rhizosphere and non-rhizosphere soil of spring barley. The obtained results indicated that the field dose of fungicides – 0.5 ppm had no effect on all the examined strains of methylo-trophic bacteria. The dose of 50 ppm, however, had varied effects, suppressing or stimulating, depending on the particular strain. The highest concentration of fungicides applied, i.e. 100 ppm in all cases suppressed the nitrogenase activity of the strains under examination.

Key words: fungicides, nitrogenase activity, methylo-trophic bacteria

Introduction

The microorganisms fixing atmospheric nitrogen substantially contribute to the growth of the total nitrogen content in the soil and indirectly influence the growth of crops. The crop growth is also obtained by more and more frequent use in the agricultural practice of chemical plant protection agents in the form of insecticides, herbicides and fungicides. The chemicals applied not only destroy the harmful organisms, but may also affect the useful elements of the soil biocenose, including the N₂-fixing bacteria. It is assumed that among the plant protection chemicals, the fungicides are those with the strongest effect on soil microorganisms (Gołębiowska and Strzelczyk 1964). The side effect of fungicide application may, in the case of free-living dinitrogen assimilators, be demonstrated by inhibition of their growth, suppression of the dinitrogen fixation activity and in symbiotic bacteria, the limitation of the symbiotic effectivity (Castro *et al.*, 1994; Strzelec and Martyniuk, 1994; Swędryńska and Sawicka, 1998; Dunfield *et al.*, 2000). The toxicity of fungicides is varied and depends on the properties of the microorganism itself, the preparation dose and chemical structure of toxic substance (Lal and Lal, 1988). The different response to the fungicide not only refers to species, but to strains as well. The varied reaction of the strains to a given fungicide is the result of their varied metabolism and different degrading capability of a given fungicide (Strzelec and Martyniuk, 1994).

Among the N₂-fixing microorganisms, the group of methylo-trophic bacteria is worth attention. These are microorganisms present everywhere in the nature, they occur in soil, water, air and in plant material. A characteristic distinguishing the group among others is the ability to utilize C1 compounds, such as methane, methanol, formate, methylamines and others (Bratina and Hanson 1992, Hanson and Hanson 1996, Rożej *et al.* 1999).

The purpose of the tests carried out was to determine the effect of the selected fungicide preparations on the nitrogenase activity of methylo-trophic bacteria -using methanol -as the only source of carbon, selected from the rhizosphere and non-rhizosphere soil of barley.

Experimental

Materials and Methods

The total of 19 methylo-trophic bacteria strains were used in the tests. The bacteria were separated in the test plot of Złotniki Agriculture Experimental Station belonging to Agricultural University in Poznań from the rhizospheres and non-rhizospheres of the Polo variety of spring barley, cultivated on grey-brown-podzolic soil composed of: 0.7% C, 12.5% loams and silts, pH 5.7. The methylo-trophic bacteria isolates were cultivated on a mineral medium, according to Urakami and Komagata (1978), with methanol

added as a single source of carbon in the quantity of $10 \text{ ml} \times \text{l}^{-1}$ of the medium. 7 strains of bacteria, isolated from the rhizosphere of spring barley, classified within the morphological group of cocci were tested—strains Nos 10, 12 and 37, bacilli—strains Nos 11, 42 and 51 and coccus-bacillus-strain No 7. 12 strains of the non-rhizosphere of barley were tested, of which cocci—strains Nos 9, 15, 42, 46, 57, 58, 60, 66 and 67, and bacilli—strains Nos 54, 62 and 75.

The following fungicides were applied: OxafunT (a.i. carboxin 37.5% + thiram 37.5%), Funaben T (a.i. carbendazim, 20% + thiram 45%), Baytan Universal 19.5 DS (a.i. triadimenol 15% + imazalil 2.5% + fuberidazole 2%).

Laboratory test were carried out, where the methylotrophic bacteria were incubated into 12 cm^3 test tubes on DN medium according to Baldini and Döbereiner (1980) modified by the addition of 5 ml methanol and plant protection chemicals in the quantities: 0.5 ppm, 50 ppm and 100 ppm. per 1 l. The culture medium inoculated with 1 ml suspension of appropriate methylotrophic bacteria strains ($10^6 \text{ cfu} \times \text{ml}^{-1}$) were incubated in 3 replications, for 3 days, at 28°C . Then the nitrogenase activity was measured by acetylene method (ARA) according to Sawicka (1983). For this purpose, 10% of the gas phase volume of acetylene was injected into each tightly sealed test tube with strains tested. After 24 hours 1 ml of this gas phase was taken from each test tube and analyzed using CHROM 5 gas chromatograph. Argon was used as a carrier. The nitrogenase activity was determined by virtue of the volume of acetylene reduced to ethylene and expressed in $\text{nmoles C}_2\text{H}_4 \times \text{ml}^{-1}$ of the culture $\times 24 \text{ h}^{-1}$, applying the theoretical conversion factor $\text{N}_2:\text{C}_2\text{H}_4 = 1:3$.

Results

The fungicides applied in the tests: Oxafun T, Funaben T and Baytan Universal in field dose – 0,5 ppm (as assumed by the Plant Protection Institute), did not affect the nitrogenase activity of methylotrophic bacteria strains examined. Regardless whether they were separated from the rhizospheric of barley or non-rhizospheric zone, the atmospheric nitrogen fixation activity affected by fungicides in all the strains examined was the same as in the control. The results obtained both for the control and the fungicides applied in the amount 0,5 ppm can be treated equally. The influence of fungicides on the nitrogenase activity of the strains examined was observed in much higher concentrations only.

In case of methylotrophic bacteria strains originating from barley rhizospheric zone (Table I) Oxafun T applied in the tests in dose of 50 ppm, i.e. 100-times above the recommended field dose, influenced atmospheric nitrogen fixation in a varied way. In most strains (Nos 37, 10, 7, 42, 41) it caused growth of nitrogenase activity between a few and several hundred per cent, and in case of 2 strains (Nos 11 and 12) the nitrogenase activity dropped by 20% and 100%, respectively. The dose 100 ppm reduced the nitrogenase activity in each case. The complete suppression of atmospheric nitrogen fixation was noticed in case of strains 12 and 7. Strain No 12 appeared to be the most sensitive to the OxafunT seed dressing compound, while strain No 10 – the most resistant one. Both strains belonged to the same morphological group of cocci. The 50 ppm Funaben T dose affected the nitrogenase activity of the examined strains, similarly to Oxafun T. It stimulated the nitrogenase activity in most strains from *ca* a dozen (No 10) to several dozen (No 12) per cent, while in strain No 7 the activity 10 times higher compared to the control (the nutrient without fungicide). The lower percentage of nitrogenase activity reduction was noted in strains Nos 37 and 42. With the application of 100 ppm dose the reduction of nitrogenase in all the bacteria strains was noted. The strongest, by 100 per cent – in strain No 12 and the weakest – in strain No 10 (26 %). The presence of 50 ppm Baytan Universal seed dressing in the incubation of methylotrophic bacteria strains stimulated the bacteria nitrogenase activity by several dozen per cent, except for strain No 7 whose growth was completely stopped by this chemical. The 100 ppm dose reduced the nitrogenase activity by several dozen to one hundred per cent – in strains Nos 12 and 7. Disregarding the type of fungicide applied, restricted or completely stopped

Table I
Effect of fungicides on nitrogenase activity of methylotrophic bacteria from rhizospheric zone of barley

Morphological group	No strain	Control nMC_2H_4 1 ml 24 h^{-1}	Oxafun T		Funaben T		Baytan Universal	
			50ppm	100ppm	50ppm	100ppm	50ppm	100ppm
% of control								
Cocci	12	0.035	0	0	191	0	134	0
	37	0.049	112	80	80	67	120	71
	10	0.080	300	93	113	74	140	29
Coccus-bacillus	7	0.040	198	0	1125	10	0	0
Bacilli	42	0.032	109	34	88	34	125	47
	41	0.032	128	72	116	63	138	41
	11	0.025	80	36	144	44	128	48

Table II
Effect of fungicides on nitrogenase activity of methylotrophic bacteria from non-rhizospheric zone of barley

Morphological group	No strain	Control nMC ₂ H ₄ 1 ml 24 h ⁻¹	Oxafun T		Funaben T		Baytan Universal	
			50ppm	100ppm	50ppm	100ppm	50ppm	100ppm
			% of control					
Cocci	9	0.049	106	96	147	92	108	59
	15	0.025	80	36	144	44	128	48
	42	0.087	87	34	55	37	46	39
	46	0.058	152	69	117	65	152	79
	57	0.049	120	82	71	31	82	22
	58	0.072	111	72	54	18	56	26
	60	0.028	300	28	371	36	343	64
	66	0.043	93	86	95	65	112	77
	67	0.056	136	75	125	71	114	79
Bacilli	54	0.111	120	53	86	76	51	44
	62	0.038	95	53	242	42	95	42
	75	0.040	98	87	108	60	178	25

nitrogenase activity was noted in bacteria originating from barley rhizosphere – always in the presence of 100 ppm dose of the preparation in the medium.

The results presented in Table II indicate that the methylotrophic bacteria originating from the non-rhizospheric zone of spring barley, like the bacteria of the rhizospheric zone, reacted in a various way to the fungicides applied in the dose of 50 ppm (the growth or drop of the nitrogenase activity). The concentration of 50 ppm Oxafun T stimulated the nitrogenase activity in the strains tested from a few (strain No 9) to several hundred per cent (strain No 60), or suppressed within a few (strain No 75) to several dozen per cent (strain No 15). The dose of 100 ppm stopped the process of N₂ fixation in all cases, in strain No 9 to the lowest extent, in graft No 60 – to the highest. In the presence of 50 ppm Funaben T dose the nitrogenase activity was stimulated in most strains, similarly from a few (strain No 75) to several hundred per cent (strain No 60). The lowest drop, however – *ca* by a few per cent was noted for strain No 66, the highest – by fifty per cent – for strain No 58. With 100 ppm of Funaben T, the suppression was between a few (strain No 9) to several dozen per cent (strain No 58).

Baytan Universal applied in a dose 100 times exceeding than recommended by The Plant Protection Institute (50 ppm) stimulated the N₂ fixation process like the preparations described above, *i.e.* from a few (strain No 9) to several hundred per cent (strain No 67) or stopped the activity between a few (strain No 62) to more than fifty per cent (strain No 42). The highest (applied in the test) concentration of Baytan in the dose of 100 ppm suppressed the process of fixing atmospheric nitrogen in each case and the boundary values of suppression were within the limit between 21% (strain No 46) and 78% (strain No 57).

In the bacteria of the non-rhizospheric zone, in contrary to the strains separated from the rhizospheric zone, none of the fungicide doses applied stopped the nitrogenase activity completely.

Discussion

Fungi are, most of all, sensitive to the fungicides used in agriculture, however the latter are not neutral to the bacteria. Fungicides, being highly active chemicals, affect both the number and the biological processes of the bacteria (Nowak, 1983; Domsh, 1992; Rózański, 1996). They may also affect the process of biological nitrogen fixation, both in the symbiotically and non-symbiotically N₂ – fixing bacteria (Lal and Lal, 1988). Scarcely any literature is available on the bacteria reactions (especially methylotrophic bacteria) to the fungicides implemented in the soil and their influence on atmospheric nitrogen fixation.

The tests on the effect of 3 selected fungicide seed dressings (Oxafun T, Funaben T and Baytan Universal) on the nitrogenase activity of methylotrophic bacteria, selected both from the rhizosphere and non-rhizosphere of spring barley, indicated that the chemicals applied in dose recommended by the Plant Protection Institute, *i.e.* in the amount of 0.5 ppm did not affect the nitrogenase activity of the selected strains. 50 ppm did influence them in a varied way, more often, however, stimulating than suppressing the nitrogenase activity.

The differences not only in terms of species but strains, in reaction to the fungicides, were also obtained by Strzelec *et al.* (1993) for symbiotic bacteria and for non-symbiotic assimilators of dinitrogen (Strzelec, 1993). The diversity of reaction to fungicides between the particular strains could be caused by the varied degrading capability of the microorganisms in relation to the chemicals and the adaptation time of the strains to the high concentrations of the chemical, related thereto. The bacteria ability to use the fungicide as nourishing substrate was pointed out by Różański (1992), Nowak (1995), Kaszubiak and Muszyńska (1996), Kaszubiak and Durska (2000). The literature related to the influence of fungicide seed dressings on N₂-fixation process are varied, depending on the type of fungicide and the dose thereof. In most cases, however, the authors state that, regardless of the type of chemical used, N₂-fixation suppression grows along with the growth of the chemical dose. The process inhibition most often takes place at concentrations from 50 to a few hundred ppm, and sometimes at a few thousand ppm only (Lal and Lal, 1988). Our results related to the effect of fungicides on the nitrogenase activity of the examined methylotrophic bacteria converge with those obtained by Pati and Govindaraju, cited by Lal and Lal (1988).

The chemical dose increase to 100 ppm always resulted in decreased nitrogenase in all the examined strains of methylotrophic bacteria. Presumably the only result of further increase of fungicide doses could be the total a setback of N₂ fixation in the examined strains.

The study revealed no significant differences in the nitrogenase activity under the influence of the fungicides used between the methylotrophic strains separated from different soil zones, or between the morphological groups the examined strains belonged to. The test results obtained indicate that the doses of selected seed dressings recommended in the agricultural practice for barley are not toxic for methylotrophic bacteria free-living in the soil and fixing atmospheric nitrogen. Largely multiplied doses, however, may cause the stimulation or reduction of nitrogenase activity in these bacteria.

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