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Antagonistic and nematocide activity of carboxylic acids of microbial origin

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INTRODUCTION & AIM

An actual direction of research in the field of plant protection is to reduce the use of toxic chemicals and replace them with biologically safe preparations. Biotechnology companies actively introduce the biological methods of plant protection, which utilise bacteria, viruses, entomopathogenic fungi and nematodes. However, it is important to note, that the widespread implementation of biopesticides based on microorganisms is limited by several factors, including the complexity of cultivation, the high cost of the preparations and the narrow scope of their applications. Biopreparations based on living microorganisms are unable to effectively combat sudden outbreaks of plant diseases that occur under unfavourable conditions. A new area of research is therefore focusing on the use of microbial metabolites as plant protection products against phytopathogens. Carboxylic acids are considered as elicitors of plant defence mechanisms, leading to increased plant resistance to bacterial and fungal pathogens after their application (Morgunov et al. 2017).

The bottleneck in the development and introduction of simple and affordable carboxylic acid-based products is the inability to produce them in sufficient quantities and of the required quality. It is known that even small impurities in chemically synthesised carboxylic acid preparations can significantly reduce their quality and even cause cytotoxicity in plants. Microbial synthesis of carboxylic acids makes it possible to obtain preparations of higher quality without harming soil ecology, the environment and human health; it is also effective from an economic point of view, as it usually reduces production costs by 2-3 times.

In this context, the present work aims to develop and test new biological products based on metabolites produced by the yeast *Yarrowia lipolytica* to protect crops against fungal and bacterial diseases.

METHOD

Succinic acid (SA), isocitric acid (ICA) and α-ketoglutaric acid (KGA) synthesized by the yeast *Y. lipolytica* were investigated. The synthesis of SA entails the combination of KGA production by *Y. lipolytica* VKM Y-2412, cultivated in a medium with ethanol as a carbon source, and subsequent decarboxylation of KGA by H_2O_2 to yield 71.7 g/L SA. The isolation of SA involved the cell separation, the decomposition of H_2O_2, the bleaching and the acidification of the filtrate with a mineral acid, the evaporation of the filtrate and extraction of SA from the residue with ethanol. The purity of crystalline SA was 100% (Kamzolova et al., 2014). ICA was produced by *Y. lipolytica* VKM Y-2373 cultivated in a medium with ethanol as a carbon source, with a yield of 70 g/L. The isolation procedure involved the cell separation, the clarification, the concentration, the acidification and crystallization processes, resulting in the formation of crystals of the monopotassium salt of ICA with a purity of 99.9% (Morgunov et al., 2018). KGA was produced by *Y. lipolytica* VKM Y-2412 cultivated in a medium with rapeseed oil as a carbon source, with a yield of 106.5 g/L. The isolation involved the cell separation, extraction of residual triglycerides, bleaching and acidification with mineral acid, concentration, precipitation of mineral salts and crystallization. The purity of KGA reached 99.1% (Morgunov et al. 2013).

The antimicrobial activity of the acids was evaluated against Gram-positive bacteria (*Staphylococcus aureus* IMV 209B, *Bacillus subtilis* RCM 912B), Gram-negative bacteria (*Escherichia coli* RCM 567B, *Erwinia carotovora* RCM 1247B) and fungi (*Trichotecium roseum* RCM 750F, *Fusarium oxysporum f. Sp. lycopersici* RCM 840F, *Fusarium napiforme* IMV 152F, *Cylindrocarpon gracile* RCM 918F, *Aspergillus flavus* RCM 25F, *Penicillium casei* IMV 542F. The nematocidic activity of the acids against phytoparasitic stem nematodes (Ditylenchus destructor) was evaluated. Ditylenchus destructor was isolated directly from potato tubers sold in the Almaty region of Kazakhstan. The nematodes were collected immediately prior to each experiment. The potato pieces were placed in sterile water in Petri dishes, and the nematodes were extracted with forceps and transferred to watch glasses containing either an acid solution (50 mg/ml) or a control solution (5% ethanol or sterile water) or comparators (5% acetic acid and 5% lactic acid). A total of 30 nematodes were placed in each glass, with a volume of 2 ml, and exposed to either the solution of the acid under investigation or the control solution or the contactors. The glasses were incubated for a period of 72 hours at a temperature of 26°C. Subsequently, the motility of all nematodes in each glass was examined and recorded as either active or inactive. Subsequently, each nematode was transferred to sterile water for a further 24-hour incubation period, after which motility was again examined. Nematodes that remained inactive after transfer to sterile water were considered dead. The above treatment procedures were repeated three times for each experiment. If the nematodes exhibited no motility and did not recover within 24 hours after transfer to water, this was deemed to indicate that the acid had a toxic effect on them. Otherwise, the effect was considered to be nematostatic (transient).

RESULTS & DISCUSSION

The results of the antimicrobial activity of the acids produced by the yeast *Y. lipolytica* are presented in Table 1. The results demonstrated that the tested acids exerted a selective effect on pathogenic microorganisms. SA exerted an inhibitory effect on the growth of three out of the ten cultures tested. The zones of inhibition of growth reached 22, 16 and 17 mm, respectively, for *S. aureus*, *E. carotovora* and *P. casei*. The ICA demonstrated the selective inhibition of the growth of *S. aureus*, *E. coli* and *T. roseum* (with zones of inhibition of growth reaching 17, 12 and 18 mm, respectively). KGA suppresses only the growth of *F. napiforme* (with zone of inhibition of growth reaching 18 mm). For comparison, the commercial lactic acid exhibited selective inhibitory effects on the growth of *E. coli*, *S. aureus*, *E. carotovora* and *B. subtilis*, whereas the commercial acetic acid demonstrated inhibitory effects on the growth of the majority of the bacteria and fungi tested.

Table 1. Antimicrobial activity of acids produced by *Y. lipolytica*

Tested microorganisms	Diameter of the growth inhibition zone (mm)				
	SA	ICA	KGA	Acetic acid	Lactic acid
Staphilococcus aureus	22.0 ± 2.1	17.0 ± 2.6	0	26 ± 1.7	22 ± 2.1
Bacillus subtilis	0	0	0	0	19 ± 1.5
Escherichia coli	0	12.0 ± 1.7	0	25 ± 2.4	18 ± 1.5
Erwinia carotovora	16.0 ± 2.0	0	0	17 ± 0.58	20 ± 2.6
Trichotecium roseum	0	18.0 ± 2.3	0	25 ± 1.0	0
Fusarium oxysporum	0	0	0	15 ± 1.0	0
Fusarium napiforme	0	0	18.0 ± 1.5	19 ± 1.2	0
Cylindrocarpon gracile	0	0	0	17 ± 2.0	0
Aspergillus flavus	0	0	0	13 ± 0.58	0
Penicillium casei	17 ± 2.1	0	0	16 ± 1.3	0
Control I (5% ethanol)	0	0	0	0	0
Control II (H_2O)	0	0	0	0	0

The nematocidic activity of the tested acids was evaluated against the potato rot nematode *D. destructor*. As shown in Table 2, after a 72-h treatment with acids, the number of inactive nematodes which lost their motility reached 72.0 % of the initial amount in the variant with SA, 46.0 % in the variant with ICA and 60.0% in the variant with KGA. After the transition into water, about 13 % of inactive nematodes restored their motility in the variant with SA or ICA. The highest number of nematodes that regained activity (37.6%) was observed in the variant with KGA. Thus, the lethality of nematodes under the effect of SA, ICA and KGA was 58.3, 33.0 and 22.4%, respectively. For comparison, the lethality of nematodes exposed to commercial acetic and lactic acids was 100% and 73%, respectively.

Table 2. Activity of acids produced by *Y. lipolytica* against nematode *D. destructor*

Parameters	% of the total number				
	SA	ICA	KGA	Acetic acid	Lactic acid
Active nematodes after a 72-h treatment	28.0 ± 2.5	54.0 ± 3.5	40.0 ± 2.5	0	26.7 ± 1.9
Inactive nematodes after a 72-h treatment	72.0 ± 5.3	46.0 ± 3.7	60.0 ± 4.4	100 ± 0	73.3 ± 1.9
Nematodes restoring motility after transfer into H_2O	13.7 ± 1.8	13.0 ± 1.6	**37.6 ± 2.4**	0	0
Lethality	**58.3**	33.0	22.4	100	73.3

CONCLUSION

The use of SA, ICA and KGA produced by *Y. lipolytica* for crop protection can be considered a promising avenue of research, as pests have not yet developed resistance to these acids. In addition, the acids produced by microorganisms are of higher purity than those synthesized chemically.

Conflict of Interest/ REFERENCES

The authors declare no conflicts of interest.

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