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INVESTIGATION THE LONG-TERM STORAGE OF AEROCOCCUS

ABSTRACT.

Methods of nondurable storage are one of the simplest, that don't mind an expensive equipment and at the same time are irreplaceable in everyday work with microorganisms.

Receipt the subcultures or periodical resowing on fresh agar medium that refers to the oldest and those that have become the traditional methods of support and preservation of bacterial cultures as in laboratory, as in industrial conditions. Interval between resowing depends on microorganism, medium that is used, temperature conditions of storage

Method of periodical resowing is simple in accomplishment and is used for a lot of microorganisms. It's accessible to everyone and easily allows controlling the quantity of strains.

A shortcoming of this method is the necessary of observance the regulations reshowing requirement in a lot of crockery, nutrient mediums, a lot of time, risk to pollute the culture, mistakes during signing the strains or attaching the wrong label, incidents of selection, risk to lose the culture. There are known the incidents of change the biological qualities of microbiological cultures or even their death.

By the help of drying the blood medium with pigmental colonies reach the effective immobilization of aerococcus cells in obtained pieces of dry nutrient medium.

Arrangement of dry pieces with immobilized aerococcus in sterile crockery prevents the contamination of aerococcus cultures with other microorganisms, covering the pieces with mineral oil prevents the destruction of aerococcus cells in a short time.

During the investigation there were determined different indices of vival activity of aerococcus: biochemical, physiological and morphological.

During the verification of storage time it was determined that the time of storage aerococcus by the declared way exceed in 10 times the time of storage the aerococcus by prototype.

Keywords: storage of microorganisms, aerococcus, retention cycle.

Formulation of the problem. Methods of nondurable storage are one of the simplest, that don't mind an expensive equipment and at the same time are irreplaceable in everyday work with microorganisms [1].

Analysis of recent research and publications. Receipt the subcultures or periodical resowing on fresh agar medium that refers to the oldest and those that have become the traditional methods of support and preservation of bacterial cultures as in laboratory, as in industrial conditions. Interval between resowing depends on microorganism, medium that is used, temperature conditions of storage [2, 3].

Method of periodical resowing is simple in accomplishment and is used for a lot of microorganisms. It's accessible to everyone and easily allows controlling the quantity of strains.

Isolation of unsolved aspects of the problem. A shortcoming of this method is the necessary of observance the regulations resowing requirement in a lot of crockery, nutrient mediums, a lot of time, risk to pollute the culture, mistakes during signing the strains or attaching the wrong label, incidents of celection, risk to lose the culture [4, 5]. There are known the incidents of change the biological qualities of microbiological cultures or even their death [6].

During frequent resowing strain-producer by the way of spontaneous dissociation often can lose or reduce the ability to producing of purpose products [7, 8].

The usage of standard mediums (beef-extract, caseic and other kinds of agars) doesn't suit for all types of microorganisms because of their physiological and biochemical peculiarities, this refers to microorganisms from the genus Aerococcus, that grow badly on simple nutrient mediums and need frequent resowing [9, 10].

By the help of experimental methods it was determined that microorganisms of genus Aerococcus grow well on nutrient mediums with animal or human blood. Usage of blood nutrient medium gives the opportunity to get the growth of aerococcus in a new quality, that looks like solid pigmented colonied of black color.

An object of the work is to examine the terms of storage aerococcus by using the new method of storage the cultures of aerococcus.

Results and discussion. Daily culture of aerococcus were sowed by lawn on 3 - 5 % blood nutrient agar in Petri dish, incubated at 37 ± 10 C during 7 -12 days until drying the nutrient medium was cut on pieces size 5 × 5 mm and put in sterile crockery (test-tubes, flasks) with further addition the sterile mineral oil up to the full cover the pieces with aerococcus and were put on refrigerator with the temperature 4 ± 10 C.

When it was necessary to get the culture of aerococcus in a necessary time a piece with immobilized aerococcus were sowed on 3 – 5% blood nutrient medium in Petri dishes. After the incubation there was get the culture of aerococcus.

By the help of drying the blood medium with pigmental colonies reach the effective immobilization of aerococcus cells in obtained pieces of dry nutrient medium.

Arrangement of dry pieces with immobilized aerococcus in sterile crockery prevents the contamination of aerococcus cultures with other microorganisms, covering the pieces with mineral oil prevents the destruction of aerococcus cells in a short time.

During the investigation there were determined different indices of vival activity of aerococcus: biochemical, physiological and morphological.

During the verification of storage time it was determined that the time of storage aerococcus by the declared way exceed in 10 times the time of storage the aerococcus by prototype (Table 1, 2, 3)

Table 1

The time of storage microorganism cultures of genus Aerococcus by the prototype and in the experiment

№ p.p.	Time of storaging the cultures (years)	Time of storaging aerococcus			
		In the control	3% blood agar	4% blood agar	5% Blood agar
1	0,5	+	+	+	+
2	1	-	+	+	+
3	2	-	+	+	+
4	3	-	+	+	+
5	4	-	-	+	+
6	5	-	-	+	+

Relationship of cause and effect of complex signs of new methods of storage aerococcus lies in: absence of necessary multiple-stage growing cultures of aerococcus for their further storage, long-term keeping in a immutable condition.

Table 2

Morphology of cells Aerococcus culture after long-term storage in comparison to referent strain of Aerococcus viridans 167

Morphology	After storage aerococcus			
	Aerococcus viridans 167 (in experiment) 0,5 y.	Aerococcus viridans 167 (3% blood agar) 3 y.	Aerococcus viridans 167 (4% blood agar) 5 y.	Aerococcus viridans 167 (5% blood agar) 5 y.
Growth on beef-extract agar (MPA)	To settle the quaternion and accumulation of irregular form, gram positive coccus	To settle the quaternion and accumulation of irregular form, gram positive coccus	To settle the quaternion and accumulation of irregular form, gram positive coccus	To settle the quaternion and accumulation of irregular form, gram positive coccus
MPA with 10% horse serum	Morphology is the same	Morphology is the same	Morphology is the same	Morphology is the same
Character of growth on: MPA	Growth is good, colonies (1-2 mm in diameter) with straight borders, convex that doesn't merge	Growth is good, colonies (1-2 mm in diameter) with straight borders, convex that doesn't merge	Growth is good, colonies (1-2 mm in diameter) with straight borders, convex that doesn't merge	Growth is good, colonies (1-2 mm in diameter) with straight borders, convex that doesn't merge
MPA+ 10% horse serum	Growth is poor. Small dotted colonies	Growth is poor. Small dotted colonies	Growth is poor. Small dotted colonies	Growth is poor. Small dotted colonies
MPB	Growth is good in the form of parietal and near-bottom sediment	Growth is good in the form of parietal and near-bottom sediment	Growth is good in the form of parietal and near-bottom sediment	Growth is good in the form of parietal and near-bottom sediment
MPA with 0,05% selenium sour sodium	Growth is good, big (2-3 mm in diameter), colonies of red colour	Growth is good, big (2-3 mm in diameter), colonies of red colour	Growth is good, big (2-3 mm in diameter), colonies of red colour	Growth is good, big (2-3 mm in diameter), colonies of red colour
MPA with 0,01% tellurium potassium	Growth is good, the colonies are medium sized (2-3 mm in diameter), colourless	Growth is good, the colonies are medium sized (2-3 mm in diameter), colourless	Growth is good, the colonies are medium sized (2-3 mm in diameter), colourless	Growth is good, the colonies are medium sized (2-3 mm in diameter), colourless
MGIA with 0,05% tellurium potassium	The growth is absent	The growth is absent	The growth is absent	The growth is absent
MPA with 3% blood	Growth is abundant. Big colonies (2-3 mm in diameter) that green blood agar	Growth is abundant. Big colonies (2-3 mm in diameter) that green blood agar	Growth is abundant. Big colonies (2-3 mm in diameter) that green blood agar	Growth is abundant. Big colonies (2-3 mm in diameter) that green blood agar

Table 3

Characteristics of aerococcus cultures after long-term storage in comparison to referent strain Aerococcus viridans 167

Characteristics of cultures	After storage aerococcus			
	A. viridans 167 (in experiment) 0,5y.	A. viridans 167 (3% blood agar) 3 y.	A. viridans 167 (4% blood agar) 5 y.	A. viridans 167 (5% blood agar) 5y
pH 9,6	Growth	Growth	Growth	Growth
45°C	Growth is absent	Growth is absent	Growth is absent	Growth is absent
Resistance to keeping on 60°C 30 min	Resistant	Resistant	Resistant	Resistant
in 15% solution H_20_2	Resistant	Resistant	Resistant	Resistant
Antibiotics MIK (mkg/ml)				
Penicilline	0,12	0,12	0,12	0,12
Oxacilline	0,12	0,12	0,12	0,12
Methicilline	0,12	0,12	0,12	0,12
Streptomycin	3,84	3,84	3,84	3,84
Lysozyme	1,92	1,92	1,92	1,92
Biclinocilline	0,12	0,12	0,12	0,12
Carbenicillin	3,84	3,84	3,84	3,84
Gramurin	1000	1000	1000	1000
Dioxidine	30,72	30,72	30,72	30,72
Nevigramon	750	750	750	750
Dimexide	500,0	500,0	500,0	500,0
Chinoxidine	1000	1000	1000	1000
Ascorbic acid	4000	4000	4000	4000
Boric acid	500,0	500,0	500,0	500,0
Chloramine	500,0	500,0	500,0	500,0
Reduction 1% methylene-blue in milk	No	No	No	No
Gelatine	Doesn't rarefy	Doesn't rarefy	Doesn't rarefy	Doesn't rarefy
Hydrolysis:				
Starch	Doesn't hydrolyze	Doesn't hydrolyze	Doesn't hydrolyze	Doesn't hydrolyze
Utilization of carbohydrates				
Arabinose	+	+	+	+
Rhamnose	_	_	_	_
Inosit	+	+	+	+
Xylose	+	+	+	+
Sorbite	_	_	_	_
Dulcitol	+	+	+	+
Glucose	+	+	+	+
Lactose	-	-	-	-
Maltose	+	+	+	+
Mannitol	+	+	+	+
Saccharose	+	+	+	+
Contents G+Ts in DNK-strains %	30-42	30-42	30-42	30-42

Additional advantages of this method are in the storage of all characteristics of aerococcus during long-term storage of all microorganisms of genus Aerococcus.

Conclusions and suggestions. By the help of experimental method it was established that microorganisms of genus Aerococcus grow well on nutrient mediums with addition animal or human blood. Usage of blood nutrient medium gives the opportunity to get the growth of aerococcus in a new quality, in the form of solid pigmented colonies of black colour.

Thanks to drying the blood medium with pigmented colonies reach effective immobilization of aerococcus cells in received pieces of dry nutrient medium.

Placement of dry pieces with immobilized aerococcus in sterile crockery prevents the contamination of aerococcus culture by another microorganisms and covering pieces with mineral oil prevents destruction of aerococcus cells in a short time.

Storage of sterile crockery with immobilized aerococcus in refrigerator in temperature 4 ± 1^0 C leads to long-term storage of aerococcus culture (3 – 5 years).

During verification the period of storage aerococcus it was determined that the time of storage aerococcus by the declared way exceed in 10 times the time of storage the aerococcus by prototype.

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