State Research Center
for Applied Microbiology and Biotechnology

Technical Activities Focused On Reorganization of Federal Culture Collection of Pathogen Microorganisms

Baranov A.M., Dunaitsev I. A., Dyatlov I.A.

Obolensk, Moscow Region, Russia

HISTORY

Microorganism Collection of State Research Center for Applied Microbiology and Biotechnology (SRCAMB) was established in 1980 to support fundamental and applied science programs.

COLLECTION FUND

- Infectious disease causative agents
- Entomopathogenic microorganisms
- Biological active substances producers
- Destructors of chemical materials
- Bacteriophages
- Somatic cell lines
- Hybridomas

FACILITIES

The Collection is located on Building # 1 of the SRCAMB and occupies about 600 m2.

FACILITIES

Collection Labs meet the requirement to biological safety levels:

BSL 1

BSL 2

BSL 3

FACILITIES

Cryoconservation and freeze-drying methods are used for a long-term storage of the collection cultures.

Storage of hybridoms in liquid nitrogen

Storage of freeze-dried bacteria.

INTRODUCTION

Most of the strains have been collected within the framework of Russian and international research projects.

The projects are focused on studying molecular mechanisms of pathogenicity, antibiotic resistance, anti-mosquito activity and phosphate-solubilizing potency of bacteria, fungi and other microorganisms.

However, the projects did not deal with comprehensive studies and long-term storage of the strains.

PURPOSE

Realization of R&D works and organizational and technical activities are directed on preservation and development of SRCAMB Collection of microorganisms to improve storage and maintenance conditions of the Culture Collection.

TASKS

- To make inventory of the basic collection; to draw up electronic catalogue of the strains
- To carry out the technical measures to guarantee appropriate conditions of collection culture storage.
- To introduce in practice modern methods for gene typing and biochemical identification of collection strains.
- To replenish the Collection with new strains of bacteria, fungi, bacteriophages and hybridomas.

METHODS

- External examination of ampoules with lyophilized material, withdrawal of the cultures with lost operational properties. Inventory and changing indistinct or incomplete labels. Filling of the electronic catalogue with inventory data.
- Biochemical testing of bacterial strains using API strips (bioMerieux, Inc.) and MIKRO-LA-TEST® plates (Czech Republic).
- Sequencing of the 16S rRNA genes to confirm some organisms identity. Molecular-genetic typing of M.tuberculosis strains by “spoligotyping" method, molecular-genetic typing of entomopathogenic fungi by RFLP-PCR and RAPD-PCR.

METHODS

- Producing of Hybridomas secreting monoclonal antibodies to bacterial pathogens by standard technique proposed by Köhler G., and Milstein C.
- Isolation of Bacteriophages possessing lytic activity against various species of pathogenic microorganisms from different samples (sewage waters, wound effluent, etc.).
- Long-term storage of the collection cultures by cryoconservation and freeze-drying methods.

RESULTS

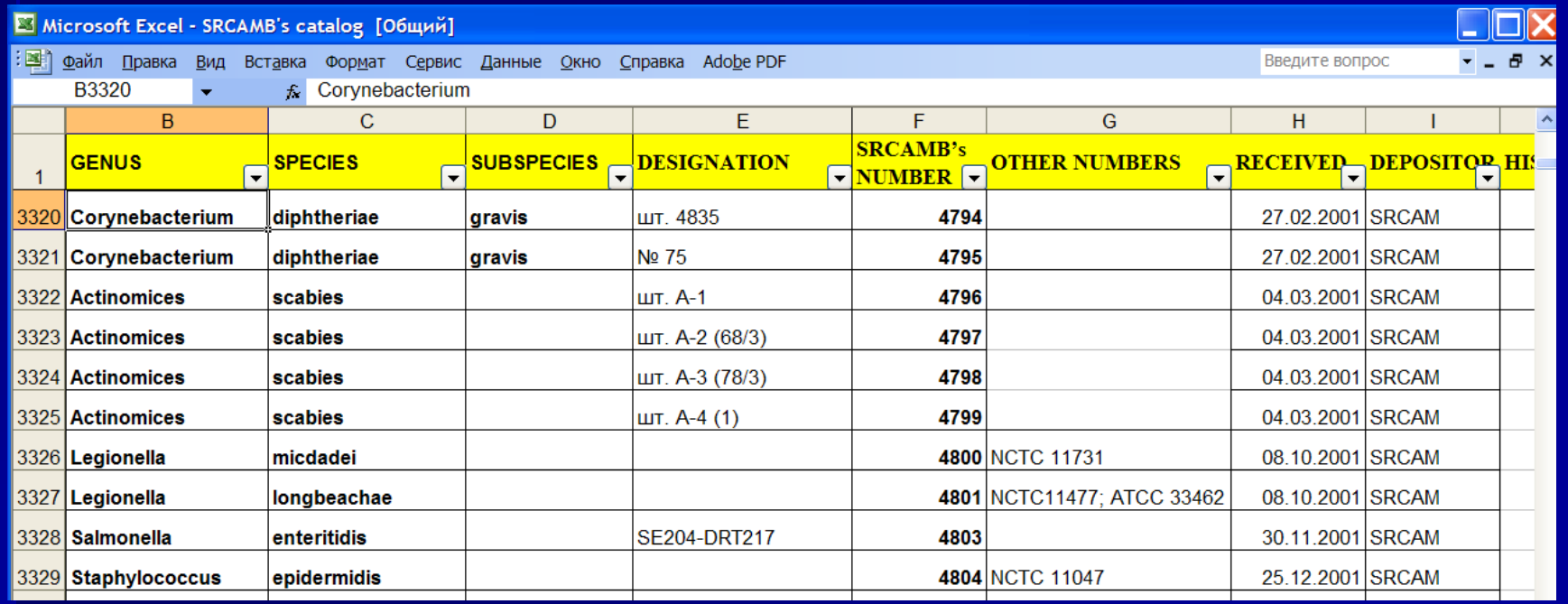
About 34 000 ampoules of bacteria of Risk Group 1-2 (BSL 1-2) from the basic Collection were inventoried and re-packed.

The inventory results

Genus	Species	Strains	Units
50	187	4555	33998

RESULTS

An electronic catalogue of the Collection cultures was made.



Microsoft Excel - SRCAMB's catalog [Общий]

Файл Правка Вид Вставка Формат Сервис Данные Окно Справка Adobe PDF

Введите вопрос

B3320 Corynebacterium

	B	C	D	E	F	G	H	I
1	GENUS	SPECIES	SUBSPECIES	DESIGNATION	SRCAMB's NUMBER	OTHER NUMBERS	RECEIVED	DEPOSITOR
3320	Corynebacterium	diphtheriae	gravis	шт. 4835	4794		27.02.2001	SRCAM
3321	Corynebacterium	diphtheriae	gravis	№ 75	4795		27.02.2001	SRCAM
3322	Actinomices	scabies		шт. A-1	4796		04.03.2001	SRCAM
3323	Actinomices	scabies		шт. A-2 (68/3)	4797		04.03.2001	SRCAM
3324	Actinomices	scabies		шт. A-3 (78/3)	4798		04.03.2001	SRCAM
3325	Actinomices	scabies		шт. A-4 (1)	4799		04.03.2001	SRCAM
3326	Legionella	micdadei			4800	NCTC 11731	08.10.2001	SRCAM
3327	Legionella	longbeachae			4801	NCTC11477; ATCC 33462	08.10.2001	SRCAM
3328	Salmonella	enteritidis		SE204-DRT217	4803		30.11.2001	SRCAM
3329	Staphylococcus	epidermidis			4804	NCTC 11047	25.12.2001	SRCAM

RESULTS

A semi-automatic system for biochemical identification of microorganisms "Automated place of microbiologist, chemotherapeutist" on the base of photometer «Multiscan Ascent» was applied as one of the methods for identification of collection strains.

The identification system.

"Multiscan Ascent", Finland

RESULTS

The system allows to identify more then 360 microorganisms, including fermentative and non-fermentative enterobacteria, streptococci, staphylococci and enterococci, anaerobic bacteria, etc. during 24-48 h after pure culture isolation. 96-well plate test-systems «PLIVA Lachema» (Czech Republic) are used.

Results of tests are comparing to a computer database. The information is obtained in a document describing:

1. The most probable genus and species of microorganisms
2. Per cent of similarity (probability), i.e., correspondence with other taxons included in the database.
3. Index of typicalness, i.e., correspondence with the type of taxon.

RESULTS

15 new hybridoma clones secreting antibodies to different pathogens were produced and deposited

Hybridomas production.

New deposited hybridomas

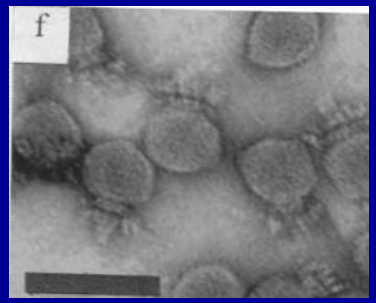
Specificity of antibodies	Clone number
E. coli O157:H7	3
spores B. anthracis STI-1	2
Pseudomonas aeruginosa	2
Salmonella enteritidis	2
Listeria monocytogenes	2
Helicobacter pylori	2
Recombinant listeriolysin-O	2

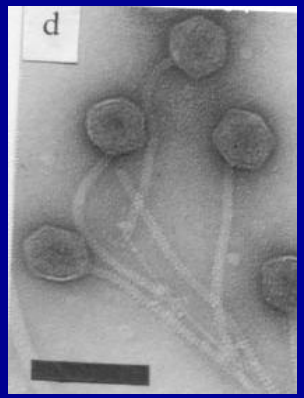
RESULTS

16 new bacteriophages, prospective therapeutic agents, were isolated and deposited

New deposited bacteriophages

Lytic activity	Strain number	Lytic activity	Strain number
Streptococcus pyogenes	1	Serratia marcescens	1
Staphylococcus aureus	1	Citrobacter freundii	2
Salmonella enteritidis	2	Hafnia alvei	1
Yersinia enterocolitica	2	Escherichia coli	1
Shigella sonnei	1	Klebsiella pneumoniae	1
Enterebacter aerogenes	1	Klebsiella oxytoca	1
Bacillus cereus	1		





Bacteriophages of SRCAMB collection

RESULTS

607 strains of M. tuberculosis were subdivided into spoligofamilies

Spolygo-family	Spolygotype	Spolygopattern
Beijing	000000000003771	
Haarlem	777637761360771	
Haarlem2	600000004020731	
Haarlem3	777777607720771	
Haarlem4	774777777420771	
LAM	777763007760771	
LAM1	777740000360771	
LAM4	777777607760731	
LAM9	777477607760771	
T	777777777060771	
T1	777777777760771	
T1 RUS2	770000777760771	
T2	770000377740000	

RESULTS

49 entomopathogenic fungous strains were typed by RFLP and RAPD-PCR methods

Strain	Genetic group
Beauveria bassiana 2055	European
Beauveria bassiana AS-622	
Beauveria bassiana 2097/2	
Beauveria bassiana 2190/1	
Beauveria bassiana VL-2231	
Beauveria bassiana Б-533	Crimean-Caucasian
Beauveria bassiana L-1399	
Beauveria bassiana L-1499	
Beauveria bassiana L-1587	
Beauveria bassiana Б-174	Far Eastern A
Beauveria bassiana Б-182	
Beauveria bassiana Б-207	Far Eastern B
Beauveria bassiana Б-208	

Genetic groups of Beauveria bassiana

RESULTS

More than 150 new strains of pathogenic bacteria from working collections of SRCAMB Laboratories and from other institutions subordinated to Rospotrebnadzor were deposited in the Collection.

RESULTS

For lyophilization of depositing cultures was used new modern equipment.

Freeze-drying process

Flasks with freeze-dried cultures

CONCLUSION

As a result of this work, the collection laboratories have been supplied with the equipment for genetic, biochemical and morphological identifications of microorganisms, for conservation and long-term storage of collection cultures.

The collection fund was inventoried and was filled up with new cultures, necessary for research of infectious causative agents.

ACKNOWLEDGEMENTS

This work was done within the framework of ISTC Project #2754.2 " Reorganization of bacterial and cell culture Collection to study infectious diseases in humans and animals".

The work was performed according to the Agreement with International Science and Technology Center (ISTC), Moscow and under financial support of DFAIT, Canada.

FOREIGN COLLABORATORS

- Foreign Affairs Canada,
  Senior Biosafety Adviser,
  Ms. Maureen Ellis
- Public Health Agency of Canada,
  Head of Special Bacteriology Section,
  Ms. Kathryn Bernard
- Center for Disease Control (CDC), Associated Director on Science,
  Dr. Stephen Morse

Thanks for your attention!