

INSTITUTE OF PLANT
PROTECTION
NATIONAL RESEARCH
INSTITUTE,
POZNAŃ, POLAND

Management of Collection of Plant Pathogens in Institute of Plant Pathogens – National Research Institute

Bank of Plant Pathogens
and Investigation on Their Biodiversity

Sylwia Stępniewska-Jarosz



History

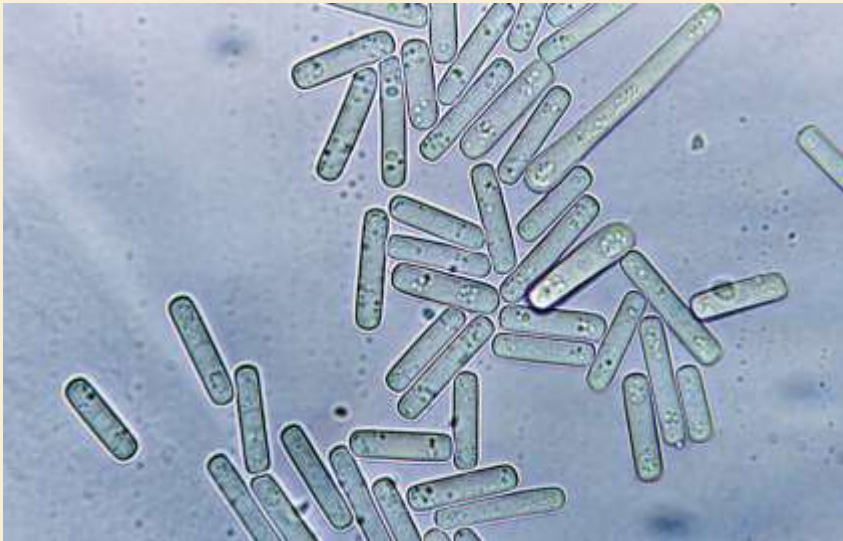
- **1994** – Institute of Plant Protection in Poznan started organizing the Bank of Plant Pathogens (BPP)
- The decision was taken in the Ministry of Agriculture and Food Economy within the frames of the „Genomic resources of cultivated plants” programme.
- **1996** – Bank of Plant Pathogens officially opened
- **2004** – name changed to

The Bank of Plant Pathogens
and Investigation on Their Biodiversity



INTRODUCTION

- **2014** - stored almost 2000 cultures of fungi (*Fusarium*, *Alternaria*, *Colletotrichum*, *Verticillium*) and nearly 200 strains of bacteria (*Clavibacter*, *Paenibacillus*, *Pseudomonas*, *Xanthomonas*, *Chryseobacterium*, *Pectobacterium*, *Erwinia* and *Rhizobium*)



- An advantage of Collection of Plant Pathogens is to keep pathogens originated mainly from **different host-plants from Poland** (genetically different from pathogens deposited in Western Europe collections)



AIM OF COLLECTION

- The aim is to provide reference strains for:
 - research,
 - education,
 - comparison
(as reference strains, standards).

Cooperation –
Main Inspectorate
of Plant Health
and Seed Inspection



COLLECTION MISSION

Collection tasks

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graph TD; A[Collection tasks] --- B[Searching for a new strains with useful characteristic]; A --- C[Classification and identification of microorganisms]; A --- D[Research on selection of effective methods of microorganisms storage in laboratory conditions];
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Searching for
a new strains
with useful characteristic

Classification
and identification
of microorganisms

Research on selection
of effective methods
of microorganisms storage
in laboratory conditions

Collection

- 77% - isolates from Poland
- The most numerous genus of fungi:
 - *Fusarium* spp. (22 species),
 - *Alternaria* spp. (11 species),
 - *Colletotrichum* spp. (6 species),
 - *Phytophthora* spp. (12 species),
 - *Verticillium* spp. (6 species),
 - *Sclerotinia* spp., *Rhizoctonia* spp.,
Botrytis spp., *Cladosporium* spp. and *Phoma* spp.



Collection

- Bacteria are represented mainly by types:
 - *Erwinia* spp. (7 species),
 - *Pseudomonas* spp. (7 species),
 - *Clavibacter* sp.,
 - *Xanthomonas* spp. (4 species),
 - *Agrobacterium* sp.,
 - *Pantoea* spp.

It is necessary to develop the catalog for collected strains with short description.

For each strain is established documentation containing:

- number in collection
- the name of the species and strain synonyms
- date and place of isolation and date of introduction to a collection
- storage method
- morphological characteristics (description of colonies, shape and size of spores, specific structures, etc.)
- photographs, drawings



BANK PATOGENÓW

Gatunek: *Fusarium avenaceum* Nr: 1657
(Fr.) Sacc.

Opis kolonii

Wykonawca: A. Pilska

Medium: PDA

Temperatura: 27°C

Kolonія 5 dni: Ø 63 mm

Wygląd 5 dni: gęsta, puszysta biała (powietrze) a przy poszukiwaniu ciemniejsza z odrobiną żółtym brzegi nieopadają. R: ciemnoczerwony

Kolonія 10 dni: Ø 80 mm

Wygląd 10 dni: gęsta, puszysta ciemnoczerwona, g. powietrze biała, brzegi nieopadają R: ciemnoczerwony

Akces

Data akcesu: 20.09.06 S119

Przyjmujący: A. Pilska

Symbol izolatu: jplmweat 8

Depozytor:

Identyfikacja: A. Pilska

Opracowanie: A. Pilska

Data izolacji: 07.2006

Miejsce izolacji: Złotówka

Roślina: jplmweat

Pożywka: PDA

Pomiary

3 × 22 (2) μm

3 × 21 (2)

3 × 21 (2)

3 × 14 (2)

3 × 51 (5) μm

3 × 57 (5)

3 × 50 (5)

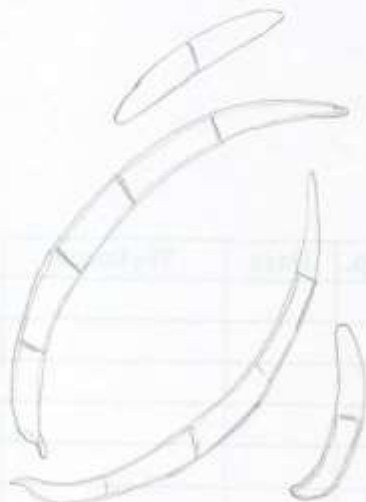
3 × 43 (4)

3 × 50 (5)

3 × 53 (5)

3 × 54 (5)

3 × (50 - 57) μm (5 komorowe)



Uwagi



Fusarium sporotrichioides chlamydospores



F. crookwellense conidia

New isolate

Documantation

Storage

Description

Photography

Under oil layer
16°C

Conservation

macroscopic

in 10% glycerol
solution - 80°C

microscopic

lyophilization

Liquid nitrogen
-196°C

THERE IS NO UNIVERSAL STORAGE METHOD!

Each pathogen is preserved by at least two complementary methods:

- on PDA medium under mineral oil layer
 - all pathogenic fungi
 - method used since the beginning of the collection
 - fungi are stored on agar slants, in universal bottles





A collection of pathogens stored in bottles
on agar slants under mineral oil at 16°C



Advantages of the method:

- Useful for sporulating and not sporulating pathogens,
- oil layer provides protection of mycelium against mites and other contaminants and prevents dehydration of the mycelium,
- sample is reusable, so there is no need to perform a number of samples,
- uncomplicated method of execution,
- does not require specialized equipment.

Disadvantages of the method:

- low physiological stability of the pathogen,
- culture is alive, constantly growing, is changing and every few years needs renovation, need for selection of the medium,
- pathogenic fungi stored in this way quite quickly lose their pathogenicity.

Storage under mineral oil

No.	Genus	Number of species	Number of isolates
1.	<i>Fusarium</i> spp.	16	548
2.	<i>Verticillium</i> spp.	6	186
3.	<i>Alternaria</i> spp.	10	169
4.	<i>Colletotrichum</i> spp.	7	97
5.	<i>Rhizoctonia</i> spp.	2	85
6.	<i>Botrytis</i> spp.	5	83
7.	<i>Phytophthora</i> spp.	11	63
8.	<i>Cladosporium</i> spp.	4	52
9.	<i>Phoma</i> spp.	14	44



Conservation by lyophilization

Lyophilization is a freeze-drying (sublimation - the process of change from solid phase to gas directly, without the liquid phase), takes place at low temperatures (-40°C) under reduced pressure. The freeze-drying process, first material is frozen at a temperature below 0°C . Then the water in the form of ice crystals is evaporated in vacuum chambers. The last step in the process is the final drying of.

The method useful for the preservation of the bacteria and sporulated fungi only (not for *Rhizoctonia*, *Pythium*, *Phytophthora*, *Sclerotinia*)

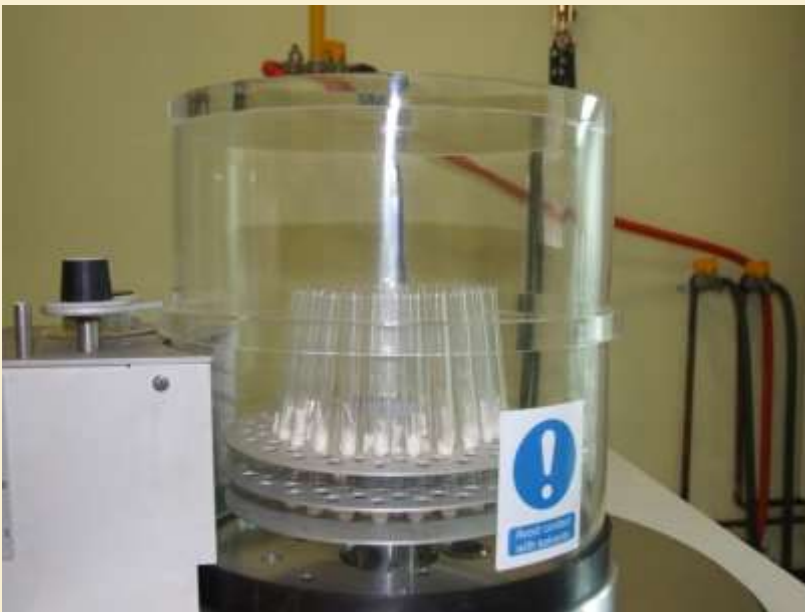
Lyophilization is carried out using a lyophilizer in centrifuge version

 **EDWARDS**
company.



Lyophilization

Complicated, long and expensive process.



- Ampoules with lyophilized microorganism are stored in an air-conditioned room at 17°C.



Advantages of lyophilization:

- preparation of a few to several identical samples of the culture,
- long periods of storage,
- microorganisms are easy to restore,
- High stability during storage,
- easy distribution of isolates,
- contamination of stored samples are not possible.

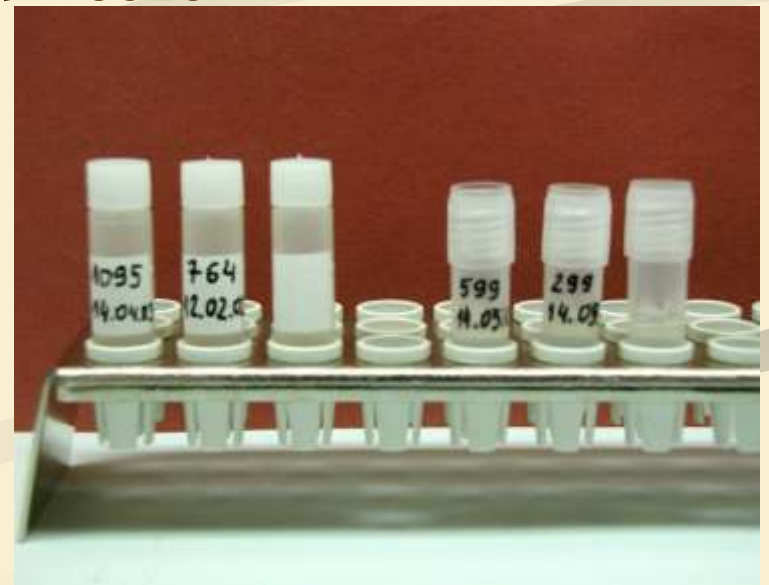
Freezing in 10% solution of glycerol

- temperature down to -75°C
- the metabolism of pathogens is kept at a low level by the use of deep-freezing
- 10% glycerol as cryoprotectant is used
- simple and inexpensive way to store fungi (sporulated and not sporulated)

This method provides a good survival rate and keeps the pathogenicity of isolates (physiological stability)

Freezing in 10% solution of glycerol

- some medium discs (diameter of 0.5 cm) overgrown with mycelium are placed in cryogenic ampoules filled with 10% solution of glycerol
- ampoules are desiccated and cooled (2 hrs/4 ° C)
- ampoules are placed in boxes and freeze (24 hours / -20°C)
- finally samples are placed in a freezer at -80°C



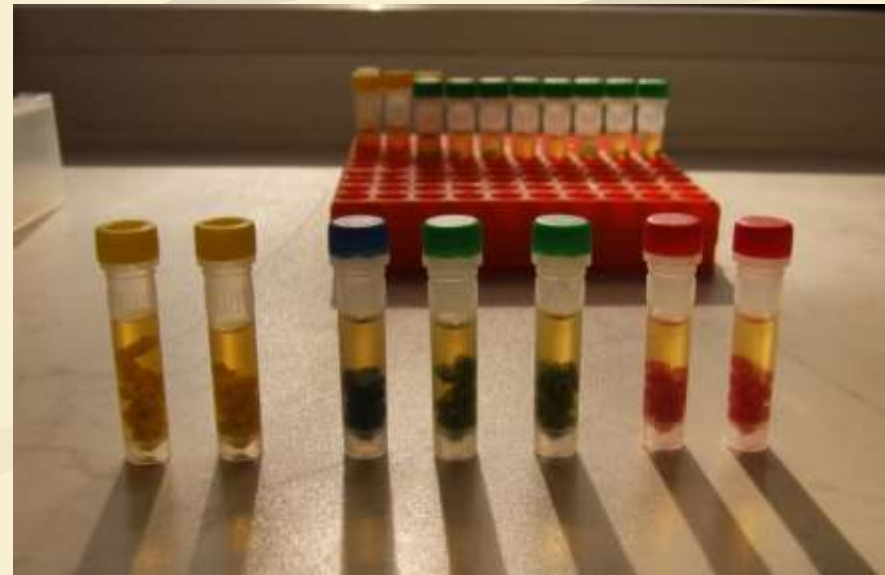
Disadvantages of freezing in 10% glycerol solution

- it is necessary to maintain a constant temperature
- samples are single use



Freezing using Bacto Protect system

- in BPP used since 1997
- convenient and easy to use system for storage of bacteria and sporulated fungi
- tubes containing approx. 25 colored ceramic beads immersed in cryoprotectant



Bacto Protect system

- single colony of bacteria/fragment of sporulated mycelium are transferred into tubes of Bacto-Protect,
- after mixing the suspension in the tube, incubated at room temperature about 5 minutes,
- microorganism covers ceramic beads by adhesion,
- cryoprotectant should be removed from tubes,
- finally samples are stored in -80°C .



Advantages of Bacto Protect system:

- quick and simple procedure
- possibility of multiple use of one tube
- ability to store isolates over 5 years
- method allows to reduce the frequent passage of strains (for new strains for further study)

Disadvantages

- the need to secure the chamber in case of power failure
- cost

Freezing in liquid nitrogen at -196°C

- Liquid nitrogen is stored in insulating storage vessel (**vacuum flask** also known as a **Dewar flask, Dewar bottle** or **Thermos**).

Isolates are stored at a temperature in the range from -196°C to -155°C , and under pressure from atmospheric to approx. 20 bar



Preparation of cultures

- 3-4 discs (\varnothing 0,5 cm) of solid medium overgrown with fungus are placed in cryogenic ampule containing 1,2ml of 10% glycerol solution. The ampule is tightly closed.
- Tubes with samples are placed in a rack and cooled for 2 hours in a refrigerator (4°C).
- The ampules are placed in a freezer NICOOL LM10 and freeze for 2 hours (cooling rate $1^{\circ}\text{C}/\text{min.}$)

- Samples are immersed in a liquid nitrogen in freezer thermos for about 10 minutes.
- Frozen ampules are placed in cryogenic holders and transferred to a container with liquid nitrogen GT 35 or XT 34 – Taylor Wharton



Advantages of storage in liquid nitrogen

- currently the best method of conservation due to the good isolates survival without losing their pathogenicity (for sporulating and not sporulating fungi),
- indefinite shelf life of isolates at a temperature of -130 do -190°C (fluctuations in temperature can cause the changes or destruction of preserved samples).

Disadvantages of isolates storage in liquid nitrogen

- the most expensive method of conservation (cost of equipment, nitrogen),
- difficulty in determination of the rate of isolates freezing,
- single use of sample,
- danger of work resulting from the physical properties of liquid nitrogen (protective clothing, room),
- the need to constantly refill the liquid nitrogen.

Comparison of preservation methods

Conservation method	Preservation temperature	Preservation time	Pathogen stability	Difficulty	Cost of materials
Preservation under oil	16°C	1-32 years	low	easy	cheap
Preservation under glycerol	16°C	6 months	average	easy	cheap
Preservation under water	16°C	2-5 years	average	easy	cheap
Lyofilisation	16°C	4-40 years	high	difficult	expensive
Freezing in 10% glycerol	-80°C	4-5 years	high	easy	cheap
Bacto-Protect System	-80°C	3-5 years	high	easy	average
Freezing in liquid nitrogen	-196°C	unlimited	high	difficult	expensive

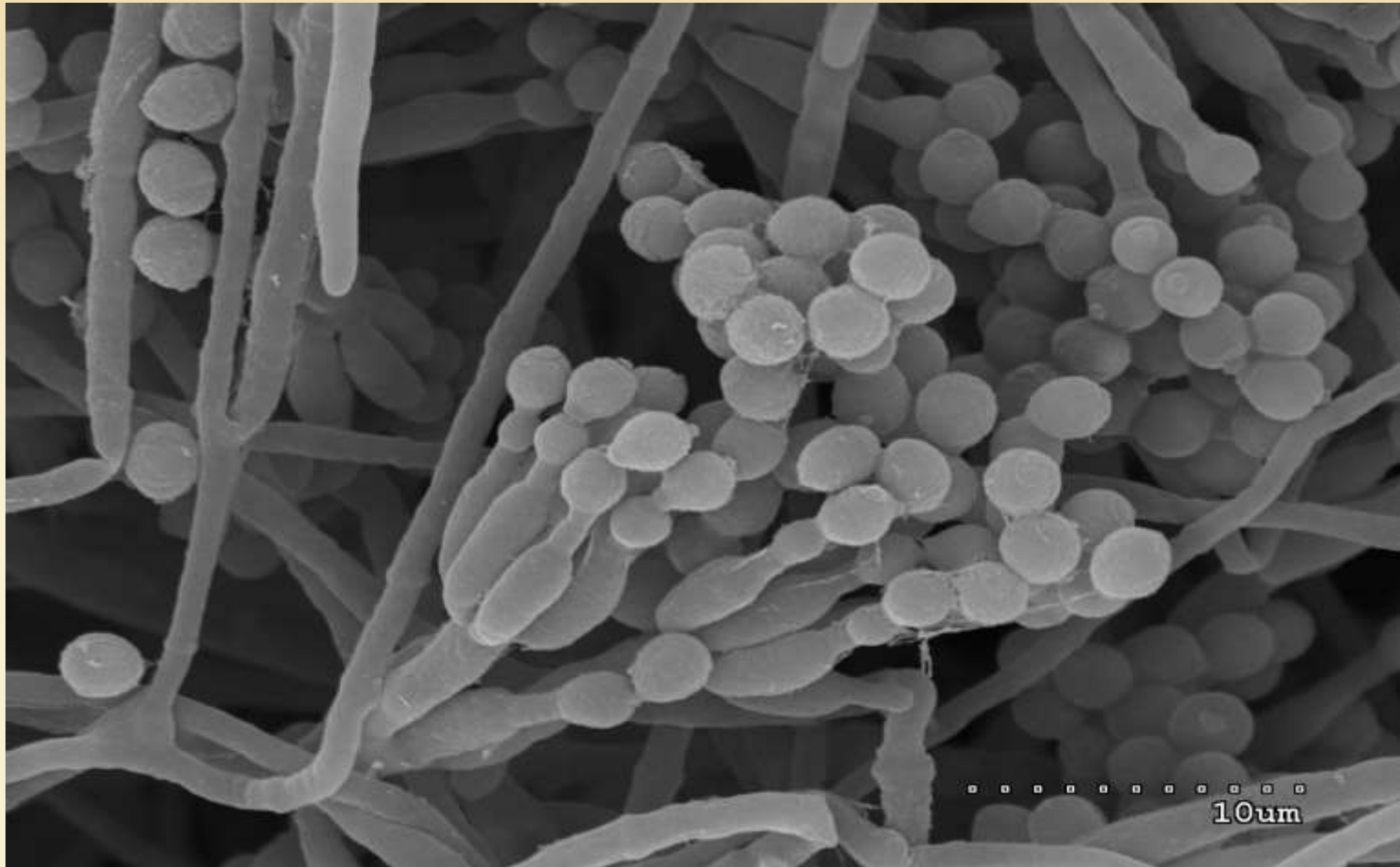


**The information
of the pathogens maintained
in the collection**

is available on the website:

<http://bankpat.expertus.com.pl/search/>





Thank you