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THE EFFECT OF SURFACTANTS OF MICROBIAL ORIGIN ON PHYTOPATHOGENIC MICROORGANISMS

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Biodegradable non-toxic surfactants of microbial origin are multifunctional preparations, which due to antimicrobial activity are promising for use in crop production to control phytopathogenic microorganisms. Studies on the prospects of using microbial surfactants to control the number of phytopathogenic microorganisms are conducted in three directions: laboratory studies of antimicrobial activity of surfactants in vitro, determination of the effect of surfactants on phytopathogens in vegetative experiments in the process of plants growing in a laboratory or greenhouse, post-harvest treatment of fruits and vegetables with solutions of microbial surfactants to extend their shelf life. The review presents literature data on antimicrobial activity of surfactants against phytopathogenic bacteria and fungi in vitro. Antimicrobial activity of surfactants is evaluated by three main parameters: minimum inhibitory concentration, zones of growth retardation of test cultures on agar media and inhibition of growth of test cultures on agar or liquid media. The vast majority of available publications relate to the antifungal activity of surfactant lipopeptides and rhamnolipids, while data on the effect of these microbial surfactants on phytopathogenic bacteria (representatives of the genera Ralstonia, Xanthomonas, Pseudomonas, Agrobacterium, Pectobacterium) are few. The researchers determined the antimicrobial activity of either total lipopeptides extracted with organic solvents from the culture broth supernatant, or individual lipopeptides (iturin, surfactin, fengycin, etc.) isolated from a complex of surfactants, or culture broth supernatant. Lipopeptides synthesized by members of the genus Bacillus exhibit antimicrobial activity on phytopathogenic fungi of the genera Alternaria, Verticillium, Aspergillus, Aureobasidium, Botrytis, Rhizoctonia, Fusarium, Penicillium, Phytophora, Sclerotinia, Curvularia, Colletotrichum, etc. in sufficiently high concentrations. Thus, the minimum inhibitory concentrations of lipopeptides against phytopathogenic fungi are orders of magnitude higher (in average 0.04–8.0 mg/mL, or 40–8000 μg/mL) than against phytopathogenic bacteria (3–75 μg/mL). However, the antifungal activity of lipopeptide-containing supernatants is not inferior by the efficiency to the activity of lipopeptides isolated from them, and therefore, to control the number of phytopathogenic fungi in crop production, the use of lipopeptide-containing supernatants is more appropriate. Rhamnolipids synthesized by bacteria of the genus Pseudomonas are more effective antimicrobial agents comparing to lipopeptides: the minimum inhibitory concentrations of rhamnolipids against phytopathogenic fungi are 4–276 μg/mL, which is an order of magnitude lower than lipopeptides. In contrast to the data on the antifungal activity of rhamnolipids against phytopathogens, there are only a few reports in the literature on the effect of these surfactants on phytopathogenic bacteria, whilst the minimal inhibitory concentrations are quite high (up to 5000 μg/mL). The advantage of rhamnolipids as antimicrobial agents compared to lipopeptides is the high level of synthesis on cheap and available in large quantities industrial waste. Currently in the literature there is little information about the effect of surface-active sophorolipids of microbial origin on phytopathogenic fungi, and all these works are mainly about the antifungal activity of sophorolipids. We note that in contrast to surfactant lipopeptides and rhamnolipids, the effective concentration of most sophorolipids, which provides the highest antimicrobial activity against phytopathogens, is higher and reaches 10,000 μg/mL.

Keywords: antimicrobial activity, phytopathogenic fungi, phytopathogenic bacteria, lipopeptides, rhamnolipids, sophorolipids.

Microbial surface-active substances (surfactants) due to their unique physicochemical and biological properties can be used in various industries, such as food, medicine, oil, as well as for environmental cleaning [1−4]. In recent years, there have been reports of the use of surfactants of microbial origin in agriculture [4−6]. In the review [7], we noted that the role of microbial surfactants as plant protection products is due to their use for bioremediation of agricultural soils, pesticide production, control of phytopathogens, participation in plant-microbial interactions and plant growth stimulation.

Studies on the prospects of using microbial surfactants to control the number of phytopathogenic microorganisms are conducted in three directions. **The first direction** includes laboratory studies of antimicrobial activity of surfactants *in vitro* with the definition of such indicators as minimal inhibitory concentrations of surfactants, zones of growth retardation of test cultures on agar media and the degree of growth inhibition in the presence of microbial surfactants [8–11]. In **the second direction** studies, the antimicrobial activity of surfactants is analyzed, treating vegetative plants (or plant seeds) with their solutions in the process of their cultivation in a laboratory or greenhouse [12–15]. **The third direction** covers work related to post-harvest treatment of fruits and vegetables with solutions of microbial surfactants in order to extend their shelf life (the so-called post-harvest biocontrol of the number of phytopathogens) [10, 16–18].

The vast majority of available literature on the control of the phytopathogens number in the presence of microbial surfactants are complex, as they include studies of antimicrobial activity of surfactants against phytopathogens both *in vitro* and *in vivo* [8, 10, 12–15].

It should be noted that literature reviews on this topic, which have appeared in recent years, usually include publications that belong to all three areas of research [6, 7, 19–22]. Besides, such reviews consider, in addition to controlling the number of phytopathogens, other aspects of the impact of microbial surfactants on plant development (participation in the induced plant resistance system; inhibition of zoospores of plant pathogens resistant to commercial chemical pesticides; participation in the formation of biofilms of microorganisms that phytopathogen antagonists, etc.) [6, 7, 19−23].

The **purpose** of this review is to analyze and summarize the literature data related to the study of antimicrobial activity against phytopathogenic bacteria and fungi *in vitro*.

The need to systematize such data is due to the fact that the researcher who studies microbial surfactants and their biological activity, in order to find the necessary information is not always easy to navigate in the works intended for specialists in crop production, in particular, plant-microbial interaction.

The available literature contains information on the action of surface-active lipopeptides [6, 8−11, 13, 15, 19, 21−23], rhamnolipids [5, 6, 12, 14, 23−25] and sophorolipids on phytopathogenic microorganisms [5 , 26−28].

Lipopeptides for controlling the number of phytopathogens

The first reports of antimicrobial activity of surfactant lipopeptides of microbial origin against phytopathogens appeared in the 80s of the twentieth century [29, 30]. Thus, in the work [29] it was found that lipopeptides synthesized by *Bacillus subtilis* B-3 were active against a wide range of phytopathogenic fungi. When tested for activity against *Monilinia fructicola* on peach fruits, these surfactants at a concentration of 1 mg/mL caused complete suppression of brown rot. The minimum inhibitory concentrations (MIC) of fengycin synthesized by *B. subtilis* F-29-3 against phytopathogenic fungi were (μg/mL): *Pyricularia oryzae* − 1.0; *Conidiobolus coronatus* − 3.16; *Curvularia lunata* − 3.16; *Fusarium* sp. − 10; *Rhizomucor miehei* − 10; *Alternaria kikuchiana* − 10; *Rhizoctonia solani* − 3.16 [30].

In the 90s of the twentieth century, several other publications appeared on the antifungal activity of lipopeptides against phytopathogens synthesized by different strains of *B. subtilis*: NB22 [31], S499 [32], B-3 [33]. The turning point in these studies was the 2000s, which were marked by the appearance of a large number of articles on the action of surfactant lipopeptides on phytopathogenic microorganisms. Such information is very briefly given in a number of general reviews on lipopeptides of microbial origin published in 2008 [34], 2010 [35] and 2015 [36, 37].

In this review, we present data that were not included in the reviews [34–37], as well as information from recent years on the antimicrobial activity of lipopeptides against phytopathogens.

In [38–51], the authors evaluated the antifungal activity of surfactant-containing supernatants. Such studies are promising from a practical point of view, as the use of the supernatant to control the number of phytopathogens makes it possible to exclude from the technological process a rather expensive stage of isolation and purification of the target product. The effect of supernatants on phytopathogenic fungi was analyzed by diffusion into agar, determining either the degree of inhibition of fungal growth (in percent), or the size of the zones of growth retardation (in mm) (Table 1).

The data given in Table 1 show that under the effect of lipopeptide-containing supernatants, the degree of inhibition of various phytopathogenic fungi was in the range of 40–100 %, and the zones of growth retardation of fungi ranged from 1 to 29 mm. However, it should be noted that most of these studies did not determine the content of lipopeptide surfactants in supernatants, so it is not possible to assess their effectiveness as antifungal agents, as well as to compare with other known microbial surfactants.

In [8−11, 44, 45, 47, 51−62] the antifungal activity of lipopeptides isolated from the supernatant and/or purified was investigated (Table 2). Some authors analyzed the effect on phytopathogenic fungi of total lipopeptides extracted with organic solvents from the supernatant of the culture broth [8, 10, 44, 45, 47, 53−58, 60], some – individual lipopeptides isolated from a complex of surfactants

Table 1

Antimicrobial activity of lipopeptide-containing supernatants against phytopathogenic fungi

Lipopeptide producer (amount of supernatant)	Phytopathogenic fungi	Antimicrobial activity		Literature
		growth inhibition, %	growth retardation zone, mm	
Bacillus subtilis M4 (50 μL/well)	*Pythium ultimum*		1−4	[39]
	Fusarium oxysporum, Rhizoctonia solani, Rhizopus sp., *Botrytis cinerea*		5−9	
Bacillus subtilis BBG100 (200 μL/well)	*Fusarium oxysporum, Pythium aphanidermatum*		8−9	[40]
	Botrytis cinerea		≥10	
Bacillus amyloliquefaciens LBM 5006 (10 μL/disk)	*Aspergillus niger* ATCC 16404		10.5	[44]
	Aspergillus phoencis		0	
	Aspergillus flavus		13.5	
	Apiosordaria sp.		13.5	
	Bipolaris sorokiniana		13.5	
	Cercosporina sojina		21	
	Diplodia sp.		15.5	
	Fusarium oxysporum f. licopersici		0	
	Fusarium graminearum		0	
Bacillus subtilis SSE4 (in the presence of 30% of supernatant in agar medium)	*Colletotrichum gloeosporioides* DOAC1690	100		[43]
	Sclerotium rolfsii DOAC 1521	92.6		
Bacillus subtilis CPA-8 (100 μL/well)	*Monilinia fructicola* CPMC1	82.1		[45]
	Monilinia laxa CPML1	90		
Bacillus subtilis ABS-S14 (45 μL/cuvette)	*Penicilium digitatum*	90.9		[47]
Bacillus sp. PPM3 (in the presence of 2 % supernatant in agar medium)	*Mucor* sp.	97.5		[48]
	Aspergillus flavus	82.7		
	Fusarium graminearum	57.1		
	Alternaria sp.	41.5		
Bacillus velezensis NWUMFkBS10.5 (60 μL/disk)	*Fusarium graminearum*		29	[49]
	Fusarium culmorum		24	
Bacillus megaterium WL-3 (100 μL/disk)	*Phytophthora infestans*	73.3	9.5	[51]

[9, 11, 51, 52, 59, 61, 62]. In the vast majority of studies indicators such as the degree of inhibition or growth retardation zones of fungi were used as a criterion for antifungal activity, and only in some studies the MIC was determined [10, 54–57, 60, 61]. Of particular note is the work [55], in which the authors analyzed the MIC of lipopeptides depending on the nature of the carbon source in the culture medium of the producer. It was found that strain AR2 synthesizes a mixture of homologues of iturin, fengycin and surfactin, and under conditions of growth on a medium with sucrose, glycerin, sorbitol and maltose, the same dominant fraction of the synthesized lipopeptide complex was C15 surfactin. However, the most active antifungal agents were lipopeptides synthesized on sucrose.

The data given in Table 2 show that lipopeptides of microbial origin have antimicrobial effect on phytopathogenic fungi in sufficiently high concentrations. Thus, the MIC of these products of microbial synthesis in relation to the genera *Fusarium, Botrytis, Rhizoctonia, Monilinia, Aspergillus, Verticillium* and others are 0.1 to 20 mg/mL (see Table 2), while the minimum inhibitory concentrations for pathogenic fungi are lower (4−32 μg/mL) [36]. In addition, the antimicrobial activity of lipopeptide-containing supernatants (see Table 1) is not inferior to the effectiveness of antimicrobial activity of lipopeptides isolated from them (see Table 2), and therefore, to control the number of phytopathogenic fungi in crop production, it is more expedient to use surfactant-containing supernatants.

The effect of lipopeptides on phytopathogenic bacteria

One of the first reports of antimicrobial activity of lipopeptides of microbial origin against phytopathogenic bacteria dates back to 1990, when Phae et al. [31] reported the ability of the lipopeptide-containing *B. subtilis* NB22 supernatant and the five components of iturine isolated from it to inhibit the growth of *Xanthomonas oryzae* (the causative agent of bacterial burn of rice) and *Pseudomonas lachrymans* (the causative agent of angular spotting of cucumber leaves). Later studies by various authors conducted during 2004–2011 [63−65] also showed that the main component of lipopeptides responsible for antibacterial activity against phytopathogens is iturine. In addition to iturin, the antimicrobial effect on phytopathogenic bacteria was caused by the lipopeptides bacillomycin [65] and locillomycin [66]. In 2020, Medeot et al. [69] reported that *B. amyloliquefaciens* MEP218 synthesizes fengycin with an antibacterial activity against *Xanthomonas axonopodis* pv. *vesicatoria* that distinguishes it from other fengycins, which are characterized mainly by antifungal action.

Generalized information about the antibacterial activity of lipopeptides [8, 31, 43, 63−70] is given in Table 3. The Table 3 does not include information on lipopeptides of *B. velezensis* 9D-6 [71], which inhibited the growth of phytopathogenic bacteria *Ralstonia solanacearum, Xanthomonas campestris* and *Xanthomonas euvesicatoria*, because in this work there are no indicators of antimicrobial activity of surfactants.

Note that in the literature there is much less information about the effect of surfactant lipopeptides on phytopathogenic bacteria compared to their antifungal activity. In addition, the minimum inhibitory concentrations of lipopeptides against phytopathogenic fungi are orders of magnitude higher (on average 0.04−8 mg/mL, or 40−8000 μg/mL) (see Table 2) than for phytopathogenic bacteria (3−75 μg/mL) (Table 3). In our review [72] on the antimicrobial activity of surfactants, we focused on the fact that lipopeptides are characterized by high antibacterial activity also against pathogens of human infectious diseases (MIC 1−32 μg/mL).

In our opinion, the different antimicrobial activity of lipopeptides against bacteria and fungi may be due to the following reasons. First, lipopeptides, like other secondary metabolites, are synthesized as a complex of similar compounds (most often iturin, surfactin and fengycin, which are characterized by different lengths of the acyl chain). Secondly, in the review [73] we noted that antifungal activity is inherent in lipopeptides with a longer (C16–C18) acyl chain, and lipopeptides with fewer carbon atoms (C7–C14) in the fatty acid residue are characterized by antibacterial activity. Third, the antimicrobial activity of lipopeptides also depends on the composition and configuration of the acyl chain, the presence of certain substituents. Fourth, in recent years, information has begun to appear in the literature that the chemical composition of synthesized lipopeptides, and hence their biological activity, depends on the cultivation conditions of the producer [73]. Therefore, the antimicrobial activity of the lipopeptide complex is determined by the ratio in its composition of certain fractions with different lengths and configurations of the acyl chain.

Table 2

The effect of lipopeptides on phytopathogenic fungi

Producer of lipopeptides	Phytopathogenic fungi	The concentration of lipopeptides	Antimicrobial activity			Literature
			Growth inhibition, %	MIC, mg/mL	Growth retardation zone, mm	
Pseudomonas fluorescens BRG100	*Rhizoctonia solani* AG 2-1	Pseudofomin 1, 0.5 mg/mL	19			[52]
		Pseudofomin 2, 0.5 mg/mL	18			
	Alternaria brassicae AB 11-1	Pseudofomin 1, 0.5 mg/mL	23			
		Pseudofomin 2, 0,5 mg/mL	51			
	Phoma lingam BJ 125	Pseudofomin 1, 0.5 mg/mL	31			
		Pseudofomin 2, 0.5 mg/mL	65			
	Sclerotinia sclerotiorum	Pseudofomin 1, 0,5 mg/mL	13			
		Pseudofomin 2, 0.5 mg/mL	60			
Bacillus subtilis BS119m	*Aspergillus flavus*	80 μg/mL	94			[53]
Bacillus amyloliquefaciens LBM 5006	*Aspergillus niger* ATCC 16404	10 μL/disk			10	[44]
	Aspergillus phoencis	«–»			16.5	
	Aspergillus flavus	«–»			14	
	Apiosordaria sp.	«–»			13	
	Bipolaris sorokiniana	«–»			15	
	Cercosporina sojina	«–»			17.5	
	Diplodia sp.	«–»			15	
	Fusarium oxysporum f. licopersici	«–»			15.5	
	Fusarium graminearum	«–»			12.5	
Bacillus subtilis CPA-8	*Monilinia fructicola* CPMC1	Butanol extract, 100 μL/well	93.4			[45]
	Monilinia laxa CPML1	«–»	93.4			
Bacillus sp. SS-12.6	*Colletotrichum acutatum*	0.5 mL of ethyl acetate extract/Petri dish	100			[8]
	Colletotrichum gloeosporioides	«–»	100			
	Monilinia fructigena	«–»	91.5			
	Botryosphaeria obtusa	«–»	81.6			
	Fusarium oxysporum	«–»	67.1			
	Penicillium expansum	«–»	77.1			
	Aspergillus flavus	«–»	74.8			
Bacillus subtilis fmbJ	*Aspergillus flavus*	Bacillomycin D, 200 mg/mL	85.7			[9]

Producer of lipopeptides	Phytopathogenic fungi	The concentration of lipopeptides	Antimicrobial activity			Literature
			Growth inhibition, %	MIC, mg/mL	Growth retardation zone, mm	
Bacillus subtilis 109GGC020	*Rhizoctonia solani*	Gageostatin A		4 μg/mL		[54]
		Gageostatin B		8 μg/mL		
		Gageostatin C	32 μg/mL			
Bacillus sp. AR2	*Alternaria alternata* MTCC 2724			0.5–0.75*		[55]
	Alternaria citri MTCC 4875			0.5–0.75*		
Bacillus subtilis ABS-S14	*Penicilium digitatum*	10 mg/mL	94.1			[47]
Bacillus subtilis SPB1	*Fusarium solani*			3		[56]
Bacillus subtilis SPB1	*Rhizoctonia bataticola,*			0.04		[57]
	Rhizoctonia solani			4		
Bacillus sp. XT1	*Botrytis cinerea*	10 mg/mL	72	8		[10]
Bacillus subtilis BS155	*Magnaporthe grisea*	40 μg/mL	33.8			[58]
Bacillus sp. CS30	*Magnaporthe grisea*	Surfactin CS30-1, 25 μg/mL	40			[59]
		Surfactin CS30-2, 25 μg/mL	50			
Bacillus sp. wsm-1	*Magnaporthe grisea*	C14 Iturin W, 20 μg/mL	100			[11]
		C15 Iturin W, 8 μg/mL	100			
Pseudomonas sp. COR52	*Pythium myriotylum* CMR1	Pseudodesmin, 50 μM	43			[62]
	Rhizoctonia solani AG2-2 CuHav-Rs18	Pseudodesmin, 50 μM	30			
Pseudomonas sp. A2W4.9	*Rhizoctonia solani* AG2-2 CuHav-Rs18	Viscosinamide, 10 μM	20			[62]
Bacillus megaterium WL-3	*Phytophthora infestans*	Surfactin, 25 mg/mL			0	[51]
	Phytophthora infestans	Iturin A, 25 mg/mL			6.1	
	Phytophthora infestans	Fengycin A, 25 mg/mL			8.1	
Bacillus subtilis 109GGC020	*Magnaporthe oryzae Triticum*	Gageopeptide A, Gageopeptide B, Gageopeptide C, Gageopeptide D, Gageopeptide B		2–10 μg/disk		[61]
Bacillus velezensis XT1	*Verticillium dahliae* V024			20		[60]

* different MICs depending on the nature of the carbon source in the culture medium.

Table 3

Antimicrobial activity of lipopeptides against phytopathogenic bacteria

Producer of lipopeptides	Phytopathogenic bacteria	The concentration of lipopeptides	Antimicrobial activity		
			MIC, μg/mL	Growth retardation zone, mm	Literature
Bacillus subtilis NB22	*Xanthomonas oryzae*	Surfactant-containing supernatant		25	[31]
		Components of iturin (peaks 1–5)	3.13–12.5		
	Pseudomonas lachrymans	Surfactant-containing supernatant		15	
		Components of iturin (peaks 1–5)	3.13–12.5		
Bacillus subtilis 6051	*Pseudomonas syringae* pv. *tomato* DC3000	Iturin	25		[63]
Bacillus subtilis OG	*Xanthomonas axonopodis* pv. *citri*	Total lipopeptides	14 mg/mL		[64]
		Iturin	10 μM/L		
	Xanthomonas campestris pv. *campestris*	Total lipopeptides	68		
		Iturin	50 μM/L		
Bacillus subtilis SSE4	*Agrobacterium tumefaciens* NTL4	Ethyl acetate extract, 10 μL/disk		16	[43]
	Xanthomonas campestris	Ethyl acetate extract, 10 μL/disk		30	
Bacillus subtilis UMAF6614	*Xanthomonas campestris* pv. *cucurbitae* NCPPB2597	Surfactant-containing supernatant		37	[65]
		Bacillomycin, 1 mg/mL		22	
		Fengycin, 1 mg/mL		–	
		Surfactin, 1 mg/mL		–	
	Pectobacterium carotovorum subsp. *carotovorum* NCPPB2349	Surfactant-containing supernatant		8	
		Bacillomycin, 1 mg/mL		12	
		Fengycin, 1 mg/mL		–	
		Surfactin, 1 mg/mL		–	

Producer of lipopeptides	Phytopathogenic bacteria	The concentration of lipopeptides	Antimicrobial activity		
			MIC, μg/mL	Growth retardation zone, mm	Literature
Bacillus subtilis UMAF6639	*Xanthomonas campestris* pv. *cucurbitae* NCPPB2597	Surfactant-containing supernatant		38	[65]
		Fengycin, 1 mg/mL		–	
		Iturin A, 1 mg/mL		45	
		Surfactin, 1 mg/ml		–	
	Pectobacterium carotovorum subsp. *carotovorum* NCPPB2349	Surfactant-containing supernatant		9	
		Fengycin, 1 mg/mL		–	
		Iturin A, 1 mg/mL		15	
		Surfactin, 1 mg/mL		–	
Bacillus sp. SS-12.6	*Xanthomonas arboricola* CFBP 2528	Ethyl acetate extract, 50 μL/well		10	[8]
	Xanthomonas arboricola IZB 313	«–»		12	
	Pectobacterium carotovorum IZB K5	«–»		4	
Bacillus subtilis 916	*Xanthomonas oryzae* pv. *oryzae*	Locillomycin A	6.3		[66]
		Locillomycin B	5.8		
		Locillomycin C	5.4		
Bacillus velezensis Y6	*Ralstonia solanacearum* GMI1000	Methanol extract, 5 μL/well	75	10	[67]
Bacillus velezensis F7	*Ralstonia solanacearum* GMI1000	Methanol extract, 5 μL/well	75	8	[67]
Bacillus amyloliquefaciens 32a	*Agrobacterium tumefaciens* C58	Total lipopeptides	2		[68]
	Agrobacterium tumefaciens B6	Total lipopeptides	4		
Bacillus amyloliquefaciens MEP218	*Xanthomonas axonopodis* pv. *vesicatoria*	Fengycin	25		[69]
Bacillus velezensis FJAT-46737	*Ralstonia solanacearum* FJAT-91	Total lipopeptides, 10 mg/mL		18.5	[70]
	Ralstonia solanacearum FJAT-77	Total lipopeptides, 10 mg/mL		14.6	

Antimicrobial activity of rhamnolipids

The first reports of the effect of microbial rhamnolipids on phytopathogenic microorganisms appeared in the 80–90s of the twentieth century [74, 75]. In [74] it was found that glycolipids (including rhamnolipids) of microbial origin inhibited the germination of conidia of the fungus *Glomerella cingulata* (the causative agent of anthracnose in various crops such as cereals and herbs, legumes, fruits, vegetables, perennial crops and trees). In 1997, Stanghellini and Miller [75] tested purified mono- and diramnolipids with a concentration of 5 to 30 μg/mL for zoosporicidal activity against three phytopathogens: *Pythium aphanidermatum, Phytophthora capsici, Plasmopara lactucaeradicis.* Lysosis of zoospores was observed under influence of rhamnolipids for less than 1 min.

Generalized data published in articles 2001–2020 on the antimicrobial activity of rhamnolipids against phytopathogenic fungi are given in Table 4. These data indicate no correlation between the concentration of rhamnolipids synthesized by different producers and the degree of inhibition of growth of phytopathogenic fungi. In our opinion, this can be explained as follows. First, it is known from the literature [72, 73] that the antimicrobial activity of surfactants of microbial origin depends on the type of test culture. Second, rhamnolipids are synthesized in the form of a complex of similar compounds, in particular, mono- and diramnolipids with different lengths of the acyl chain, and the antimicrobial activity of the target product depends on the ratio of these chains [73]. The data presented in the works [24, 88, 90] support the second assumption. Thus, Rodrigues et al. [88] found that rhamnolipid-containing supernatant of *P. aeruginosa* #112 and a solution of extracted from it total rhamnolipids (surfactant concentration 3 g/L) caused inhibition of growth of *Aspergillus niger* MUM 92.13 by 75.5 and 28.6 %, respectively, and isolated from the mixture mono- and diramnolipids with a concentration of 1.5 g/L – by 46.2 and 40 %, respectively. Monnier et al. [90] showed that the inhibition of growth of *Leptosphaeria maculans* under the effect of 0.5 g/L of semi-purified commercial rhamnolipids was 60 % (if the preparation contained 66 and 34 % of mono- and rhamnolipids) and 73 % in the presence of 41 and 59 % mono- and diramnolipids, respectively.

In [24], it was found that the MIC of rhamnolipids (ready-made preparations synthesized by *Pseudomonas aeruginosa* were used) for *Zymoseptoria tritici* were higher than 1500 μM. At the same time, some chemically synthesized derivatives of rhamnolipids containing 12 carbon atoms in the acyl chain were characterized by higher antimicrobial activity (133-450 μM) relative to this test culture.

The data given in Table 4 show that rhamnolipids with antifungal properties can be obtained from cheap and available in large quantities industrial waste [76−78, 83, 88]. In the review [72], we noted that in the literature there is little information about the antimicrobial activity of surfactants synthesized on such substrates.

In contrast to the data on the antifungal activity of rhamnolipids against phytopathogens, information on the effect of these surfactants on phytopathogenic bacteria is quite limited. Thus, in 2000 [91] it was found that the MIC of rhamnolipids synthesized by *P. aeruginosa* B5 against phytopathogens *Erwinia carotovora* pv. *carotovora*, *Ralstonia solanacearum* and *Xanthomonas campestris* pv. *vesicatoria*, which affect most of the agricultural plants, were >50 μg/mL. In 2012, Sanchez et al. [12] showed that the cultivation of *Pseudomonas syringae* pv. *tomato* DC3000 and *Pseudomonas syringae* pv. *tomato* AvrRPM1 in the presence of 0.2 and 1 mg/mL rhamnolipids did not inhibit the growth of phytopathogenic bacteria. In 2016, Leite et al. [92] found that under the action of 15 μL of supernatant (rhamnolipid concentration 0.57 g/L) obtained after culturing *P. aeruginosa* P1R16 on olive oil, the growth retardation zone of *Ralstonia solanacearum* 1226 was 22 mm. Finally, in 2020 [25] it was shown that the MIC of diramnolipid for *Xanthomonas campestris,* synthesized by *P. aeruginosa* RTE4 on glucose (2 %) was 5 mg/mL.

However, there is enough information in the literature about the antimicrobial activity of rhamnolipids against pathogenic for humans bacteria [23, 28, 72, 93]. Note that the MIC of rhamnolipids against human pathogens are orders of magnitude lower (50–500 μg/mL) than for phytopathogenic bacteria (above 5000 μg/mL). Nevertheless, the conclusion about the antimicrobial activity of rhamnolipids against phytopathogens is made only on the basis of several works available in the literature.

Effect of sophorolipids on phytopathogenic microorganisms

The first reports of antimicrobial activity of the surface-active sophorolipids on phytopathogenic fungi appeared in the early 2000s, when Kim et al. [94] established the ability of these surfactants at a concentration of 300 mg/L to inhibit the growth

Table 4

The effect of rhamnolipids on phytopathogenic fungi

Producer	Carbon source in the culture medium, concentration	Test culture	Antimicrobial activity			Lite-rature
			Surfactant concentra-tion, μg/mL	Growth inhibition, %	MIC, μg/mL	
Pseudomonas aeruginosa AT10	Soybean oil refinery wastes, 5 % (volume fraction)	*Botrytis cinerea*			18	[76]
		Rhizoctonia solani			18	
Pseudomonas aeruginosa 47T2 NCBIM 40044	A mixture of waste olive and sunflower oil in a ratio of 1:1, 40 g/L	*Botrytis cinerea*			170	[77]
		Colletotrichum gloeosporioides			276	
		Rhizoctonia solani			109	
		Fusarium solani			75	
		Penicillium funiculosum CECE 2914			16	
Pseudomonas aeruginosa LBI	Soapstock, 2 %	*Alternaria alternata*			4	[78]
		Penicilium funiculosum CECT 2914			32	
Pseudomonas aeruginosa PNA1	Not specified	*Pythium myriotylum* CMR1	2.5	65.2		[79]
Pseudomonas aeruginosa (strain not specified)	Not specified	*Botrytis cinerea* T4	100	60		[80]
Pseudomonas aeruginosa ZJU211	Rapeseed oil (6 %, volume fraction)	*Fusarium graminearum*	30	11.9		[81]
		Mucor circinelloides	«–»	31.4		
		Mucor spp	«–»	41.7		
		Botrytis cinerea	«–»	42.2		
		Fusarium oxysporum	«–»	77.6		
Pseudomonas aeruginosa DS9	Glucose, 2 %	*Fusarium sacchari*	2000	63		[82]
Serratia rubidaea SNAU02	Maduka oil meal	*Fusarium oxysporum*	500	31 mm		[83]
		Colletotrichum gloeosporioides	500	24 mm		
Pseudomonas aeruginosa (strain not specified)	Soybean oil	*Phytophthora sojae*	100	30		[84]
Pseudomonas aeruginosa DS9	Glucose, 4.5 %	*Colletotrichum falcatum* Went (ITCC 4803)	100	86.6		[85]
Pseudomonas aeruginosa ZJU211	Not specified	*Alternaria alternata*	250	40.2		[86]

Producer	Carbon source in the culture medium, concentration	Test culture	Antimicrobial activity			Literature
			Surfactant concentra-tion, μg/mL	Growth inhibition, %	MIC, μg/mL	
Pseudomonas aeruginosa SS14	Glucose, 2 %	*Fusarium verticillioides* FS7	200	82.2		[14]
Pseudomonas aeruginosa ZJU211 (CCTCC M209237)	Rapeseed oil (6 %, volume fraction)	*Verticillium dahliae* ATCC 7611	60	73		[87]
Pseudomonas aeruginosa #112	Molasses of sugar cane	*Aspergillus niger* MUM 92.13	3000	28.6		[88]
		Aspergillus carbonarius MUM 05.18	3000	22.6		
Pseudomonas aeruginosa 41B	Not specified	*Botrytis cinerea* B630	60	54		[89]
Pseudomonas aeruginosa (strain not specified)	Not specified	*Leptosphaeria maculans*	500	73		[90]
Pseudomonas aeruginosa (strain not specified)	Not specified	*Zymoseptoria tritici*			>1500 μM	[24]
Pseudomonas aeruginosa RTE4	Glucose, 2 %	*Fusarium solani*			10000	[25]
		Corticium invisium			20000	

of *B. cinerea* KCTC 6973 by 57 %. To obtain sophorolipids, the producer *Candida bombicola* ATCC 22214 was grown in glucose medium (100 g/L).

Note that at present there is little information in the literature about the effect of sophorolipids on phytopathogenic microorganisms [26, 27, 95–97]. Moreover, all these works are exclusively about the antifungal activity of sophorolipids, and only one article [97] reported their ability to inhibit the growth of phytopathogenic bacteria.

Yoo et al. [95] found that sophorolipids synthesized by *C. bombicola* ATCC 22214 in glucose medium (100 g/L) at a concentration of 100 mg/L inhibited the growth of *Pythium ultimum* KACC 40705 by 90 %, and at a concentration of 500 mg/L inhibited by 80 % the growth of another member of the genus *Pythium* (*P. aphanidermatum* KACC 40156), as well as members of the genus *Phytophthora* (*P. capsici*, *P. nicotianae* KACC 40906, *P. infestans* KACC 40718).

The efficacy of sophorolipids synthesized by *Wickerhamiella domercqiae* Y2A in medium with glucose (80 g/L) and rapeseed oil (80 g/L) against nine strains of phytopathogenic fungi of the genera *Penicillium, Aspergillus, Botrytis* and *Mucor*, which cause mold on apples, pears, oranges, peaches and dates. Thus, at a concentration of sophorolipids 1 g/L inhibition of growth of these fungi was (%): 60−75, under action of 3 g/L – 82−91, and 10 g/L – 96−100.

Schofield et al. [97] reported that sophorolipid derivatives (with different degrees of lactonization and acetylation, as well as different acyl chain lengths) and combinations of sophorolipid derivatives showed antifungal activity against 18 fungal pathogens (*Alternaria tomatophilia*, *Alternaria solani*, *Alternaria alternata*, *Aspergillus niger*, *Aureobasidium pullulans*, *B. cinerea*, *Chaetomium globosum*, *Fusarium asiaticum*, *Fusarium austroamericana*, *Fusarium cerealis*, *Fusarium graminearum*, *Fusarium oxysporum*, *Penicillium chrysogenum*, *Penicillium digitatum*, *Penicillium funiculosum*, *P. infestans*, *P. capsici, Ustilago maydis*) and seven bacterial phytopathogens (*Acidovorax carotovorum*, *Erwinia amylovora*, *Pseudomonas cichorii*, *Pseudomonas syringae*, *Pectobacterium carotovorum*, *Ralstonia solanacearum* and *Xanthomonas campestris*). MIC ranged from 2.5 to 10 mg/mL for individual sophorolipid derivatives and from 0.009 to 10 mg/mL for combinations of these surfactants.

In 2017 [26], antifungal activity of sophorolipids synthesized by *Rhodotorula babjevae* YS3 on glucose medium (10 g/L) was reported. The MIC for *Colletotrichum gloeosporioides* ITCC 6434 was 62 μg/mL, *Fusarium verticilliodes* MTCC 10556, *Fusarium oxysporum f.* sp. *pisi* ITCC 4814 – 125 μg/mL, while for *Corynespora cassiicola* ITCC 6748 MIC was significantly higher (> 2000 μg/mL).

Chen et al. [27] found that sophorolipids synthesized by *Wickerhamiella domercqiae* Y_{2A} in glucose medium (8 %) have antifungal activity against phytopathogenic fungi (Table 5). The data given in Table 5 indicate that the maximum level of growth inhibition of test cultures (68−98 %) was achieved at the highest of the studied surfactant concentrations (10 mg/mL).

It should be noted that, in contrast to surfactant lipopeptides and rhamnolipids (see Tables 2–4), the effective concentration of most sophorolipids, which provides the highest antimicrobial activity against phytopathogens, is higher and reaches several mg/mL.

Table 5

The effect of *Wickerhamiella domercqiae* Y_{2A} sophorolipids on phytopathogenic fungi [27]

Test culture	Inhibition of growth (%) at the concentration of sophorolipids (mg/mL)		
	0.5	3	10
Pyricularia oryzae AX1105	55.88	82.15	89.50
Rhizoctorzia solani	75.46	84.25	87.05
Pythium ultimum ACCC 36075	97.2	98.2	98.12
Fusarium oxysporum ACCC 36468	59.09	66.16	69.70
Fusarium concentricum	61.14	67.88	68.16
Fusarium sp. ACCC 36194	58.11	68.67	71.27
Phytophthora infestans	69.51	73.98	74.98
Gaeumannomyces graminis var. *Tritici*	50.83	68.01	73.06

* * *

Thus, the analysis of literature data showed that surfactants of microbial origin (lipopeptides, rhamnolipids and sophorolipids) cause antimicrobial action on phytopathogenic fungi and bacteria, and the vast majority of studies relate to the antifungal effect of these surfactants. However, the problem of today is the fight against bacteriosis of crops, which we have focused on in our previous works [7, 98].

Despite a sufficient number of studies on the effect of rhamnolipids on phytopathogenic fungi, published during 2001–2020 [14, 24, 25, 76−90], such studies are still less than those with lipopeptides. In our opinion, this is primarily due to the fact that in recent years lipopeptides have been actively studied as useful for plants products of metabolism of rhizospheric and endophytic bacteria of the genus *Bacillus* [7, 19−22, 50, 67, 71].

Nevertheless, rhamnolipids have a significant advantage as antimicrobial agents over lipopeptides because the MIC values of rhamnolipids relative to phytopathogenic fungi (4–276 μg/mL) are lower than lipopeptides (40–8000 μg/mL). In addition, rhamnolipids with antifungal properties can be obtained from cheap and available in large quantities industrial waste [76−78, 83, 88]. Note that the level of microbial synthesis of rhamnolipids (up to 40 g/L, [99]) is significantly higher than lipopeptides (usually not more than 1–3 g/L, [100]).

Much less (compared to rhamnolipids and lipopeptides) in the literature there are reports of the effect of sophorolipids on phytopathogenic microorganisms, in particular, only on their antifungal activity. One of the reasons for the low interest of researchers in sophorolipids as antimicrobial agents against phytopathogens is the rather high minimum inhibitory concentrations of these surfactants (up to 10,000 μg/mL).

ВПЛИВ ПОВЕРХНЕВО-АКТИВНИХ РЕЧОВИН МІКРОБНОГО ПОХОДЖЕННЯ НА ФІТОПАТОГЕННІ МІКРООРГАНІЗМИ

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Резюме

Біодеградабельні нетоксичні поверхнево-активні речовини мікробного походження є препаратами мультифункціонального призначення, які завдяки антимікробній активності є перспективними для використання у рослинництві для боротьби з фітопатогенними мікроорганізмами. Дослідження, присвячені перспективам використання мікробних ПАР для контролю чисельності фітопатогенних мікроорганізмів, проводяться у трьох напрямах: лабораторні дослідження антимікробної активності поверхнево-активних речовин *in vitro*, визначення впливу поверхзнево-активних речовин на фітопатогени у вегетаційних дослідах у процесі вирощування рослин в лабораторії чи теплиці, післяврожайна обробка фруктів та овочів розчинами мікробних ПАР з метою подовження терміну їх зберігання. В огляді наведено дані літератури про антимікробну щодо фітопатогенних бактерій і грибів активність поверхнево-активних речовин *in vitro.* Антимікробну активність поверхнево-активних речовин оцінюють за трьома основними показниками: мінімальна інгібуюча концентрація, зони затримки росту тест-культур на агаризованих середовищах і інгібування росту тест-культур на агаризованих або в рідких середовищах. Переважна більшість наявних публікацій стосуються антифунгальної активності поверхнево-активних ліпопептидів та рамноліпідів, у той час як дані про дію цих мікробних ПАР на фітопатогенні бактерії (представників родів *Ralstonia*, *Xanthomonas, Pseudomonas, Agrobacterium, Pectobacterium*) є небагаточисельними. Дослідники визначали антимікробну активність або сумарних ліпопептидів, екстрагованих із супернатанту культуральної рідини органічними розчинниками, або індивідуальних ліпопептидів (ітурин, сурфактин, фенгіцин та ін.), виділених з комплексу поверхнево-актив-

них речовин, або супернатанту культуральної рідини. Ліпопептиди, синтезовані представниками роду *Bacillus* проявляють антимікробну дію на фітопатогенні гриби родів *Alternaria*, *Verticillium, Aspergillus*, *Aureobasidium, Botrytis*, *Rhizoctonia, Fusarium*, *Penicillium*, *Phytophora, Sclerotinia, Curvularia, Colletotrichum* та ін. у достатньо високих концентраціях. Так, мінімальні інгібуючі концентрації ліпопептидів щодо фітопатогенних грибів є на порядки вищими (в середньому 0,04–8 мг/мл, або 40–8000 мкг/мл), ніж щодо фітопатогенних бактерій (3–75 мкг/мл). Разом з тим антифунгальна активність ліпопептидвмісних супернатантів не поступається за ефективністю активності виділених з них ліпопептидів, а отже, для контролю чисельності фітопатогенних грибів у рослинництві доцільнішим є використання саме ліпопептид-вмісних супернатантів. Рамноліпіди, синтезовані бактеріями роду *Pseudomonas*, є ефективнішими антимікробними агентами порівняно з ліпопептидами: мінімальні інгібуючі концентрації рамноліпідів щодо фітопатогенних грибів становлять 4–276 мкг/мл, що на порядок нижче, ніж ліпопептидів. На відміну від даних про антифунгальну щодо фітопатогенів активність рамноліпідів у літературі є всього кілька повідомлень про дію цих поверхнево-активних речовин на фітопатогенні бактерії, при цьому мінімальні інгібуючі концентрації є достатньо високими (до 5000 мкг/мл). Перевагою рамноліпідів як антимікробних агентів порівняно з ліпопептидами є високий рівень синтезу на дешевих і наявних у великих кількостях промислових відходах. На даний час у літературі є небагато інформації про дію поверхнево-активних софоліпідів мікробного походження на фітопатогенні гриби, причому у всіх цих роботах мова йде переважно про антифунгальну активність софоліпідів. Зазначимо, що на відміну від поверхнево-активних ліпопептидів і рамноліпідів ефективна концентрація більшості софоліпідів, що забезпечує найвищу антимікробну щодо фітопатогенів активність, є вищою і досягає10000 мкг/мл.

Ключові слова: антимікробна активність, фітопатогенні гриби, фітопатогенні бактерії, ліпопептиди, рамноліпіди, софоліпіди.

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