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Original article

ANTIMICROBIAL AND CYTOTOXIC CHARACTERISTICS OF ANTIBIOTIC STREPTOFUNGIN

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Background. Streptomycetes were and remain a source of new antimicrobial compounds of various nature. A new wave of interest in such research is associated with the possibilities of applied genomics to reveal the hidden biosynthetic potential of streptomycetes, and therefore the discovery of new practically valuable antimicrobial products. The problem of antibiotic resistance of pathogens can be solved by creating compositions of active substances of different nature to overcome the protective mechanisms of pathogens.
Objective. The purpose of the work was to establish and evaluate the antimicrobial and cytotoxic characteristics of the new antibiotic streptofungin, synthesized by *Streptomyces albus* 2435 (CMIM-S-668) and its mutant strains 2435/M, UN44, 4S, US101, AE6, 105, 80/5.
Methods. To establish the characteristics of streptofungin, the antagonistic activity of selected *S. albus* producer strains was determined (by the radial streak method), the minimum inhibitory concentration of the antibiotic (by the serial dilution method), and cytotoxicity was determined by the MTT test with resazurin.
Results. The activity of the antibiotic streptofungin against Candida fungi (*C. albicans*, *C. utilis*) is shown. Minimum inhibitory concentrations of streptofungin were determined for *C. albicans* ATCC 10231 (10 μg/ml), *B. subtilis* ATCC 6633 (200 μg/ml) and *P. aeruginosa* ATCC 9027 (500 μg/ml). According to the resazurin test, streptofungin does not show a cytotoxic effect in a wide range of concentrations from 2.5 to 500 μg/ml, and therefore can be considered potentially permissible for humans and animals in the studied concentrations.
Conclusions. The antagonistic activity of mutant strains of *S. albus* culture is due to the action of a complex of antimicrobial products that have a different antimicrobial spectrum and mechanism of action. The obtained results give reasons to consider streptofungin as a promising pharmaceutical substance with antifungal action, as well as to consider the possibility of its combination with the bacteriolytic enzyme complex of the same culture for the development of an antimicrobial agent with a wide spectrum of action.
Keywords: antimicrobial activity; cytotoxicity; antibiotics; pathogens; antimicrobial substances; streptomycetes.

Introduction

Over the past decades, antibiotics have remained among the main active pharmaceutical ingredients (APIs) used in the development of antimicrobial drugs. The relevance of the search for new antibiotics is noted by the World Health Organization as one of the components of the fight against resistant pathogens, despite ongoing discussions about the effectiveness of this approach in addressing the global problem of antibiotic resistance [1, 2].

Chemically synthesized and modified antibiotic substances occupy a significant place in the list of APIs, but microorganisms remain a natural "depository" where researchers turn to find producers of new antibiotics. The practical significance of such research is given by the latest opportunities of applied genomics to reveal the often hidden biosynthetic potential of microorganisms – the use of highly sensitive screening methods, genetic engineering of producers, the use of combinatorial biosynthesis, etc. [3–5].

Among known producers of antibiotics, actinobacteria have long been predominant, and the new possibilities mentioned above have renewed interest in them, primarily in streptomycetes [6, 7]. This was facilitated by the decoding of the complete genome of the typical species *Streptomyces albus* with the identification of tens of gene clusters of the secondary metabolites synthesis, as well as its use as a host for the heterologous expression of genes for the synthesis of a number of antibiotics (moenomycin, staphamycin, triocoralin, etc.) [8, 9].

The analysis of the mentioned information by the authors of the presented work in previous years caused a new look at the natural strain *Streptomyces albus* 2435 (formerly *Streptomyces recifensis var. lyticus*) and its mutants (2435/M, UN 44, etc.), which for a long time were studied only as producers of a complex of bacteriolytic enzymes active to varying degrees against such pathogens as *S. aureus*, *P. aeruginosa*, *E. coli*, *Proteus rettgeri*, etc. [10, 11]. The result of such consideration was the discovery of the ability of *Streptomyces albus* producer to synthesize a complex of antifungal and antibacterial antibiotics (derivatives of phthalaldehyde), which may be interesting as a potential API [12]. Additional interest in complex development using this producer is the possibility of simultaneously obtaining two products (enzymes and antibiotics) and combining them into a broad-spectrum antimicrobial agent. Such combination of APIs with different mechanisms of action is noted as an effective technique for increasing the effectiveness of antimicrobial drugs against resistant pathogens, and most importantly, for inhibiting the development of their antibiotic resistance in general [13, 14].

Therefore, the purpose of the work was to establish the antimicrobial and cytotoxic characteristics of the new antibiotic streptofungin, which is synthesized by the *Streptomyces albus* strains.

Materials and Methods

The selected strains of *Streptomyces albus* culture from the museum of the Department of Industrial biotechnology and biopharmacy, Igor Sikorsky Kyiv Polytechnic Institute, was used in the work: strain 2435 (CMIM S-668), strain 2435/M (IMV Ac-5001), strain UN44 (IMV Ac-5030), and strains 4S, US101, AE6, 105, 80/5 (work museum of the Department).

This culture synthesizes a complex of biologically active substances, which includes glycosidases, lytic endopeptidases, muramidases, non-lytic proteinases, amylases, as well as complex of antifungal and antibacterial antibiotics – streptofungin [11, 12].

To determine the antagonistic activity of *Streptomyces albus* producer strains and the minimum inhibitory concentration (MIC) of the obtained antibiotic used the test-strains from the museum of the antibiotic department of the Zabolotny Institute of Microbiology and Virology of the NAS of Ukraine: *Staphylococcus aureus* ATCC 6538, *Candida albicans* ATCC 10231, *Candida utilis* LIA-01, *Bacillus subtilis* ATCC 6633, *Proteus vulgaris* ATCC 6896, *Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC8739.

Method of antagonistic activity determination. On Petri dishes with Gause medium to the culture of *Streptomyces albus* (a circle with a diameter of 1-2 cm), test-strains were seeded radially with strokes, using their suspension at a concentration of 10^9 cells/ml in a physiological solution. The seeded cups were incubated for 24 hours at a temperature of 37 °C. After that, the growth inhibition zones of the test-strains were measured from the edge of the producer colony to the beginning of the growth of the test-strain.

Samples of antibiotic streptofungin were obtained according to the following scheme: cultivation of the producer was performed in 750 ml rolling flasks with 150 ml of nutrient medium based on glucose and soya flour, for 72 h at 28 ± 1 °C and stirring at 180 rpm [12]. Nutrient medium (g/l): glucose – 6.0; soybean flour – 8.0; NaCl – 14.0; $CaCl_2$ – 4.5; $MgSO_4Ч7H_2O$ – 5.8; $MnCl_2$ – 0.04; K_2HPO_4 – 1.5; H_2O – up to 1 l. After completion of the biosynthesis process, and antibiotic was extracted with chloroform in a ratio of 1:1, and the resulting extract was dried by the vacuum method.

Methodology for determining of the minimum inhibitory concentration (MIC). A series of antibiotic dilutions was made using its 1% (10 mg/ml) solution. A series of Petri dishes was prepared, in each of which 10 ml (total volume) of pre-melted and cooled to 40 °C MPA (Meet-peptone agar) medium was added, containing the appropriate amount of the antibiotic itself (serial dilution method). The following final antibiotic concentrations were obtained in Petri dishes: 1, 5, 10, 20, 50, 100, 200, 500 μg/ml. The cups were dried at room temperature for 2 hours and a suspension of test-cultures (concentration 5×10^8 cells/ml) was applied to the surface of the medium with a bacteriological loop. The sown cups were incubated in a thermostat for 24 hours at a temperature of 37 °C. MPA medium without antibiotic used as reference. The maximum dilution of the antibiotic where there is no growth of the test-strain is the MIC.

Antibiotic concentration was determined by the Bouguer–Lambert–Bere formula [15].

$$C = \frac{1}{E_{1\text{sm}}^{1\%} \cdot b} \cdot D \cdot P,$$

where C – antibiotic concentration, mg/ml; $E_{1\text{sm}}^{1\%}$ – 2.1 (extinction of a 1% alcohol solution of an antibiotic, the optical path length is 1 cm); b – thick-

ness of the substance layer in the cuvette (1 cm); D – optical density of the antibiotic solution at a $\lambda = 275$ nm; P – total dilution of the sample.

Method of determining the cytotoxicity of an antibiotic preparation [16]. Cells of the MDBK lines (Madin–Darby epithelial cells obtained from bull kidneys) and A549 (epithelial cells obtained from human lungs) were previously grown in a CO_2 incubator at 37 °C on a standard Minimum Essential Medium (MEM Eagle, Sigma-Aldrich) nutrient medium with growth factors in 96-well plates for a day to form a monolayer. The nutrient substrate was carefully deleted, and the cells were washed twice with phosphate-salt buffer (FSB, pH 7.2, Sigma #P4417) and left in 200 μl of FSB for introduction of the antibiotic sample.

The antibiotic solution was prepared in 96% ethanol at the rate of 10 mg/ml. From this solution, appropriate volumes were added to MDBK and A549 cells, so that the final concentration of it was 2.5–500 μg/ml. Cells treated in this way were kept in a CO_2 incubator for an hour. After that, the cells were repeatedly washed twice from the antibiotic in FSB and left in 200 μl of FSB for introduction of resazurin.

A stock solution of resazurin (sodium resazurinate 85.6%, pure, China) was prepared at the FSB at a rate of 0.15 mg/ml. The solution was filtered through a filter with a pore diameter of 0.2 μm and stored in an opaque bottle at 4 °C. Before use, the resazurin solution was warmed to room temperature and 20 μl was added for every 100 μl of FSB in the wells of the plate. The plate with cells and resazurin was placed in a CO_2 incubator for 2-3 hours. After that, the optical density of the solutions in the wells of the plate was determined at 538 nm using a plate photocolorimeter Multiskan FC Microplate Photometer (Thermo Scientific, USA).

The obtained data were processed statistically using the Statistica v.10 program (StatSoft Inc., USA). The reliability of the differences between the average values of the effectiveness indicators of different concentrations of the substance under study was established using the method of variance analysis in accordance with the *t*-criterion. The samples were compared using two methods, which differ in their mathematical approach and therefore complement each other: the LSD method (method of least significant differences) and the Tukey-HSD (method of true significant differences). Differences between mean values were considered significant at $p \leq 0.05$.

Results

At the previous stages of work with the *Streptomyces albus* culture a collection of mutants with increased and/or modified biosynthetic ability of the bacteriolysins synthesis was obtained by various methods from the original strain (2435) [17]. The revealed ability of the culture to produce antibiotics led to the urgency of additional study of the spectrum of the culture antimicrobial metabolites action in general and the properties of the actual new streptofungin antibiotic complex.

The results obtained at the first stage of the study of the antagonistic activity of *S. albus* against selected test-cultures (typical causative agents of inflammatory processes) indicate a similar spectrum of antimicrobial action, but differences in the productivity of different strains or the ratio of individual antimicrobial metabolites (Table 1).

All strains are antagonistic to *S. aureus* and the represented Candida species. The highest antagonism towards *S. aureus* was found by *S. albus* strains UN44, AE6 and 105, forming zones of growth inhibition from 11 to 15 mm. Regarding re-

Table 1: Antagonistic activity of *S. albus* strains

Strains of *S. albus*	Test-cultures				
	S. aureus ATCC 6538	*C. albicans* ATCC 10231	*C. utilis* LIA-01	*B. subtilis* ATCC 6633	*P. vulgaris* ATCC 6896
	Zone of growth inhibition, mm				
2435	6 ± 0.06	9 ± 0.1	16 ± 0.2	14 ± 0.2	0
2435/M	9 ± 0.2	15 ± 0.3	11 ± 0.07	7 ± 0.06	0
UN44	11 ± 0.2	23 ± 0.5	11 ± 0.1	0	0
4S	7 ± 0.1	14 ± 0.4	2 ± 0.03	3 ± 0.02	0
US101	10 ± 0.2	25 ± 0.9	4 ± 0.1	11 ± 0.04	0
AE6	15 ± 0.5	30 ± 1.2	20 ± 0.5	0	12 ± 0.3
105	14 ± 0.2	7 ± 0.07	9 ± 0.3	25 ± 0.9	0
80/5	10 ± 0.3	18 ± 0.4	13 ± 0.2	6 ± 0.09	0

presentatives of Candida, there is a noticeable difference in antagonistic activity not only between strains, but also a difference in the ability to delay the growth of different species. In general, high antifungal activity was found in most of the studied strains of *S.albus* (zones of growth inhibition of *C. albicans* 20–30 mm and *C. utilis* from 11–20 mm), among which strain AE6 is the most active.

The mutants with a high level of antagonism against *S. aureus* and the presented *Candida* species under these conditions did not show the ability to suppress *B. subtilis* (spore-forming bacteria), which was characteristic of the original strain 2435. And one of the mentioned mutants (strain AE6) turned out to be the only one that suppressed the growth of *P. vulgaris*.

At the next stage of the work, deep cultivation of the *S. albus* 2435 strain was carried out, extraction of the antibiotic from the culture liquid with chloroform (1:1) and a sample of the streptofungin preparation by vacuum drying was obtained for further analysis [12].

The impact of varying concentrations of streptofungin was assessed concerning to the spectrum of microbial test-cultures strains as recommended in the European Pharmacopoeia (https://pheur.edqm.eu/subhome/11-3), and the presented research results allow us to establish one of the defining characteristics of any antibiotic – the minimum inhibitory concentration (MIC) (Table 2).

Streptofungin was the most active against *C. albicans*, and at the concentration of 10 μg/ml in the medium, there was no growth of the test-strain. As for the spore culture of *B. subtilis*, a similar picture was observed on the medium with a streptofungin concentration of 200 μg/ml, and the growth of *P. aeruginosa* was absent at an antibiotic concentration of 500 μg/ml.

It is obvious that streptofungin can be attributed to antifungal antibiotics by its specificity, and its MIC against a typical strain of *C. albicans* indicates its high activity. Such pharmaceutical substances as antimycotics are considered highly active and promising for development if their MIC is 4–16 μg/ml [18].

Along with such an important characteristic for an antibiotic as MIC, another indicator that determines its practical prospects is toxicity for the human. Analysis of these two characteristics can provide an answer to the question of the potentiality of a certain substance as an API.

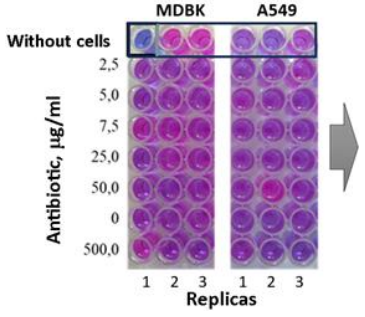
Therefore, at the next stage, the cytotoxicity of the antibiotic streptofungin was investigated on cell lines MDBK (Madin–Darby epithelial cells obtained from bull kidneys) and A549 (epithelial cells obtained from human lungs) using the resazurin test.

The interaction of cells with resazurin leads to a change in the color of the solution from the original (blue) to pink (Fig. 1), which indicates the presence of metabolic activity and corresponds to the concentration of living cells [16].

Table 2: Activity of streptofungin against test-cultures strains

Test-cultures strains	Antibiotic concentration, μg/ml					
	1	10	50	100	200	500
B. subtilis ATCC 6633	+	+	+	+	–	–
C. albicans ATCC 10231	+	–	–	–	–	–
E. coli ATCC 8739	+	+	+	+	+	+
P. aeruginosa ATCC 9027	+	+	+	+	+	–
S. aureus ATCC 6538	+	+	+	+	+	+

Notes. "+" – the presence of growth of the test strain; "–" – no growth of the test strain.



a

Cell lines		MDBK			A549		
Replicas		1	2	3	1	2	3
Without cells		0.529	0.901	0.899	0.616	0.611	0.718
Antibiotic, μg/ml	2.5	0.736	0.797	0.808	0.634	0.668	0.774
	5.0	0.811	0.794	0.741	0.695	0.651	0.663
	7.5	0.899	0.942	0.803	0.652	0.683	0.647
	25.0	0.773	0.842	0.759	0.626	0.706	0.662
	50.0	0.768	0.802	0.766	0.665	0.839	0.724
	0	0.815	0.796	0.782	0.615	0.628	0.705
	500.0	0.871	0.862	0.779	0.759	0.686	0.664

b

Figure 1: Color change of resazurin in the presence of MDBK and A549 cells pretreated with streptofungin in different concentrations (a) and corresponding optical density (b). The black frame and gray priming marks the wells and the corresponding optical indicator for the wells without cells and antibiotic

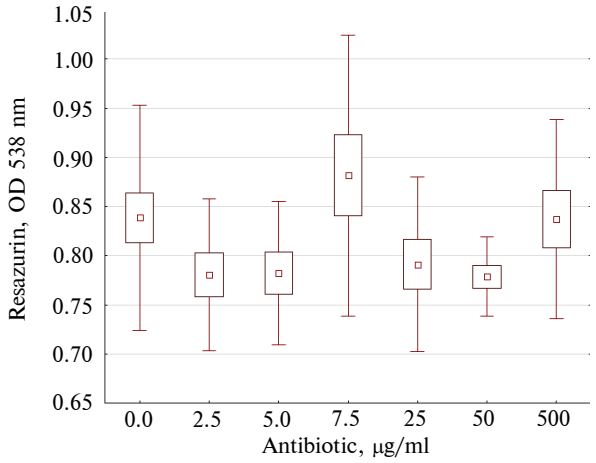


Figure 2: The effect of the antibiotic streptofungin on the viability index of Madin–Darby epithelial cells: □ – mean; ▯ – mean ± *SE*; ⌶ – mean ± 2*SD*

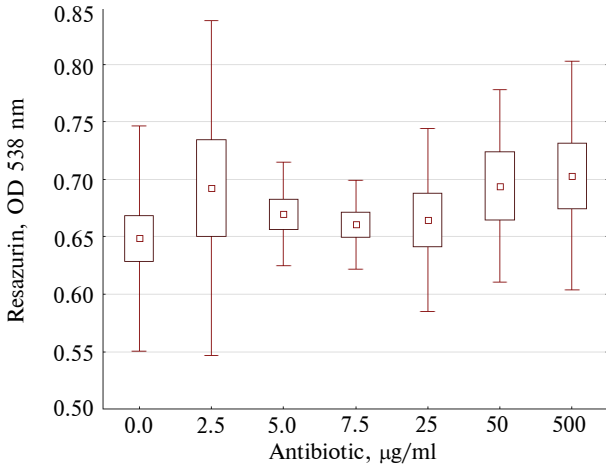


Figure 3: The effect of the antibiotic streptofungin on the viability index of A549 cells: □ – mean; ▯ – mean ± *SE*; ⌶ – mean ± 2*SD*

Taking into account the specific features of light filters installed on different devices, we analyzed the light absorption of all available light filters (405, 450, 492, 538, 620 nm) and determined that the highest optical absorption values were recorded at wavelengths of 538 and 620 nm, however, at the wavelength of 538 nm, the optical value increases linearly together with the metabolic activity of the cells, while at a wavelength of 620 nm these indicators are reversed.

According to statistical processing, the activity of MDBK cells (without antibiotic treatment) was 0.84 ± 0.06; the activity of A549 cells was slightly lower and amounted to 0.65 ± 0.05. Under the action of the antibiotic, the activity of MDBK cells mostly decreased (except of concentrations of 7.5 and 500 μg/ml) by 7-8% (Fig. 1), but this decrease was not of a significant ($p > 0.5$) (Fig. 2).

The data represent the corresponding results of the statistical analysis of the data for the detection of a significant difference between the average activity indicators of the cells without treatment with the antibiotic (0 μg/ml) and treated with antibiotics at different concentrations (2.5–500 μg/ml).

For A549 cells, treatment with the antibiotic had a weak stimulatory effect, as the average values increased by a maximum of 14% at a compound concentration of 50 μg/ml, but these changes were not of a reliable nature (Fig. 3).

Thus, according to the data of the resazurin test, the studied antibiotic in concentrations of 2.5–500 μg/ml did not show a cytotoxic effect, and therefore it can be considered a potentially permissible compound for humans and animals in the studied concentrations.

Discussion

The object of the presented research is the culture of the streptomycete *Streptomyces albus*, which has been studied for more than one decade in a numbers of scientific laboratories and used in development, primarily as a producer of a complex of bacteriolytic enzymes [11, 12, 17]. Subsequently, its ability to positively influence the growth of some agricultural plants, as well as inhibit the development of acarids, was shown [19]. All this, as well as the return of interest in streptomycetes in general among researchers over the last ten years [5–9, 20], caused the authors to turn to the analysis of the antibiotic activity of the culture. The result was the establishment of the culture's ability to synthesize a complex of antibiotics (called streptofungin), its nature and methods of isolation [12]. Interesting from a practical point of view is the possibility of simultaneous production of two different antimicrobial products of this producer – a complex of enzymes and a complex of antibiotics – in one producing cycle due to their different nature, localization, and therefore methods of isolation from the culture liquid.

Such approaches are modern and relevant, as shown, for example, using some antibiotic-producing streptomycetes, which simultaneously synthesize peptidase complexes. The main advantage of streptomycete peptidases is their thermal stability and a wide pH range of action. Peptidase preparations are obtained as a by-product from the culture liquid of *S. fradiae*, *S. griseus*, and *S. rimosus* during the biosynthesis of antibiotics [21].

Therefore, at the next stage of our work, the task was to determine the main characteristics of streptofungin, which is actually a complex of a number of individual antibiotics that can be isolated together (as the sample obtained for this study) or can be extracted in various organic solvent systems shown by the authors [12] and obtained as separate substances. The latter can obviously be promising plans, especially if the main characteristics of streptofungin are established, which will show its potential as a pharmaceutical substance.

The relevance of the indicated direction of research is confirmed by the introduction of new antibiotics into production, despite the discussed problems of antibiotic resistance and the high cost of such developments. Thus, the fifth generation of cephalosporins on the pharmaceutical market is represented by Zeftera (ceftobiprole medokaril) and Zinfor (ceftaroline). The peculiarities of the drugs are their activity against methicillin-resistant *Staphylococcus aureus* and penicillin-resistant causative agent of streptococcal pneumonia. In the USA the new antibacterial drug Zerbax, a combination of ceftolozane (5th generation cephalosporin) and tazobactam (beta-lactamase inhibitor) was registered and approved by the FDA [22].

The study of the antagonistic activity of different strains of the *S. albus* culture showed their close specificity (see Table 1), and the result of stepwise selection using various mutagens contributed to an increase in the synthesis of certain substances in antimicrobial complexes, but did not fundamentally affect their qualitative changes. From a practical point of view, this makes it possible to choose producers for different developments with increased target antimicrobial activity against different pathogens or with broad specificity. At the same time, the question of the activity of the final form of the same product of biosynthesis of different strains (for example, streptofungin), even in the case of a certain quantitative difference in their biosynthetic ability, is a question of the purification and concentration methods used for isolation.

We note that when analyzing the antagonistic activity of the producer of several antimicrobial compounds at the same time, especially of different nature (as in the case of the studied culture), it is difficult to separate the contribution of these components to the result. Also, of course, it will depend on the concentration of the component that is secreted into the agar nutrient medium.

A clear confirmation of such a "cautious" attitude to the conclusions regarding the biosynthetic capacity of cultures is the example of the strain *S. albus* UN 44, which in this study did not show antagonism against *B. subtilis* (see Table 1): in our previous work [12], fractions of antibiotics synthesized by it, active against another reference test strain of *B. subtilis*, were isolated, but at the same time, antagonism against it was not detected on the agar medium either. Obviously, the question is the amount of antibiotic produced and secreted by the culture when grown on an agar medium and the effective concentration in relation to different test strains.

The mutant strains of *S. albus* used in the study were obtained using various mutagens and their combinations, which determines the differences in the spectrum of their antagonistic activity. So, most remarkable is the biosynthetic activity of the *S. albus* AE6 (the only one among others, obtained by HNO_2 treatment), which lost the ability to inhibit of *B. subtilis* (spore-forming bacteria), and synthesizes metabolites active against *P. vulgaris*. According to our previous analysis of the effect of various mutagens on the *S. albus* genome, which leads to the rearrangement of its nucleotide sequences, only in this type of mutants was the appearance of a fragment of 650 pairs of nucleotides, which can determine the above-mentioned changes in metabolism [17].

S. albus UN44 that obtained by treatment with a combination of HNO_2 and N-methyl-N-nitrosourea, like the AE6 strain, lost its ability to inhibit spore-forming bacteria, but does not synthesize metabolites active against proteus (see Table 1). However, it is obvious that both mutants have an increased synthesis of antibiotic, which determines the maximum growth inhibition zones of *C. albicans* (23–30 mm), and therefore this ability may also be related with the effect of the HNO_2 to the culture genome.

Important characteristic of any antibiotic, which will determine its practical value as an API, is the MIC, which we determined for streptofungin in relation to several test strains. The activity of streptofungin at low concentrations (less than 10 μg/ml) against *C.albicans* makes it a promising antimicrobial agent, and the limited range of antifungal agents in general adds to this importance. Regarding higher MICs for pathogens such as *P. aeruginosa* and spore bacteria (*B. subtilis*), this issue can be further explored with a streptofungin substance of a higher degree of purification than the currently obtained test sample.

A comparison of the streptofungin MICs and other recently isolated antibiotics from streptomycetes shows their close values, and some studies

show the possibility of combining different compounds and lowering their MICs. For example, echinomycin from *Streptomyces* sp. LS462 demonstrated antifungal activity (MIC of 6.25 μg/mL) and synergistic antifungal activity with a significantly reduced dose (up to 60-fold) in combination with posaconazole on *C. albicans* 5314 [23].

Therefore, the activity of streptofungin against *P. aeruginosa*, *B. subtilis* may be manifested differently in combination with other antimicrobial compounds, including bacteriolysins of the producer *S. albus* itself. Such a difference in the spectrum of activity of various antimicrobial metabolites of the culture is noticeable if you compare its antagonistic activity (see Table 1) and the activity of streptofungin (see Table 2) against *S.aureus*: significant antagonism (10–30 mm zone of growth inhibition) is shown by all strains, and streptofungin even at a concentration of 500 μg/ml does not affect the growth of the test strain. The answer is clear, because staphylolytic activity is the leading specificity of the enzyme complex of the culture, the strains of which were selected based on this feature [11, 17]. Therefore, the shown antagonism against *S. aureus* is due exclusively to the action of bacteriolysins produced by the culture, which are also active against *P. aeruginosa*.

The different spectrum of antimicrobial activity of *S. albus* products along with different mechanisms of its manifestation opens the possibility of creating antimicrobial compositions based on them. The presence of enzymes in such a composition will not only determine a certain spectrum of antimicrobial action, but also "help" the antibiotic to act, destroying individual defense mechanisms of pathogens. For example, we previously showed the ability of the *S. albus* enzyme complex to destroy the *P. aeruginosa* biofilm due to the content of proteinases, which can allow streptofungin in much lower concentrations (than shown in Table 2) to inhibit its reproduction.

But no one substances can be used as an antimicrobial agent if its effective doses against microbial pathogens are toxic to humans. For example, the antibiotic cycloserine isolated from streptomycetes *S. orchidaceus*, *S. garyphalus*, and *S. lavendulae* inhibits the biosynthesis of peptidoglycan of the cell wall of bacteria and is active against G+ and G– microorganisms. However, its use is limited due to high toxicity, which causes serious negative side effects [24].

High toxicity (including against human cancer cell lines) was shown by three new complexes of angucycline-type antibiotics isolated from *Streptomyces* sp. XZHG99T, which obviously have potential mainly as antitumor agents despite their significant activity against *Mycobacterium smegmatis* and *S. aureus* [25].

Therefore, in this work we analyzed the cytotoxicity of streptofungin in doses that were determined as MIC for different test strains. The chosen method of studying the cytotoxicity of an antibiotic with resazurin allows to detect a wider range of its effect on cells than the known MTT test, since resazurin is restored (changing its color) by a greater number of living cell enzymes. The established effect of streptofungin on the used epithelial cell lines MDBK and A549 allows us to talk about its lack of cytotoxicity in concentrations effective against microbial pathogens, and therefore about its potential as an API in antimicrobials.

Conclusions

Antagonistic activity of investigated *S. albus* strains is due to the action of two different antimicrobial products – antibiotic complex streptofungin and complex of lytic enzymes, which have a different antimicrobial spectrum. The leading activity of streptofungin is determined against Candida fungi, and lytic enzymes actively destroy bacterial cells, primarily *S. aureus*.

Minimum inhibitory concentrations of the antibiotic streptofungin were established for *C. albicans* ATCC 10231 (10 μg/ml), *B. subtilis* ATCC 6633 (200 μg/ml) and *P. aeruginosa* ATCC 9027 (500 μg/ml). According to the resazurin test, streptofungin in concentrations of 2.5–500 μg/ml does not show a cytotoxic effect in relation to MDBK and A549 epithelial cells, and therefore can be considered potentially permissible for humans and animals in the studied concentrations.

The obtained results give reason to consider the new antibiotic streptofungin as a promising API with antifungal action, and its combination as an antimicrobial agent with the *S. albus* enzyme complex can create an antimicrobial composition with a wide spectrum of action and enhance the effectiveness of each of these components.

Interests disclosure

Tetiana Todosiichuk is the member of the Editorial Council of *Innovative Biosystems and Bioengineering* and was not involved in the editorial evaluation or decision to accept this article for publication. The other authors have no conflicts of interest to declare.

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АНТИМІКРОБНІ ТА ЦИТОТОКСИЧНІ ВЛАСТИВОСТІ АНТИБІОТИКА СТРЕПТОФУНГІН

Проблематика. Стрептоміцети були й залишаються джерелом нових антимікробних сполук різної природи. Нова хвиля зацікавленості у таких дослідженнях пов'язана з можливостями прикладної геноміки щодо розкриття прихованого біосинтетичного потенціалу стрептоміцетів, а отже, відкриття нових практично цінних антимікробних продуктів. Проблема антибіотикорезистентності збудників може бути вирішена створенням композицій діючих речовин різної природи для подолання захисних механізмів патогенів.
Мета. Встановлення та оцінка антимікробних і цитотоксичних характеристик нового антибіотика стрептофунгіну, що синтезується *Streptomyces albus* 2435 (ЦКПМ-S-668) і його мутантними штамами 2435/M, UN44, 4S, US101, AE6, 105, 80/5.
Методи. Для встановлення характеристик стрептофунгіну визначали антагоністичну активність селекціонованих штамів-продуцентів *S. albus* (методом радіальних штрихів), мінімальну інгібуючу концентрацію антибіотика (методом серійних розведень), цитотоксичність – МТТ-тестом із резазурином.
Результати. Показано активність антибіотика стрептофунгіну щодо грибів р. *Candida* (*C. albicans*, *C. utilis*). Встановлено мінімальні інгібуючі концентрації стрептофунгіну щодо *C. albicans* ATCC 10231 (10 мкг/мл), *B. subtilis* ATCC 6633 (200 мкг/мл) і *P. aeruginosa* ATCC 9027 (500 мкг/мл). За даними резазуринового тесту стрептофунгін не проявляє цитотоксичної дії у широкому діапазоні концентрацій від 2,5 до 500 мкг/мл, а тому може вважатися потенційно безпечним для людини і тварин у вивчених концентраціях.
Висновки. Антагоністична активність мутантних штамів культури *S. albus* обумовлена дією комплексу антимікробних продуктів, які мають різний антимікробний спектр і механізм дії. Отримані результати дають підстави розглядати стрептофунгін як перспективну фармацевтичну субстанцію антифунгальної дії, а також можливість його поєднання з бактеріолітичним ферментним комплексом цієї ж культури для розробки антимікробного засобу з широким спектром дії.

Ключові слова: антимікробна активність; цитотоксичність; антибіотики; збудники інфекцій; антимікробні речовини; стрептоміцети.