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Cryopreservation potential of *Streptomyces lucensis* and *Streptomyces violaceus* actinomycete collection strains as producers of glycosidase inhibitors

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Abstract: The article presents a study into the effect of long-term, low-temperature (-80 and -150 °C) storage on the properties of *Streptomyces lucensis* RNCIM As-1743 and *Streptomyces violaceus* RNCIM As-1734 actinomycete collection strains acting as producers of glycosidase inhibitors. The titre (CFU in 1 cm³ of the initial inoculum) and the inhibitory activity of strains were determined with respect to pancreatic α -amylase in the solutions obtained by *Streptomyces* culture on a corn starch hydrolysate. For *Streptomyces*, a high survival rate (91–100 %) was established after storage at temperatures of -80 and -150 °C using a 15 % glycerol solution in terms of a cryoprotector. *Streptomyces violaceus* strain was identified to be the most resistant to long-term storage at low temperatures. Its inhibitory activity turns to be completely retained after storage at temperatures of -80 and -150 °C. In *Streptomyces violaceus* strain, the maximum activity level of 2250±200 IU/cm³ for an inhibitor of pancreatic α -amylase is observed on the 1st day of subculture, while *Streptomyces lucensis* RNCIM As-1743 demonstrates the highest activity value on the 3rd day to reach a value of 3660±200 IU/cm³ following storage at a temperature of -80 °C. The studied *Streptomyces* strains are chromogenic. The most intense chromogenesis is noted during the culture of *Streptomyces violaceus* strain stored at a temperature of -150 °C. The cryopreservation of *Streptomyces violaceus* and *Streptomyces lucensis* actinomycete strains was established to provide high (10⁷–10⁸) cell survival and preservation of their inhibitory activity at a high level when exposed to temperatures of -80 and -150 °C with a 15 % glycerol solution as a cryoprotector. Experimental data indicate the low-temperature storage method to be promising for *Streptomyces lucensis* and *Streptomyces violaceus* collection cultures.

Keywords: *Streptomyces lucensis* RNCIM As-1743 strains, *Streptomyces violaceus* RNCIM As-1743 strains, low-temperature storage, viability, inhibitory activity, chromogenesis

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Возможности криоконсервирования коллекционных штаммов актиномицетов *Streptomyces lucensis* и *Streptomyces violaceus* – продуцентов ингибиторов гликозидаз

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Резюме: Исследовано влияние длительного низкотемпературного хранения (-80 и -150 °C) на свойства коллекционных штаммов актиномицетов *Streptomyces lucensis* ВКПМ Ас-1743 и *Streptomyces violaceus* ВКПМ Ас-1734 – продуцентов ингибиторов гликозидаз. Определен титр (КОЕ в 1 см^3 исходного инокулята) и ингибиторная активность штаммов по отношению к панкреатической α -амилазе в растворах, полученных при культивировании *Streptomyces* на гидролизате кукурузного крахмала. Установлен высокий (91–100 %) уровень выживаемости *Streptomyces* после хранения при температурах -80 и -150 °C с применением 15 %-го раствора глицерина в качестве криопротектора. Выявлено, что штамм *Streptomyces violaceus* наиболее устойчив к условиям длительного хранения при низких температурах. Он полностью сохранил ингибиторную активность после хранения при температурах -80 и -150 °C. Максимальный уровень активности ингибитора панкреатической α -амилазы у штамма *Streptomyces violaceus* отмечается на 1-е сутки рекультивирования и составляет (2250 ± 200) ИЕ/ см^3 , у *Streptomyces lucensis* ВКПМ Ас-1743 – на 3-и сутки рекультивирования и достигает значения 3660 ± 200 ИЕ/ см^3 после хранения при температуре -80 °C. Исследуемые штаммы *Streptomyces* являются пигменто-образующими. Наиболее интенсивное пигментообразование отмечено при культивировании штамма *Streptomyces violaceus*, хранившегося при -150 °C. Проведенные исследования показали, что криоконсервация штаммов актиномицетов *Streptomyces violaceus* и *Streptomyces lucensis* при температурах -80 и -150 °C с применением 15 %-го раствора глицерина в качестве криопротектора обеспечивает высокую (10^7 – 10^8) степень выживаемости клеток и сохранение их ингибиторной активности на высоком уровне. Экспериментальные данные свидетельствуют о перспективности метода низкотемпературного хранения для коллекционных культур *Streptomyces lucensis* и *Streptomyces violaceus*.

Ключевые слова: штаммы *Streptomyces lucensis* ВКПМ Ас-1743 и *Streptomyces violaceus* ВКПМ Ас-1734, низкотемпературное хранение, жизнеспособность, ингибиторная активность, пигментообразование

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INTRODUCTION

Actinobacteria of the *Streptomyces* genus manifest themselves as producers of more than half of the most common biologically active compounds applied in medicine, veterinary, agriculture and other industries. Thus, maintaining the viability of *Streptomyces* cultures during long-term storage constitutes an important research area and makes a significant theoretical and practical contribution to the problem of preserving an ever-increasing biological diversity.

For most microorganism collections, the preservation of strains in working condition with the retention of their valuable properties is carried out using periodic subculture methods, storage under a layer of mineral oil, in dry sterile soil or sand, in saline solutions, as well as in a lyophilised state or at low temperatures [1].

The primary method for long-term microorganism storage consists in cryopreservation. Among the advantages of cryogenic storage are the low probability of culture infection, preservation

of microorganism properties in a stable state, low time and material costs, the possibility of using frozen cultures as a direct inoculum and eliminating the risk of genetic changes in cultures during storage. In addition, according to the published studies, microorganism cultures stored at low temperatures in a frozen state appear to be less damaged and demonstrate a higher level of viability as opposed to dried and lyophilised cultures [1, 2].

The cryostability of microorganisms is determined by their taxonomic affiliation, physiological state, cell number, cryopreservation modes, the presence of a protective environment, as well as the temperature and heating rate. Different cryostability is observed in microbial organisms of not only different genera and species, but also strains. Gram-positive bacteria are known to be usually more resistant to freezing in contrast to gram-negative bacteria [3]. It is thought that microorganisms are most resistant to freezing at the end of the logarithmic growth stage or at the beginning of the stationary phase. According to a number of studies, increased cryoresistance is also characteristic of spore-forming microorganisms [4].

Protective substances – or cryoprotectors – can be applied in order to increase the resistance of cells to low temperatures. Two main cryoprotectant types can be distinguished. The first includes glycerin and dimethyl sulphoxide (DMSO) either easily to pass through the cell membrane and provide both intracellular and extracellular protection against freezing. The second type of cryoprotectors involves the following substances providing a protective effect on the outer surface of the cell membranes: sucrose, lactose, glucose, mannitol, sorbitol, dextran, polyvinylpyrrolidone and polyglycol. According to some studies, the first type of protectors (glycerin and DMSO) have been shown to be more effective and applicable for a wide range of bacteria [5]. In this case, the protective action of glycerin is explained by its prevention of excessive salt concentration levels causing a violation of biochemical processes in microorganism cells during freezing [3].

A distinctive feature of many *Streptomyces* consists in their chromogenetic ability by forming carotenoid pigments, black-brown melanins and blue-violet anthocyanins. Thus, the colour of the substrate and aerial mycelium is determined by a variety of pigments. Chromogenesis in microorganisms has a certain physiological significance through the protection of cells from natural ultraviolet radiation, participation in biochemical reactions and antibiotic effect [6]. Therefore, the study of stressful effects on *Streptomyces* bacteria appears to be of great practical importance.

The methods are known for long-term, low-temperature storage of *Streptomyces hygroscopicus* RIA 1433 *Nonomuraea* Sp antibiotic producers (-70 °C) [4], strains of *Streptomyces aureovorticillus*, *Streptomyces aureofaciens* and *Streptomyces*

griseus actinobacteria (-196 °C) [7], as well as collection cultures of *Rhodococcus* actinobacteria (-85 °C) [8].

Streptomyces lucensis RNCIM As-1743 and *Streptomyces violaceus* RNCIM As-1734 collection cultures of the All-Russian Research Institute of Food Additives (VNIIPD) involves the producers of glycosidase inhibitors – biologically active substances and potential food micro-ingredients [9]. Currently, no studies on effectiveness evaluation of the long-term storage at extremely low temperatures in relation to these actinobacteria were conducted.

The present study is aimed at the effect of low temperatures (-80 and -150 °C) on the viability of *Streptomyces lucensis* RNCIM As-1743 and *Streptomyces violaceus* RNCIM As-1734 actinomycetes and their preservation of inhibitory activity during storage.

EXPERIMENTAL PART

The object of the study involves *Streptomyces lucensis* RNCIM As-1743 and *Streptomyces violaceus* RNCIM As-1734 actinomycete strains selected at VNIIPD and deposited in the All-Russian collection of industrial microorganisms of the Research Institute for Genetics and Selection of Industrial Microorganisms [10, 11].

The studied actinobacteria strains were cultured before freezing on the slant starch-containing Czapek medium at a temperature of 29 °C for 7 days.

Cell separation was carried out by flushing the culture from a slant agar starch-containing Czapek medium using 10 cm³ of nutrient fermentation medium. In order to increase the resistance of cells to the effects of low temperatures, a cryoprotector of 15 % glycerol solution was added to the nutrient medium for fermentation. In the study, bacterial suspensions were applied with a population density of 10⁷–10⁸ CFU/cm³.

Cryo- (Greiner Bio-One, Germany) and Eppendorf tubes were used for freezing spore suspensions at a temperature of -80 °C and -150 °C, respectively. All tubes were exposed to +4 °C for 2 h with subsequent placement in low-temperature freezers. Cells were frozen in stages: up to -30 °C at a controlled cooling rate of 1 °C/min followed by lowering the temperature to -150 °C at a rate of 15–30 °C/min.

The samples of *Streptomyces* actinomycete strains were frozen and stored in an MDF-1156ATN ULTRA-LOW temperature freezer (SANYO Electric Co. Ltd) at a temperature of -150 °C and in the "Station of low-temperature automated storage of biological samples at -80 °C" Unique Research Installation (LiCONiC Instruments, Liechtenstein) of the Departmental collection of beneficial microorganisms for agricultural purposes of the Research Institute of Agricultural Microbiology [12]. The storage duration comprises 9 months.

The recovery process for frozen cells was carried out by thawing at a temperature of 37 °C for

3 minutes. Then the cells were placed on Czapek agar medium in test tubes. After 7 days of incubation at a temperature of 29 °C, the viability of the cultures was determined and culturing was carried out.

The viable cell count (CFU/cm³) of *Streptomyces* actinomycetes was determined by serial dilutions of thawed spore suspensions, followed by inoculation on Petri dishes with agar Czapek starch-containing medium. After exposing the cultures for 3 days at a temperature of 29 °C, the number of cultured microcolonies was calculated. Percentage of survived CFU of actinobacteria was determined relative to the number of CFU counted before cryopreservation.

Streptomyces lucensis and *Streptomyces violaceus* culturing was carried out periodically in a 750 cm³ flask with a corn starch hydrolysate using a Multitron incubation shaker (INFORS, Switzerland) with a stirring speed of 160±20 rpm at a temperature of 29±1 °C for 96 h [10, 11]. The fermentation medium of pH = 7.0 was composed by corn starch hydrolysate with dextrose equivalent (DE = 25±5 %), soy flour, sodium chloride, disubstituted potassium phosphate and magnesium sulphate heptahydrate at concentrations of 20, 5.0, 3.0, 1.0 and 0.5 g/dm³, respectively [13]. For the hydrolysis of corn starch, an Amilosubtilin G3x enzyme preparation with an amylolytic activity of 850 u/g was used (GOST 23635-90).

The inhibitory activity of cells survived after storage was determined in the obtained solutions by culturing *Streptomyces lucensis* and *Streptomyces violaceus* using the colorimetric method in reference to pancreatic amylase (test enzyme). An amount of an inhibitor suppressing 1 unit of pancreatic α-amylase by 50 % at a temperature of 37 °C and pH = 7 was assigned as a unit of inhibitory activity [9]. Prior to determination, the intrinsic amylase of *Streptomyces* strains was inactivated in the solution by heat treatment at 98±1 °C. The inhibitory effect was studied in reference to pancreatic amylase (Sigma, USA, enzyme activity 34517 u/g) [14].

The chromogenesis property was evaluated using a UV-1800 spectrophotometer (Shimadzu, Japan) by determining the optical density of the solutions obtained by *Streptomyces* culturing on a starch hydrolysate at a wavelength of 560 nm.

Statistical processing data on inhibitory activity and viability was performed using the Microsoft Office Excel 2010.

RESULTS AND DISCUSSION

Previous studies have proved the low temperature storage of *Streptomyces* to cause no adverse effect on the viability and inhibitory activity of cultures. The studied strains of *Streptomyces lucensis* RNCIM As-1743 and *Streptomyces violaceus* RNCIM As-1734 actinomycetes with a population density of 10⁷–10⁸ CFU preserved a high

viability and inhibitory activity with reference to pancreatic α-amylase after 4 months of storage in a 15% glycerol solution at a temperature of -12 and -18 °C [15].

As a result of these studies, the *Streptomyces lucensis* RNCIM As-1743 and *Streptomyces violaceus* RNCIM As-1734 collection strains were established to tolerate the freezing process at extremely low temperatures (-80 and -150 °C).

According to our data, high viability is retained in the cells of the studied cultures stored in a 15 % glycerol solution at temperatures of -80 and -150 °C after 9-month storage. Thus, after 9 months of storage at temperatures of -80 and -150 °C, the survival rate of the studied actinobacteria cells stored at a population density of 10⁷ and 10⁸ CFU was preserved throughout the entire storage time and amounted to 91–100 %. For *Streptomyces lucensis* RNCIM As-1743, the number of viable cells in frozen suspensions ranged from 1.94·10⁸ to 1.97·10⁸ CFU/cm³, as opposed to *Streptomyces violaceus* RNCIM As-1734 amounted from 5.24·10⁷ to 5.30·10⁷ CFU/cm³. On both accounts, the obtained indicators meet the requirements for low-temperature storage for bacterial cultures.

In a comparative aspect, by the end of the storage period, the number of viable cells in the studied strains of *Streptomyces* stored on Czapek dense agar medium in test tubes at a temperature of +4 °C without subculturing decreases by 73.8 and 64.5 % and comprises 1.40·10⁷ and 7.41·10⁷ CFU/cm³ for *Streptomyces violaceus* and *Streptomyces lucensis*, respectively (Table).

The viability study into *Streptomyces lucensis* RNCIM As-1743 and *Streptomyces violaceus* RNCIM As-1734 collection cultures established these *Streptomyces* actinomycete strains to be highly resistant to cryopreservation and remain viable under high stress conditions. The long-term, low-temperature resistance of the studied strains is apparently due to the biological characteristics of *Streptomyces lucensis* and *Streptomyces violaceus*.

Collection and industrial strain microorganisms are acknowledged to demonstrate population variability in addition to loss of cell viability during storage. In this case, the dominant phenotype is replaced by another with altered properties and productive activity, resulting in the loss of strain priority properties [7].

The studied *Streptomyces* strains manifest themselves as producers of a pancreatic α-amylase inhibitor. For this reason, maintaining a high level of biosynthetic activity of strains acting as producers of biologically active substances during low-temperature storage turns to be a prerequisite. Therefore, in addition to studies on the effect of cryopreservation on the viability and inhibitory activity of actinomycete cultures during long-term storage was evalu-

ated. Throughout the storage period, inhibitory activity referenced to pancreatic α -amylase was monitored. Figures 1–3 demonstrate the data on

inhibitory activity of *Streptomyces* strains stored at ultra-low temperatures of -80 and -150 °C when culturing on a corn starch hydrolysate.

Viability of the studied *Streptomyces violaceus* and *Streptomyces lucensis* cultures exposed to cryopreservation at -80 and -150 °C

Жизнеспособность исследуемых культур *Streptomyces violaceus* и *Streptomyces lucensis* после криоконсервации при -80 и -150 °C

Streptomyces Strain	Storage temperature, °C	Number of viable cells, CFU / cm ³				Cell survival, %
		before storage deposition	3 months after	6 months after	9 months after	
<i>Streptomyces violaceus</i>	+4		$(4.16 \pm 0.48) \cdot 10^7$	$(3.32 \pm 0.38) \cdot 10^7$	$(1.40 \pm 0.18) \cdot 10^7$	26.2
	-80	$(5.35 \pm 0.48) \cdot 10^7$	$(5.39 \pm 0.50) \cdot 10^7$	$(5.22 \pm 0.52) \cdot 10^7$	$(5.30 \pm 0.50) \cdot 10^7$	99.1
	-150		$(5.42 \pm 0.55) \cdot 10^7$	$(5.38 \pm 0.61) \cdot 10^7$	$(5.24 \pm 0.48) \cdot 10^7$	97.9
<i>Streptomyces lucensis</i>	+4		$(9.24 \pm 0.98) \cdot 10^7$	$(8.05 \pm 0.88) \cdot 10^7$	$(7.41 \pm 0.72) \cdot 10^7$	35.5
	-80	$(2.09 \pm 0.25) \cdot 10^8$	$(2.06 \pm 0.25) \cdot 10^8$	$(1.98 \pm 0.22) \cdot 10^8$	$(1.91 \pm 0.21) \cdot 10^8$	91.4
	-150		$(1.92 \pm 0.17) \cdot 10^7$	$(1.87 \pm 0.22) \cdot 10^7$	$(1.99 \pm 0.22) \cdot 10^7$	95.2

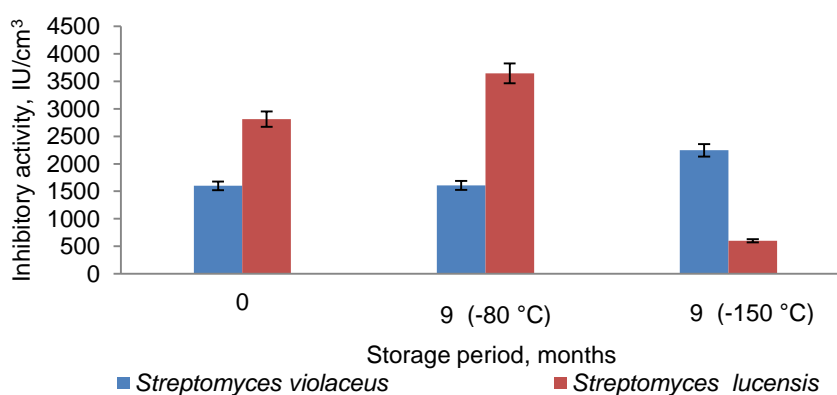


Fig. 1. Inhibitory activity of actinobacteria *Streptomyces violaceus* VKPM AC-1734 and *Streptomyces lucensis* VKPM AC-1743 before laying for storage (0 months) and after 9 months of storage at -80 and -150 °C

Рис. 1. Ингибиторная активность актинобактерий *Streptomyces violaceus* ВКПМ Ас-1734 и *Streptomyces lucensis* ВКПМ Ас-1743 до закладки на хранение (0 мес.) и после 9 мес. хранения при -80 и -150 °C

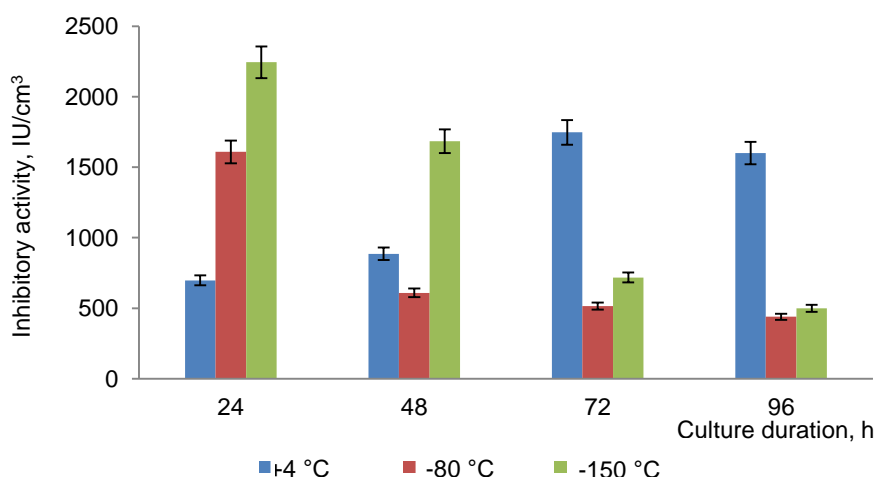


Fig. 2. Relationship between the cryogenic conservation temperature and the inhibitory activity of the strain *Streptomyces violaceus* VKPM Ac-1734 in recultivation process

Рис. 2. Влияние температуры криогенного консервирования на динамику ингибиторной активности штамма *Streptomyces violaceus* ВКПМ Ас-1734 в процессе рекультивирования

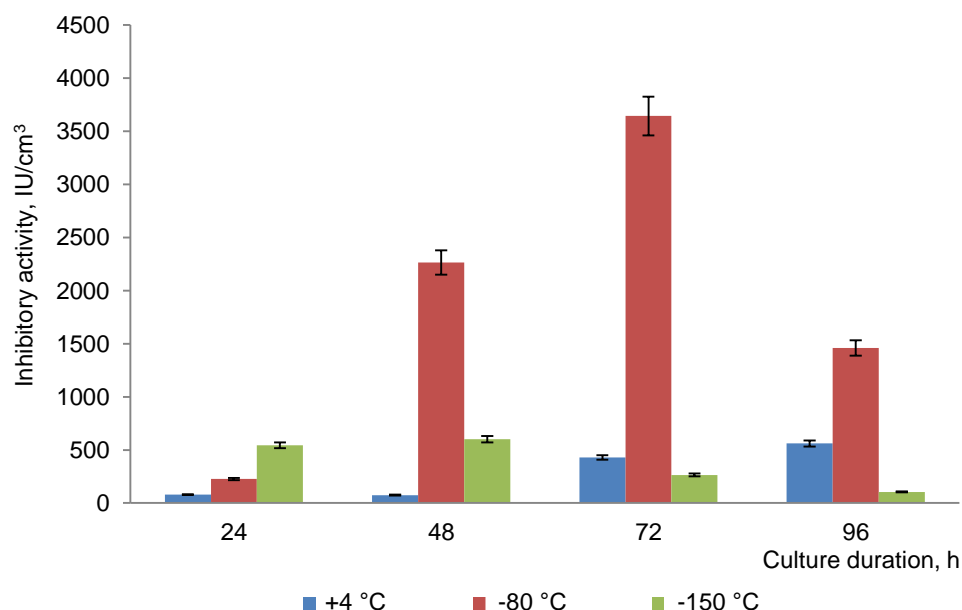


Fig. 3. Relationship between the cryogenic conservation temperature and the dynamics of the inhibitory activity of the strain *Streptomyces lucensis* VKPM Ac-1743 in recultivation process

Рис. 3. Влияние температуры криогенного консервирования на динамику ингибиторной активности штамма *Streptomyces lucensis* ВКПМ Ас-1743 в процессе рекультивирования

As a result of the studies, a high level of bio-synthetic activity is established to be preserved in both strains after 9-month storage at temperatures of -80 and -150 °C. *Streptomyces violaceus* strain was identified to be the most resistant to long-term storage at low temperatures, as opposed to *Streptomyces lucensis*. As referenced to pancreatic α -amylase, the inhibitory activity of *Streptomyces violaceus* is completely retained at temperatures of -80 and -150 °C; this contrasts with the *Streptomyces lucensis* strain, in which with the inhibitory activity decreased by 78 % by the end of the storage period at a temperature of -150 °C as compared to the value prior to cryopreservation.

At a storage temperature of -150 °C, the inhibitory activity of the *Streptomyces violaceus* strain remained at a higher level as compared to the temperature of -80 °C. In *Streptomyces violaceus* strain, the maximum activity level for an inhibitor of pancreatic α -amylase is observed on the 1st day of reculturing and comprises (2250±200) IU/cm³ after storage at -150 °C, while *Streptomyces lucensis* RNCIM As-1743 demonstrates the highest activity on the 3rd day of reculturing to reach a value of 3660±200 IU/cm³ following storage at a temperature of -80 °C. The *Streptomyces lucensis* cells reduced following cryopreservation at a temperature of -150 °C is characterised by low activity glycosidase inhibitor production on the 1st–2nd day of subculture equal to 600 ± 70 IU/cm³.

Unlike the strain of *Streptomyces violaceus* stored under similar conditions, the *Streptomyces lucensis* strain stored on a Czapek dense nutrient medium for a long time (9 months) at a temperature of +4 °C without subculture is also noted to almost completely lose the ability to synthesise a glycosidase inhibitor (see Fig. 2, 3).

According to the obtained data, the reaction of the *Streptomyces lucensis* strain to the low-temperature effect (-150 °C) is represented by the almost complete loss of the inhibitor synthesis ability.

A sharp increase in inhibitory activity of producers exposed to abiotic stress on the first day of subculture is apparently due to the fact that, when the temperature drops from negative to positive (from -80 and -150 to +37 °C compared to rise from +4 to +37 °C when thawing cultures after storage), the restoration of biochemical reactions in response to stress exposure is sharply activated. During the first days following anabiosis, the culture synthesises its own amylases to assimilate the carbon source (glucose, maltose and dextrans representing products of starch hydrolysis in starch hydrolysate) followed by a secondary metabolite, amylolytic enzyme inhibitor, is produced under conditions of carbon starvation. The inhibitor activity decreases with increasing duration of fermentation, possibly due to its structural modification. This is acknowledged for the studied glycosidase inhibitors synthesised by streptomycetes during the fermentation of carbohydrate-containing media

and being pseudo-oligosaccharides represented by the multiple forms in their chemical nature [16, 17].

The adaptation to culture conditions turns out to be slower, when subculturing the studied streptomycetes stored at a temperature of +4 °C.

The *Streptomyces* strains under study are recognised to be chromogenic. In relation to the presented experiments, the "chromogenic" indicator is possible to be used as a criterion for assessing the adaptation of *Streptomyces* culture to external conditions. In other words, when the temperature moves from negative to positive, the restoration of biochemical reactions in response to stressful effects is intensified. The synthesis of secondary metabolites is activated, including pigments and enzyme inhibitors.

In accordance to the results of the studies, following 7 days of streptomycetes culture incubation on Czapek dense agar medium in test tubes at a temperature of 29 °C, a clear difference is observed in the staining of aerial mycelium strains. For *Streptomyces lucensis* RNCIM As-1743, the aerial mycelium pigment is white with a greyish tint, while the staining of *Streptomyces violaceus* RNCIM As-1734 is pink-violet (Fig. 4).

After the first culture on agar medium of cells was reduced following storage at low temperatures (-80 and -150 °C), a more intense chromogenesis was observed in *Streptomyces violaceus* RNCIM As-1734 strain. The colour of the aerial mycelium of the strain takes on a dark purple hue (tubes 4–6 in Fig. 4). The effect of low temperatures probably results in a partial destruction of proteins, with the products composing a substrate for tyrosinase

catalysing the oxidation of phenols. More intense chromogenesis of the *Streptomyces violaceus* RNCIM As-1734 culture is probably due to higher tyrosinase activity compared to *Streptomyces lucensis* RNCIM As-1743 strain [14]. The most intense pigment diffusion into the medium is noted after storage of the conidia of *Streptomyces violaceus* strain at a temperature of -150 °C.

In order to determine the pigment concentration varying, as proved by studies, in dependency with the storage temperature and the duration of streptomycete culturing after removal from the anabiosis, the optical density of solutions was measured as characterised by an increase at the maximum absorption of the synthesised pigment by the end of the culture process: after storage at a temperature of 80 °C, the optical density increased from 0.42 AU on the 1st day to 1.5 AU at the end of the process; after storage at a temperature of -150 °C, an increase from 0.85 to 1.9 AU, respectively, is detected. The data obtained indicate an increase in the concentration of chromogenic substances in the culture fluid. Apparently, the actinorhodin pigment is synthesised, inherent to the cultures of *Streptomyces coelicolor*, *S. violaceoruber* and *S. lividans*, with the absorption maximum ranging from 540 to 640 nm [18, 19]. This pigment changing colour from red to blue in dependence to the conditions of microorganism culturing is used by researchers as an indicator of changes in pH of the medium. The results of the studies demonstrated the pH to vary from 7 to 4.5 for the culture fluid where benzoisochromanquinone pigments are synthesised as characteristic for actinomycetes with a generic affiliation to *Streptomyces* [20, 21].



Fig. 4. Conidia development of *Streptomyces lucensis* (1, 2, 3) and *Streptomyces violaceus* (4, 5, 6) strains on the Czapek's medium with starch for 7 days of growth before freezing (1, 4) and after storage at -80 °C (2, 5) and -150 °C (3, 6)

Рис. 4. Развитие конидий штаммов *Streptomyces lucensis* (1, 2, 3) и *Streptomyces violaceus* (4, 5, 6) на среде Чапека с крахмалом в течение 7 суток роста до замораживания (1, 4) и после хранения культур при -80 °C (2, 5) и -150 °C (3, 6)

According to the study results, the intensely pigmented *Streptomyces violaceus* RNCIM As-1734 strain is more resistant to temperature changes; this is additionally confirmed by the above results of studies on the effect of ultra-low temperatures on the strain inhibitory activity (see Fig. 1).

A study of the viability of *Streptomyces lucensis* RNCIM As-1743 and *Streptomyces violaceus* RNCIM As-1734 collection cultures identified these strains of *Streptomyces* actinomycetes to be highly resistant to cryopreservation and remain viable and biosynthetic activity under high stress conditions. The resistance of the studied *Streptomyces* strains to low-temperature exposure for a long time is apparently due to the biological characteristics of these actinobacteria.

CONCLUSION

The cryopreservation study of *Streptomyces violaceus* and *Streptomyces lucensis* actinomycete strains showed that the process supports high (10^7 – 10^8) cell survival and preservation of inhibito-

ry activity at a high level under the exposure to temperatures of -80 and -150 °C using a 15 % glycerol solution as a cryoprotector.

The *Streptomyces violaceus* RNCIM As-1734 strain is identified to be the most resistant to storage at low temperatures, retaining both viability and complete inhibitory activity at the studied temperatures. A higher level of the inhibitory activity of the strain is determined to be preserved at a storage temperature of -150 °C, as opposed to -80 °C.

The most intense pigment diffusion into the medium is noted following storage of the *Streptomyces violaceus* strain at a temperature of -150 °C.

The obtained experimental data indicate that low-temperature storage of *Streptomyces* collection cultures supported by the Research Institute for Genetics and Selection of Industrial Microorganisms is a promising method.

The investigated modes of freezing and storage guarantee the preservation of the viability and functional activity of the studied cultures.

REFERENCES

1. Savkina OA, Ternovskoi GV, Lokachuk MN, Pavlovskaya EN, Safronova VI. Cryopreservation is a promising method for storing industrially valuable strains of lactic acid bacteria and yeast. *Sel'skokhozyaistvennaya biologiya* = Agricultural Biology. 2014;4:112–119. (In Russian) <https://doi.org/10.15387/agrobiology.2014.4.112rus>
2. Chukparova AU. Otsenka sokhraneniya zhiznesposobnosti shtammov mikroorganizmov pri nizkotemperaturnoi konsekvatsii = Assessment of retention of the microorganism viability during low-temperature conservation. In: *Biologicheski aktivnye veshchestva mikroorganizmov: proshloe, nastoyashchee, budushchee: materialy Vserossiiskogo simpoziuma s mezhdunarodnym uchastiem* = Biologically active substances of microorganisms – past, present, future: Proceedings of the All-Russian Symposium with International Participation. 27-29 January 2011, Moscow. Moscow: MAKSPress, 2011, p. 131. (In Russian)
3. Filippova SN, Surgucheva NA, Gal'chenko VF. Long-term storage of collection cultures of actinobacteria. *Microbiology*. 2012;81(5):630–637. <https://doi.org/10.1134/S0026261712050062>
4. Sineva ON, Kulikova NG, Filippova SN, Terekhova LP. Storage of Actinobacteria of the Genera *Streptomyces* and *Nonomuraea* by Low Temperature Preservation. *Antibiotiki i khimioterapiya* = Antibiotics and chemotherapy. 2014;59(11-12):11–15. (In Russian)
5. Pokhilenko VD, Baranov AM, Detushev KV. Methods of long-term storage of collection cultures of microorganisms and development trends. *Izvestiya vysshikh uchebnykh zavedenii. Povolzhskii region*. = University Proceedings. Volga region. Medical Sciences. 2009;4:99–121. (In Russian)
6. The formation of microorganisms pigments and aromatic substances. Glow Microbes. Available from: https://studopedia.su/6_31038_obrazovanie-mikroorganizmami-pigmentov-i-aromaticeskikh-veshchestv-svechenie-mikrobov.html/ [Accessed 05th Juli 2019]. (In Russian)
7. Tsutsaeva AA, Anan'ina AE, Balyberdina LM, Stepanyuk LV, Pavlenko NV. Long-term storage of industrial microbial strains. *Mikrobiologiya* = Microbiology. 2008;77(5):696–700. (In Russian)
8. Kamenskikh TN, Kalashnikova EA, Ivshina IB. Features of cryopreservation of alkanotrophic actinobacteria of the genus *Rhodococcus*. *Vestnik Permskogo universiteta. Seriya: Biologiya* = Bulletin of Perm University. Biology. 2010;1:15–20. (In Russian)
9. Sharova NYu. Amylase inhibitors from *Streptomyces lucensis* VKPM Ac-1743 and *Streptomyces violaceus* VKPM Ac-1734. *Applied Biochemistry and Microbiology*. 2015;51(1):58–63. <https://doi.org/10.1134/S0003683815010159>
10. Sharova NYu, Nikiforova TA, Pozdnyakova TA. Actinomycete strain *Streptomyces violaceus* – producer of glycosidase inhibitor. Patent RF, no. 2346042; 2006. (In Russian)
11. Sharova NYu, Pozdnyakova TA, Khodkevich OA. Actinomycete strain *Streptomyces lucensis* – producer of glycosidase inhibitor. Patent RF, no. 2355755; 2008. (In Russian)
12. Safronova VI, Tikhonovich IA. Organization of authorized long-term storage of useful agricultural microorganisms in the departmental collection of the Russian Agricultural Academy. *Sel'skokhozyaistvennaya biologiya* = Agricultural Biology. 2012;47(3):32–36. (In Russian)
13. Printseva AA, Sharova NY, Vybornova TV, Manzhieva BS. Features of low-temperature storage of streptomyces strains – producers of glycosidase

inhibitors. *Food systems*. 2019;2(1):20–22. <https://doi.org/10.21323/2618-9771-2019-2-1-20-22>

14. Sharova N.Yu., Vybornova T.V., Printseva A.A., Manzhieva B.S. The properties of the conidia of strains of the actinomycete *Streptomyces lucensis* and *Streptomyces violaceus* during storage at low temperatures. *Food systems*. 2018;1(3):27–32. <https://doi.org/10.21323/2618-9771-2018-1-3-27-32>

15. Vybornova T.V., Sharova N.Yu., Printseva A.A. Low-temperature storage and the viability preservation of *Streptomyces*. *Tekhnika i tekhnologiya pishchevykh proizvodstv* = Food Processing: Techniques and Technology. 2018;48(3):41–49. (In Russian) <https://doi.org/10.21603/2074-9414-2018-3-34-40>

16. Sharova N.Yu. Biosynthesis of amylase inhibitor by Streptomyces cultures. *Applied Biochemistry and Microbiology*. 2016;52(8):723–732. <https://doi.org/10.1134/S000368381608007X>

17. Sharova N.Yu., Khodkevich O.A., Pozdnyakova T.A. Cultural-morphological and physiological-biochemical properties of new strains in actinomycetes – producers of glycosidase inhibitors. *Vestnik*

Rossiiskoi akademii sel'skokhozyaystvennykh nauk = Proceedings of Russian Agricultural Academy. 2009;3:74–76. (In Russian)

18. Palanichamy V., Hundet A., Mitra B., Reddy N. Optimization of cultivation parameters for growth and pigment production by *Streptomyces* spp. isolated from marine sediment and rhizosphere soil. *International Journal of Plant, Animal and Environmental Sciences*. 2011;1(3):158–170.

19. Danevčič T., Boric M., Stopar D. Microbial Ecophysiology of *Vibrio ruber*. *Food Technology and Biotechnology*. 2014;52(2):198–203.

20. Thakur R., Rai V. Isolation and Biochemical characterization of thermophilic *Streptomyces* from soil of Raipur District of Chhattisgarh, India. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*. 2011;2(4):795–808.

21. Mohanasrinivasan V., Sriramkalyan P., Pishita N., Devi C.S., Selvarajan E., Suganthi V. et al. Fermentative production of extracellular pigment from *Streptomyces coelicolor* MSIS1. *Research journal of biotechnology*. 2013;8(4):30–40.

БИБЛИОГРАФИЧЕСКИЙ СПИСОК

1. Савкина О.А., Терновской Г.В., Локачук М.Н., Павловская Е.Н., Сафронова В.И. Криоконсервация – перспективный метод хранения промышленно ценных штаммов молочнокислых бактерий и дрожжей // *Сельскохозяйственная биология*. 2014. N 4. С. 112–119. <https://doi.org/10.15387/agrobiology.2014.4.112rus>

2. Чукпарова А.У. Оценка сохранения жизнеспособности штаммов микроорганизмов при низкотемпературной консервации // *Биологически активные вещества микроорганизмов: прошлое, настоящее, будущее: материалы Всероссийского симпозиума с международным участием (Москва, 27–29 января 2011 г.)*. М.: МАКС Пресс, 2011. С. 131.

3. Filippova S.N., Surgucheva N.A., Gal'chenko V.F. Long-term storage of collection cultures of actinobacteria // *Microbiology*. 2012. Vol. 81. N 5. P. 630–637. <https://doi.org/10.1134/S0026261712050062>

4. Синева О.Н., Куликова Н.Г., Филиппова С.Н., Терехова Л.П. Хранение культур актинобактерий – представителей родов *Streptomyces* и *Nonomuraea* методом низкотемпературной консервации // *Антибиотики и химиотерапия*. 2014. Т. 59. N 11-12. С. 11–15.

5. Похиленко В.Д., Баранов А.М., Детушев К.В. Методы длительного хранения коллекционных культур микроорганизмов и тенденции развития // *Известия высших учебных заведений. Поволжский регион*. 2009. N 4 (12). С. 99–121.

6. Образование микроорганизмами пигментов и ароматических веществ. Свечение микробов // *Студопедия [Электронный ресурс]*. URL: https://studopedia.su/6_31038_obrazovanie-mikroorganizmami-pigmentov-i-aromaticheskikh-veshchestv-svechenie-mikrobov.html (05.07.2019).

7. Цуцаева А.А., Ананьина А.Е., Балыбердина Л.М., Степанюк Л.В., Павленко Н.В. Опыт долгосрочного хранения промышленных штаммов микроорганизмов // *Микробиология*. 2008. Т. 77. N 5. С. 696–700.

8. Каменских Т.Н., Калашникова Е.А., Ившина И.Б. Особенности криоконсервации алканотрофных актинобактерий рода *Rhodococcus* // *Вестник Пермского университета. Серия: Биология*. 2010. Вып. 1 (1). С. 15–20.

9. Sharova N.Yu. Amylase inhibitors from *Streptomyces lucensis* VKPM Ac-1743 and *Streptomyces violaceus* VKPM Ac-1734 // *Applied Biochemistry and Microbiology*. 2015. Vol. 51. Issue 1. P. 58–63. <https://doi.org/10.1134/S0003683815010159>

10. Пат. № 2346042, Российская Федерация, МПК C12N 9/24, C12N 1/20, C12R 1/465. Штамм актиномицета *Streptomyces violaceus* – продуцент ингибитора гликозидаз / Н.Ю. Шарова, Т.А. Никифорова, Т.А. Позднякова; заявитель и патентообладатель ГУ ВНИИПАКК. № 2006138251/13; заявл. 30.10.2006; опубл. 10.02.2009. Бюл. № 4.

11. Пат. № 2355755, Российская Федерация, МПК C12N 1/20, C12N 9/24, C12N 9/26. Штамм актиномицета *Streptomyces lucensis* – продуцент ингибитора гликозидаз / Н.Ю. Шарова, Т.А. Позднякова, О.А. Ходкевич; заявитель и патентообладатель ГУ ВНИИПАКК. № 2008101164/13; заявл. 09.01.2008; опубл. 20.05.2009, Бюл. N 14.

12. Сафронова В.И., Тихонович И.А. Организация авторизованного долгосрочного хранения полезных микроорганизмов сельскохозяйственного назначения в ведомственной коллекции Россельхозакадемии // *Сельскохозяйственная биология*. 2012. Т. 47. N 3. С. 32–36. <https://doi.org/10.15389/agrobiology.2012.3.32rus>

13. Printseva A.A., Sharova N.Y., Vybornova T.V., Manzhieva B.S. Features of low-temperature storage of streptomyces strains – producers of glycosidaz inhibitors // Food systems. 2019. Vol. 2. N 1. P. 20–22. <https://doi.org/10.21323/2618-9771-2019-2-1-20-22>

14. Sharova N.Yu., Vybornova T.V., Printseva A.A., Manzhieva B.S. The properties of the conidia of strains of the actinomycete *Streptomyces lucensis* and *Streptomyces violaceus* during storage at low temperatures // Food systems. 2018. Vol. 1. N 3. P. 27–32. <https://doi.org/10.21323/2618-9771-2018-1-3-27-32>

15. Выборнова Т.В., Шарова Н.Ю., Принцева А.А. Исследование влияния низких температур на сохранение жизнеспособности штаммов *Streptomyces* в процессе хранения // Техника и технология пищевых производств. 2018. Т. 48. N 3. С. 41–49. <https://doi.org/10.21603/2074-9414-2018-3-34-40>

16. Sharova N.Y. Biosynthesis of amylase inhibitor by Streptomyces cultures // Applied Biochemistry and Microbiology. 2016. Vol. 52. Issue 8. P. 723–732. <https://doi.org/10.1134/S000368381608007X>

17. Шарова Н.Ю., Ходкевич О.А., Поздняков

ва Т.А. Культурально-морфологические и физиолого-биохимические свойства новых штаммов актиномицетов – продуцентов ингибиторов гликозидаз // Вестник Российской академии сельскохозяйственных наук. 2009. N 3. С. 74–76.

18. V. Palanichamy, Aachari Hundet, Bhaskar Mitra, Narayana Reddy. Optimization of cultivation parameters for growth and pigment production by *Streptomyces* spp. isolated from marine sediment and rhizosphere soil // International Journal of Plant, Animal and Environmental Sciences. 2011. Vol. 1. Issue 3. P. 158–170.

19. Danevčič T., Boric M., Stopar D. Microbial Ecophysiology of *Vibrio ruber* // Food Technology and Biotechnology. 2014. Vol. 52. N 2. P. 198–203.

20. Rama Thakur, Vibhuti Rai. Isolation and Biochemical characterization of thermophilic *Streptomyces* from soil of Raipur District of Chhattisgarh, India // Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2011. Vol. 2. Issue 4. P. 795–808.

21. Mohanasrinivasan V., Sriramkalyan P., Ipsita N., Devi C.S. Selvarajan E., Suganthi V., et al. Fermentative production of extracellular pigment from *Streptomyces coelicolor* MSIS1 // Research journal of biotechnology. 2013. Vol. 8. Issue 4. P. 30–40.

Contribution

Tatyana V. Vybornova, Natalya Yu. Sharova, Anastasia A. Printseva carried out the experimental work, on the basis of the results summarized the material and wrote the manuscript. Tatyana V. Vybornova, Natalya Yu. Sharova, Anastasia A. Printseva have equal author's rights and bear equal responsibility for plagiarism.

Conflict of interests

The authors declare no conflict of interests regarding the publication of this article.

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