CLAMP ROT PATHOGENS OF SUGAR BEET AND NEW METHODS OF THEIR CONTROL

Kolomiets E.I..[1], *Rapoport A.I..*[2], *Sviridov A.V.*[3], *Kilchevskaya O.S.*[1], *Kuptsov V.N.*[1], *Romanovskaya T.V.*[1]

[1]Institute of Microbiology, National Academy of Sciences of Belarus

[2] Institute of Microbiology and Biotechnology, Latvian University

[3] Grodno State Agrarian University

Introduction. Clamp rot of sugar beet is a widely distributed pathology in Belarus. Root crop disease during winter storage in clamps and piles resulting in heavy yield losses may be caused by a series of phytopathogenic microorganisms (Dementyeva 1985; Belaj 1988). Composition of species responsible for spread of this pathology in the Republic remains to be studied. Biopesticides based on antagonistic bacteria characterized by a broad spectrum of antimicrobial activity and ability to control development of phytopathogens may act as an alternative to chemical agents suppressing root crop infection in the course of storage limited by sanitary standards. Biological control of phytopathogens engaging bacteria-antagonists as an active principle of biopesticides is designed to ensure efficient plant protection and generation of ecologically safe products. Biopreparations distinguished by enhanced capability to inhibit progress of clamp rot have not been registered in Belarus. Elaboration of domestic biopesticide "Betaprotectin" showing high antagonistic activity to species – sources of clamp rot pathogens typical for local climatic conditions is an extremely relevant problem.

Materials and Methods. Phytopathogenic fungi isolated from infected tissues of sugar beet roots and identified at Grodno State Agrarian University, as well as bacteria-antagonists isolated from various natural sources at laboratory of biological control agents, Institute of Microbiology, National Academy of Sciences, Belarus were chosen as objects of studies. Fungal isolates were identified according to [Pidoplichko 1977]. The fungi were grown on Petri plates and in shaken flasks on potato-glucose medium. Effect of antagonistic bacteria on invasion rate of mycelium colonizing a slice of sugar beet tissue was evaluated by 5-point scale [Sviridov et al 2008]. Bacteria were cultured on modified Meynell medium in laboratory fermentor ANKUM-2M (200 rpm, 1 l air/1 l medium·min, 34°C, 72 h). Inoculum exposed to thermal treatment at 65 °C for 30 min was used to assess impact of stress factors on antimicrobial activity of bacteria growing in laboratory fermentor. Antifungal activity of bacteria was determined by wells technique [Segi 1983].

Efficiency trials of antagonistic bacterial preparations against clamp rot pathogens were performed at large-scale storage piles filled with sugar beet hybrid varieties Sylvano and Mars

(Grodno region). Industrial tests of preparation “Betaprotectin” were carried out at large-scale clamps and piles loaded with hybrids Sylvano and Korab. “Betaprotectin” application dose equaled 0.5 l/ton and expense of working solution 3.0 l/ton. Root treatment was conducted during harvesting. Untreated root crops served as the control. Biopreparation efficiency was estimated in accordance with recognized phytopathological methods [Poliakov et al 1984].

The studies covered 2006-2009 year span. The obtained findings were processed by dispersion analysis [Dospehov 1985].

Results and Discussion. The following fungal cultures causing clamp rot of sugar beet cultivars in Belarus were isolated: *Fusarium redolens Wollenweber, Fusarium culmorum Smith et Saccardo, Penicillium expansum Link, Botrytis cinerea Persoon et Fries, Gliocladium catenulatum Gilman et Abbott, Sclerotinia sclerotiorum (Lib.) de Bary, Alternaria tenuis Nees, Phoma betae Frank.*

It was found in laboratory experiments modeling infection of beet root slices with isolated phytopathogens that *Gl. catenulatum, F. redolens, F. culmorum, P. expansum, Sc. sclerotiorum* displayed elevated attacking potential – mycelium productivity 2.0-2.7 points (table 1). *Ph. betae* and *Al. tenuis* proved less aggressive – the level of mycelium development in these variants was insignificant – from 0 to 1.3 points.

The important initial stage in elaboration of biological method for sugar beet protection from clamp rot is search of microbial strains capable to control growth of pathological agents. Antifungal activity of selected bacterial cultures against isolated phytopathogens was evaluated (table 2). Majority of antagonists caused delay, inhibition or full absence of growth in phytopathogenic fungi. Strains *B. subtilis* 10/19, *B. subtilis* M-22, *B. subtilis* G-3 showed maximal activity towards fungal pathogens in laboratory experiments in vitro. Analysis of small-scale experimental data of biopreparations effect on sugar beet root preservation in field piles during winter storage in vivo demonstrated (table 3) the highest efficiency of antagonistic bacteria *B. subtilis* M-22, *B. subtilis* 14 S, *B. subtilis* G-3. Under their influence spread of clamp rot on sugar beet roots was reduced 1.5-1.7 times. Maximum biological efficiency (36.9-39.2 %) was recorded for preparation derived from bacterial strain *B. subtilis* M-22.

The obtained results were used for elaboration of biopesticide “Betaprotectin”. Its production technology is based on highly active strain *B. subtilis* M-22 deposited at Belarusian collection of nonpathogenic microorganisms, Institute of Microbiology, National Academy of Sciences of Belarus under registration number BIM B-439 D.

In conformity with data collected during cultivation of bacteria *B. subtilis* BIM B-439 D in laboratory fermentor it was found (figure 1) that with inoculum exposed to thermal treatment the peak antagonistic activity of the culture was reached by 48 h of fermentation, which is 12 h

faster than the control values. The offered procedure envisages directed regulation of bacterial antagonistic activity under the impact of physical stress factors. Use of spore inoculum of bacteria *B. subtilis* BIM B-439 D subjected to thermal treatment provides increase in antimicrobial activity of liquid biopreparation by 1.2-1.4 times.

Production trials of "Betaprotectin" efficiency were realized with 2 hybrid varieties of sugar beet filling large-scale storage clamps and piles. In compliance with obtained results (table 4) "Betaprotectin" reduced deleterious effect of pathology on stored sugar beet roots 2-fold in comparison with the control, and spread of clamp rot was significantly inhibited. Biological efficiency of "Betaprotectin" application ranged from 17.5 % to 39.6 %, and its economic efficiency – from 5.1 % to 35.8 %. Root crop preservation rate reached 94.1 %.

Conclusion. Phytopathogenic fungi responsible for clamp rot of sugar beet in Belarus were isolated and identified. Degree of their aggressiveness was defined. Bacterial culture distinguished by high antagonistic activity towards isolated phytopathogens was selected to form the basis of biopreparation controlling diseases of sugar beet in storage. The method aimed at increasing antimicrobial activity of bacteria by exposure to physical stress factors was proposed. Elevated biological and economic efficiency of novel biopesticide was demonstrated.

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Key words: bacteria-antagonists, *Bacillus subtilis,* biopesticide "Betaprotectin", storage piles, clamps, root crops, sugar beet, phytopathogens

Summary

It was found that pathogens of clamp rot in sugar beet cultivated in Belarus are the following fungal species: *Fusarium redolens, Fusarium culmorum, Penicillium expansum, Botrytis cinerea, Gliocladium catenulatum, Sclerotinia sclerotiorum, Alternaria tenuis, Phoma betae. Gl. catenulatum, F. redolens, F. culmorum, P. expansum, Sc. sclerotiorum* proved more aggressive, *Ph. betae* и *Al. tenuis* – less aggressive. Experiments in vitro and in vivo have revealed that strain *Bacillus subtilis* BIM B-439 D – main component of new biopesticide "Betaprotectin" was characterized by maximal antagonistic activity against clamp rot pathogens. According to small-scale experimental data, "Betaprotectin" biological efficiency reached 36.9-39.2 %. Technology of manufacturing new biopreparation comprises a procedure of directed regulation of bacterial antagonistic activity under the impact of physical stress factors – application of spore inoculation material exposed to thermal treatment raised antimicrobial activity of *B. subtilis* BIM B-439 D liquid culture by 1.2-1.4 times. Deleterious effect of pathology during storage of root crops in large-scale clamps and piles fell 2-fold upon "Betaprotectin" treatment as compared to the control. Spread of clamp rot slowed down likewise. Biological efficiency of biopesticide application ranged from 17.5 % to 39.6 %, economic efficiency – from 5.1 % to 35.8 %. Root crop preservation rate reached 94.1 %.

Table 1 – Effect of antagonistic bacteria on invasion rate of mycelium on the slice of beet root

Preparation	Rate of fungal mycelium invasion, pts						
	Pathogens *						
	1	2	3	4	5	6	7
Ps. aurantiaca 9	1.0	1.0	1.0	2.3	1.0	1.0	0
B. subtilis 10/19	2.0	0.7	0.5	2.0	2.0	1.3	0.3
B. subtilis 12 A	2.0	0.7	0.3	2.0	1.0	1.3	0.3
B. subtilis 14 S	0.7	2.7	2.0	2.0	1.0	1.3	0.7
B. subtilis M-22	1.3	1.0	1.0	2.1	0.7	1.3	0.7
Isolate G-3	0.7	1.0	0.3	2.0	1.0	1.0	0.7
Isolate G-6	1.0	1.6	1.3	2.3	1.7	1.0	0.3
Control	4.0	3.8	2.5	4.0	4.0	4.0	1.3

Note: -* 1 – *F. redolens,* 2 – *F. culmorum,* 3 – *P. expansum,* 4 – *Gl. catenulatum,* 5 – *Sc. sclerotiorum,* 6 – *Al. tenuis,* 7 – *Ph. betae*

Table 2 – Antifungal activity of studied bacterial cultures

Cultures	Diameter of growth inhibition zones in test-cultures *, mm							
	1	2	3	4	5	6	7	8
Ps. aurantiaca 9	24.0	23.0	29.5	38.0	27.5	28.0	20.0	32.0
B. subtilis 10/19	30.0	24.0	32.0	46.0	23.5	37.0	29.0	47.0
B. subtilis 12 A	22.0	20.0	30.0	47.0	29.5	45.0	22.0	45.0
B. subtilis 14 S	21.0	19.0	33.0	40.0	26.5	33.0	25.0	36.0
B. subtilis M-22	26.0	27.5	27.0	38.0	37.0	40.0	24.0	41.0
Isolate G-3	25.0	23.0	21.0	48.0	18.5	43.0	21.0	30.0
Isolate G -6	27.0	21.5	28.0	44.0	17.5	34.0	21.0	31.0
$НСР_{05}$	2.3	1.1	2.0	1.7	1.5	4.1	1.5	1.8

Note: -* test-cultures: 1 – *F. redolens,* 2 – *F. culmorum,* 3 – *P. expansum,* 4 – *B. cinerea,* 5 – *Gl. catenulatum,* 6 – *Sc. sclerotiorum,* 7 – *Al. tenuis,* 8 – *Ph. betae*

Table 3 – Efficiency of biopreparation during sugar beet storage

Preparation	Clamp rot distribution, %	Spread of clamp rot, %	Deleterious effect of pathology, %	Biological efficiency, %	Root crop preservation, %
Sugar beet hybrid Sylvano					
Ps. aurantiaca 9	50.0	15.0	6.8	16.9	93.2
B. subtilis 10/19	50.0	15.6	6.8	13.8	93.2
B. subtilis 12 A	50.0	16.9	8.5	6.2	91.5
B. subtilis 14 S	35.0	12.2	5.8	32.3	94.2
B. subtilis M-22	35.0	11.4	4.9	36.9	95.1
B. subtilis Г-3	31.7	11.7	5.2	35.4	94.8
B. subtilis Г-6	46.7	15.3	7.3	15.4	92.7
Control	55.0	18.1	8.7	-	91.3
Sugar beet hybrid Mars					
Ps. aurantiaca 9	63.3	19.7	9.3	4.1	90.7
B. subtilis 10/19	55.0	15.8	7.6	23.0	92.4
B. subtilis 12 A	38.3	16.1	9.2	21.6	90.8
B. subtilis 14 S	56.7	15.8	7.0	23.0	93.0
B. subtilis M-22	45.0	12.5	5.1	39.2	94.9
B. subtilis Г-3	50.0	14.4	6.6	29.7	93.4
B. subtilis Г-6	45.0	16.9	9.5	17.6	90.5
Control	61.7	20.6	9.3	-	90.7

Table 4 – Efficiency of “Betaprotectin” action against clamp rot of sugar beet roots

Place of storage	Hybrid	T/C*	Clamp rot spread, %	Deleterious effect of pathology, %	Biological efficiency, %	Economic efficiency, %	Crop root preserva-tion, %
Clamp	Korab	T	24.7	5.9	27.6	5.1	94.1
-//-	-//-	C	34.2	10.8			89.2

-//-	Sylvano	T	48.6	22.0	17.5	16.9	78.0
-//-	-//-	C	58.9	34.6			65.4
Pile	Korab	T	42.8	19.5	39.6	18.6	80.5
-//-	-//-	C	70.8	49.1			50.9
-//-	Sylvano	T	35.8	14.5	37.0	35.8	85.5
-//-	-//-	C	56.9	30.4			69.6

Note: -* T –treatment, C – untreated control

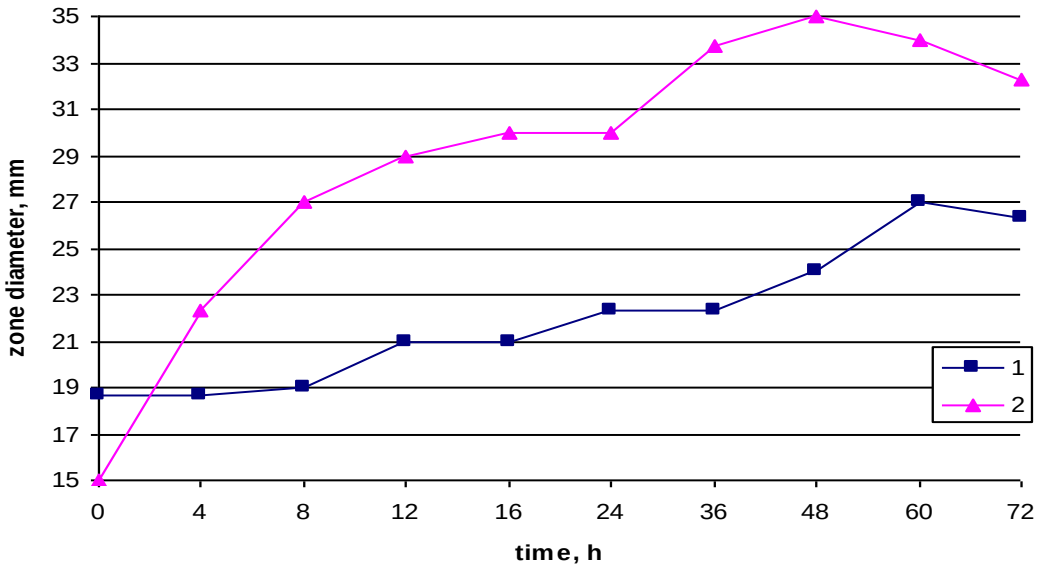

Figure 1 – Dynamics of changes in antagonistic activity of *B. subtilis* BIM B-439 D evaluated via diameter of growth inhibition zone of fungus *F. redolens*, using untreated (1) and heated (2) inoculation material