

Comparison of microbial preservation methods: a narrative review

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Abstract

Many microorganisms are used to produce antibiotics, vaccines, and medicines for various diseases, and preserving these microorganisms and viruses can ensure that the development process is streamlined. We have reviewed the short-term and long-term microbial and viral preservation methods including novel and emerging technologies. Short-term preservation methods of microorganisms are designed to maintain the viability of the organisms for periods ranging from a few days up to one year. The novel microfluid systems allow for the creation of microenvironments that support the growth and proliferation of specific microorganisms and the isolation of individual cells or small populations of microorganisms for studying microbial behavior and interactions. Long-term preservation involves storing the organisms for an extended period, ranging from months to decades, while retaining their viability and genetic stability. The mineral oil or liquid paraffin storage, storage in distilled water, storage in sterile soil, lyophilization, and cryopreservation are well known, and encapsulation of nanoparticles to preserve microorganisms, electrospinning, and electro spraying and supercooling are novel and emerging methods. Each short and long-term microbial and viral preservation method has advantages and disadvantages, and, based on the requirement, the appropriate method can be chosen.

Keywords Microorganisms, viruses, short-term, long-term, preservation, novel methods.

Introduction

Bacteria, fungi, and viral agent preservation is maintaining microorganisms and viruses in a viable and stable state for an extended period.¹ It is always required to provide the specific environmental and nutritional requirements to maintain viability.^{2,3}

Many microorganisms are used to produce antibiotics, vaccines, and medicines for various

diseases, and preserving these microorganisms and viruses can ensure that the development process is streamlined and carried over. Preserved microorganisms and viruses play a critical role in agriculture, where they help to fix nitrogen in soil, decompose organic matter, and promote plant growth.^{2,4,5} In clinical microbiology and research laboratories, preserving pathogens or isolates for future reference and analysis is important.⁵

Different methods and techniques are used for preserving pathogens or isolates, and the choice of preservation method depends on various factors such as the type of microorganism, the intended duration of storage, and the intended future applications.¹⁻⁷ The studies and comprehensive reviews providing the most appropriate methods and the duration of preservation for each microbe and virus are scant. Also, comparative reviews with novel methods are less available.

Here, we have reviewed the short-term and long-term microbial and viral preservation methods and compared and contrasted the novel and emerging technologies with previous methods.

Received: 20 August 2024; revised: 17 December 2024; accepted: 23 December 2024.

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Article downloaded from www.germs.ro

Published December 2024

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ISSN 2248 - 2997

ISSN - L = 2248 - 2997

Microorganism and virus preservation methods

Microorganism preservation can be broadly divided into short-term and long-term preservation (Figure 1).

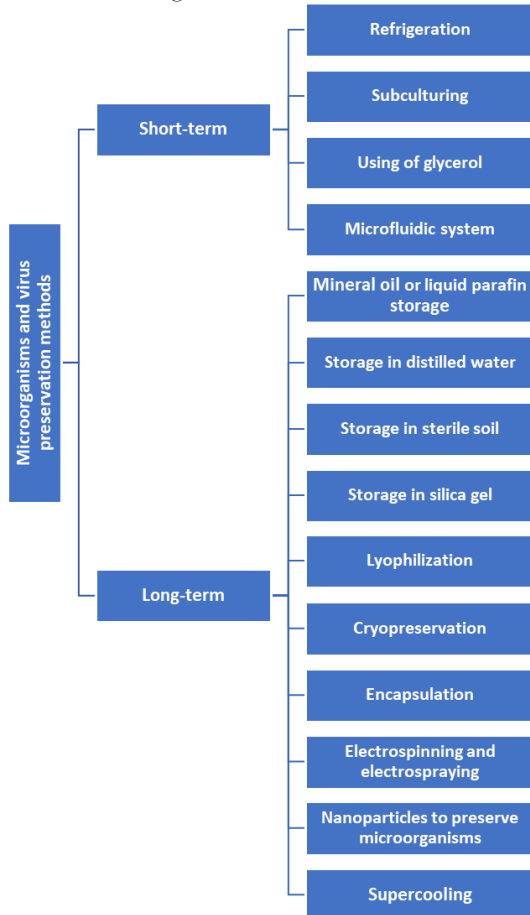


Figure 1. Microorganisms and viruses short- and long-term preservation methods

1. Short-preservation methods

Short-term preservation methods for microorganisms are designed to maintain their viability for periods ranging from a few days to one year.

1.1. Refrigeration

Refrigeration involves storing the microorganisms at temperatures between 2-8°C, which can slow their metabolic rate, preventing or delaying their growth and prolonging their viability.^{3,4}

S. pneumoniae, *H. influenzae*, and *N. meningitidis* are highly susceptible to low temperatures. The cold temperature negatively

affects the metabolic processes, enzymatic reactions, and overall cellular functions required for the survival of the bacterium.⁷

Influenza viruses, including seasonal influenza strains and the more well-known strains like H1N1 and H3N2, are highly labile and require specialized storage methods such as ultra-low temperature freezers or preservation in liquid nitrogen for long-term viability.³

1.2. Subculturing

Subculturing, which involves transferring a small amount of the original culture to a sterile and fresh medium, is another commonly used method for the short-term preservation of microorganisms.^{1,4} Table 1 displays the turnover time and subculturing frequency of commonly used microbes.

Preservation of bacteria

Preservation can be challenging when working with fastidious bacteria with specific nutritional and environmental requirements.^{8,9} Subculturing under antibiotic pressure can result in the emergence of antibiotic-resistant subpopulations due to random mutations or horizontal gene transfer. Additionally, subculturing without specific nutrients can adapt bacterial subpopulations that can utilize alternative resources. The genetic changes during subculturing can affect the expression or functionality of virulence factors in pathogens, resulting in subpopulations with different levels of pathogenicity.⁸

Preservation of fungi

At 4°C, most filamentous fungi may survive for at least one to two years. Superficial fungi such as *Malassezia furfur* can be sub-cultured on Dixon's Agar or Leeming-Notman agar. They should be incubated at 30°C for 5 to 7 days to promote optimal growth. For dimorphic fungi such as *Histoplasma capsulatum*, subcultures are typically grown on Sabouraud-dextrose agar (SDA) at room temperature (25°C) for a period of 2 to 4 weeks.¹⁰ Dermatophytes, including *Microsporum audouinii*, are commonly sub-cultured on Sabouraud glucose agar at a temperature of 26°C. The cultures should be incubated for 2 to 4 weeks. Yeast species like *Cryptococcus neoformans* can be subcultured on SDA and incubated at

Table 1. Turnover time and subculturing frequency of commonly used microbes

Microorganism/virus	Turnover time	Subculturing frequency
Bacteria (<i>E. coli</i>)	24-48 hours	Daily
Yeast (<i>Saccharomyces cerevisiae</i>)	24-48 hours	Daily
Fungi (<i>Aspergillus niger</i>)	5-7 days	Weekly
Viruses (influenza virus)	No subculturing	No subculturing

30°C for 48 to 72 hours.¹⁰ Opportunistic fungi, such as *Candida albicans*, are sub-cultured on SDA and incubated at 30°C for 24 to 72 hours.¹¹

Preservation of viruses

Some common types of cultures used for virus preservation include cell cultures, embryonated eggs, animal models, insects, and artificial matrices. Cell cultures can be used for many types of viruses, including herpes simplex virus (HSV), cytomegalovirus, human papillomavirus, hepatitis B virus, and human immunodeficiency virus. Influenza virus is commonly preserved using embryonated eggs. Animal models can preserve rabies virus by maintaining it in animal models, such as mice or hamsters. Dengue virus and yellow fever virus can be preserved by maintaining them in mosquito hosts.^{1,12}

1.3. Using glycerol

Preservation of bacteria

The viability of different organisms in glycerol can vary. *E. coli* and *S. pneumoniae* can remain viable for up to 5 months,⁴ while *H. influenzae* can remain viable for up to 4 months. On the other hand, *N. meningitidis* can remain viable for 6 weeks, and *N. gonorrhoeae* remains viable for 3 weeks. *Listeria monocytogenes* can be stored in 20% glycerol at -80°C.¹³

Preservation of fungi

The concentration of glycerol used is typically between 10-20% v/v.¹⁴ Examples of fungi that can be preserved using glycerol are *Aspergillus* spp. and *Penicillium* spp.¹⁵

Preservation of viruses

The higher glycerol concentration might have a virucidal effect because it dehydrates the viruses. Therefore, optimizing the glycerol concentration for each virus and cell type is important.

The RNA of the rabies virus was successfully preserved in 50% glycerol at -70°C for up to one

year.¹⁶ Glycerol is effective in preserving the viability of a wide range of viruses, including enveloped viruses, HSV-I, and non-enveloped viruses, poliovirus type 1 and the concentration of 85% glycerol appears to be preferred to preserve them because it does not affect the breakdown of extracellular DNA.^{12,13}

1.4. Microfluidic system

Microfluidic systems allow for the creation of microenvironments that support the growth and proliferation of specific microorganisms and the isolation of individual cells or small populations of microorganisms for studying microbial behavior and interactions.¹⁷ One example of a microfluidic system for microbial preservation is the microfluidic cell trap array, which uses microfabricated channels and traps to capture and preserve individual microorganisms. While microfluidic systems have the potential to provide more precise and efficient preservation methods compared to traditional techniques, more research is needed to optimize these systems and ensure their long-term viability for microbial preservation. Bacteria and fungi can be preserved using this technique, such as *E. coli* and *Saccharomyces cerevisiae*, ranging from days to weeks or even longer.¹⁸

Short-term preservation methods of microbes, with advantages and disadvantages, are displayed in Table 2.

2. Long-term preservation methods

Long-term preservation of microorganisms involves storing the organisms for an extended period, ranging from months to decades while retaining their viability and genetic stability.

2.1. Mineral oil or liquid paraffin storage

The method involves the addition of mineral oil or liquid paraffin to the culture medium, covering the top layer of the medium and the microorganisms, forming a barrier between the

Table 2. Short-term preservation methods of microbes with advantages and disadvantages

Preservation method	Method	Advantages	Disadvantages
Refrigeration	Preservation under the temperature between 2-8°C for weeks to a few months	Cost-effective; easy to use; can preserve a wide range of microorganisms	Cultures can lose viability faster than frozen stocks; susceptibility to contamination
Subculturing	Preservation under the temperature range for subculturing fungi (between 25°C and 30°C for a period of 1 to 4 weeks) or bacteria (between 35°C and 37°C for a period of 24 to 48 hours)	Useful for maintaining genetic and physiological characteristics of the original strain	Time-consuming; labor-intensive; not suitable for large-scale preservation
Glycerol preservation	The typical temperature range for glycerol preservation of fungi, bacteria, and viruses between -80°C and -196°C for weeks to a few months	Long-term storage; viability maintained; widely used	Risk of contamination; requires specialized equipment; can alter characteristics of microorganisms
Microfluidic systems	More research need	Controlled environment; well-suited for high-throughput screening; precise and efficient preservation	More research is needed to optimize and ensure long-term viability; may not be suitable for all microorganisms; requires specialized equipment

organisms and oxygen. This creates an oxygen-free environment that prevents the growth and multiplication of microorganisms, preserving their viability for an extended period.¹⁶

One of the most significant benefits is its ability to maintain the viability of microorganisms for months to years. When stored under mineral oils, pathogenic bacteria, such as *E. coli* and *S. aureus*, may lose virulence or pathogenicity over time.¹⁹

Preservation of bacteria

Using this method, some bacterial genera, such as *Bacillus* spp., can be preserved for up to 8-12 years, and *Mycobacterium* spp. and *Azotobacter* spp. for 7-10 years.^{11,20}

However, it is important to note that preserving bacterial cultures using paraffin oil is not foolproof. Therefore, it is crucial to maintain a clean and sterile environment when working with bacterial cultures, regardless of the preservation method used.²⁰

Preservation of fungi

At room temperature or between 15° and 20°C, cultures can be stored even for a few years

(2, 4 or, in extreme circumstances, up to 32 years). This approach is suitable for mycelial or nonsporulating fungi cultures that cannot be frozen or dried using freeze-drying technology. The main drawback of the method is that the fungi keep growing, which opens the possibility of selection for mutants.¹⁸

A 10 mm layer of paraffin or oil covers fungi cultures grown on agar slants. Periodically check the oil level in the tubes, add additional oil if needed. *Pleurotus* spp., *Aspergillus* spp., *Trichophyton mentagrophytes*, and *C. albicans* can be preserved using the paraffin oil.^{18,21}

2.2. Storage in distilled water

Sterile distilled water is a medium for the long-term preservation of microorganisms because it is sterile and free from any nutrients that might encourage the growth of the microorganisms. However, over time, the distilled water can become depleted of oxygen and nutrients, leading to a decline in the viability of the microorganisms.²²

Preservation of bacteria

The researchers found that bacteria could survive longer in water with a neutral pH (pH 7) compared to acidic or alkaline water. A wide range of bacteria can be stored, including *P. fluorescens*, *Erwinia* spp., *Xanthomonas campestris*, *Salmonella* spp., *Yersinia enterocolitica*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *S. aureus*, *Rhizobium* spp. and *Pseudomonas* spp. Suspending the organisms before inoculation in a screw-capped tube containing phosphate-buffered saline at pH 7.2 would be useful.^{8,11,21}

Preservation of fungi

The main concerns with this technique are using an appropriate volume of water to inoculum blocks, which should be at least 40 times more significant, and avoiding evaporative water loss from poorly sealed storage tubes by periodically adding sterile water to maintain the necessary water level. Fungi such as *C. albicans*, *Cladosporium* spp., *Penicillium* spp., *Cephalosporium* spp., *A. niger*, and *A. fumigatus* can be preserved.^{17,23}

2.3. Storage in sterile soil

The method of preserving spore-forming bacteria and fungi by storing them in sterile soil is widely accepted. This method involves placing the microorganisms in a dormant state within the sterile soil. The soil is sterilized, and a spore suspension is added using aseptic techniques. Studies have shown that this method is highly effective in preserving the viability of spore-forming microorganisms, with some organisms remaining viable for up to 70-80 years.²⁴

Preservation of bacteria

This technique has been used to store some bacteria species that produce spores, particularly Clostridia. Additionally, it has been utilized to store bacilli and *Azotobacter* spp. non-sporulating bacteria that may not survive well in the lyophilization process can also be stored in soil using this method (*E. coli*). *Bacillus subtilis*, *Pseudomonas fluorescens*, and *Rhizobium* spp. can be preserved using this method.^{16,23}

Preservation of fungi

This low-maintenance and economical technique is suitable for *Fusarium* spp., *Penicillium* spp., *Alternaria* spp., *Rhizopus* spp., *Septoria*

spp., *Rhizoctonia* spp., and *Pseudocercospora* spp. Dryness-induced dormancy, however, might take time to develop, and morphological differences in some fungi can be observed.^{14,24}

2.4. Storage in silica gel

Sterile silica gel is a widely used medium for storing bacteria and fungi. The process involves sterilizing screw-cap tubes partially filled with silica gel in an oven. After cooling, a skim-milk suspension containing spores and cells of the target microorganism is added over the silica gel and cooled again. The tubes are then dried at 25°C, followed by cooling, and stored in closed containers containing desiccants to prevent moisture absorption. Organisms can survive for 1-2 years.²⁵

Preservation of bacteria

P. denitrificans, *E. coli*, and *Azotobacter vinelandii* can be preserved using this silica gel method.^{12,16}

Preservation of fungi

Fusarium spp., *S. cerevisiae*, and *A. nidulans* can be stored.^{14,24,25} Fungal spores stored on silica gel have the ability to remain viable for over ten years, making this method inexpensive, simple, and reliable for several fungi, including entomopathogens.^{14,24}

2.5. Lyophilization

Lyophilization, also known as freeze-drying, can be divided into three steps: freezing, sublimation, and desorption.²⁶ It involves removing the water from the microorganisms by subjecting them to a vacuum and low temperature. This process helps to prevent damage caused by ice crystal formation and allows the microorganisms to be stored for long periods at room temperature.^{1,13,26}

Preservation of bacteria

Common ingredients used to maintain viability during lyophilization include mannitol, skim milk, and bovine serum albumin.²⁷ Cryoprotectants, such as sucrose and trehalose, help preserve the biomolecules' structure during the process.²⁸ Typically, a basic freeze-drying solution is made using 20% skim milk and 5-10% sucrose. Probiotics, including *Bifidobacteria*, can be preserved using this freeze-drying method.²⁸ Certain bacteria, such as *Clostridium botulinum*,

Aquaspirillum serpens, and *Helicobacter pylori*, cannot be preserved using lyophilization.¹ These bacteria have unique physiological and structural characteristics that make them particularly vulnerable to the stresses induced by lyophilization.

Preservation of fungi

The freeze-drying method suits coelomycetes, hyphomycetes, basidiomycetes, ascomycetes, and some thermophilic fungi such as *Thermothelomyces* spp. and *Humicola* spp..²⁹ However, it is generally ineffective for fungi with large vacuolar volumes and contains a lot of water, such as some Oomycetes and Entomophthorales. It does not adequately preserve non-sporulating fungi (vegetative hyphae), and some species of yeast (*Brettanomyces*, *Dekkera*, *Lipomyces*, *Leucosporidium*, *Bulleera*, *Sporobolomyces*).¹ Store the lyophilized ampoules at approximately 4°C, although they can be kept at ambient temperature, if necessary, their viability will likely decline sooner than if refrigerated.^{13,17,29}

Preservation of viruses

This is the most satisfactory method of keeping viruses alive for a very long time. Depending on the unique design of the freeze-drying equipment, there are several changes in the technical procedures. The most straightforward and efficient method only requires one vacuum stage for small volumes of virus and small numbers of samples because the glass ampoules are inserted directly onto the branching exhaust manifold of the freeze dryer.¹²

2.6. Cryopreservation

The preservation of biological materials at cryogenic temperatures, typically -80°C or -196°C, is referred to as cryopreservation (liquid nitrogen). Cryopreservation is useful for the long-term preservation of microorganisms that are difficult to culture or grow and those that are sensitive to low temperatures. Cryopreservation also requires cryoprotective agents to protect the microorganisms from damage caused by ice crystal formation during freezing. Low temperatures protect proteins and DNA from denaturation and damage while slowing cellular water movement. As a result, the biochemical and physiological activities of the cells are

effectively halted, and the cells are protected for extended periods. Cell preservation at -20°C is not recommended to be used for long-term storage. A reservation at -80°C is adequate, but -196°C is ideal because it largely prevents DNA mutations from occurring.^{22,30}

Preservation of bacteria

The bacterial cryopreservation protocol involves preparing the bacterial culture in a suitable medium, washing the cells, suspending them in a cryoprotective agent, and freezing them in small vials. The cryoprotective agent helps to protect the cells from damage during the freezing process.³¹

To select the appropriate freezing medium, researchers should consider the growth requirements of the bacteria and the medium's composition. Additionally, they should take precautions to prevent contamination during the cryopreservation process, such as using sterile techniques and thoroughly cleaning equipment.^{27,28}

Preservation of fungi

In commercial cryopreservation systems, polystyrene beads can be used as carriers to cryopreserve sporulating *A. fumigatus* cultures at -80°C and conidia of entomopathogenic fungi. For *S. cerevisiae* cultures, porous ceramic beads can be employed at -70°C. This technique can be used to preserve fungi that are difficult to keep alive, such as unculturable, obligate parasites and mutualists such as *Halophytophthora*, *Saprolegnia*, and *Aphanomyces* spp., as well as some Basidiomycota and Glomeromycota members.^{23,26}

Preservation of viruses

Viruses can be cryopreserved at -20°C to -70°C and in liquid nitrogen. Viruses can remain infectious at temperatures as low as -60°C, and to preserve their infectivity, it is best to rapidly freeze small volumes (0.1-0.5 mL) of virus suspension. However, if retaining infectivity is not necessary, the sample can be stored at -20°C for many years without losing its antigenicity, though infectivity may decrease. A useful method for preserving specific virus antigens for diagnostic purposes is to store acetone-fixed virus-infected cells on glass coverslips at -20°C for long-term storage. Baculoviruses or pox viruses can be

preserved at -20°C. Virus-like particles, certain DNA viruses, and non-enveloped viruses can be kept stable at 4°C for an extended period. Because RNA and most encapsulated viruses are extremely heat-labile, long-term storage requires rapid freezing and storing at -80°C.^{15,26,32}

2.7. Encapsulation

Encapsulation involves coating the microorganism with a protective layer to improve its stability and prevent loss of activity during storage and transportation. The protective layer can comprise various materials, such as alginate, gelatin, polyethylene glycol or polyvinyl alcohol.^{26,33}

The techniques used for immobilizing cells through encapsulation can be broadly categorized into two types depending on the size of the polymeric bead produced: macro-encapsulation and microencapsulation. Macroencapsulation involves trapping cells within polymeric beads ranging in size from a few millimeters to centimeters. Microencapsulation produces beads within the size range of 1-1000 µm. In the case of microencapsulation, it has been observed that the viability of cells at the center of larger beads tends to decrease over time.³³

Encapsulation can protect microorganisms from environmental stresses such as temperature, pH, and osmotic pressure, improving their stability during storage and transportation. However, the encapsulation process can reduce the microorganisms' activity, and the capsule may limit the diffusion of substrates and products in and out of the capsule, which can affect the performance of the biosensor.

Fluorescent dyes, such as SYTO-green, ethidium bromide, and 6-carboxyfluorescein, have been used to assess the viability of encapsulated cells. These dyes stain the nucleic acid of dead bacteria, allowing differentiation from live bacteria with intact cytoplasmic membranes.

Several types of microorganisms can be effectively encapsulated using different encapsulation techniques. Some bacteria suitable for encapsulation include *Pantoea agglomerans*, *E. coli*, *B. subtilis*, *Pseudomonas* spp., *Lactobacillus*, and *Bifidobacterium* spp. Yeast strains like

Saccharomyces boulardii, *Saccharomyces cerevisiae*, and *Hirsutella rhossiliensis*, an endoparasitic fungus, can also be encapsulated successfully. The shelf life of encapsulated viruses is highly dependent on the stability of the viral particles and the encapsulation method employed.^{15,26,33}

2.8. Electrospinning and electrospraying

Electrospinning and electrospraying are emerging microencapsulation techniques used to preserve the viability of sensitive microorganisms. They involve atomization processes using an electrically charged jet of polymer solution to create nanoscale and microscale fibers or particles. These techniques have shown promise in food applications and as efficient methods for preserving microorganisms. A polymer solution containing microorganisms is placed in a syringe with a needle at the tip, and a high-voltage electric field is applied, which causes the polymer solution to form a charged jet that is pulled toward a collector, forming nanofibers. The microorganisms become encapsulated within these fibers, which act as protective carriers during storage. The electrospraying electric field causes the solution to break into fine droplets, forming nanoparticles or microcapsules that can protect the microorganisms.³⁴

Both electrospinning and electrospraying provide high surface area-to-volume ratios and high permeability, making them practical for preserving microorganisms. The thin polymeric fibers produced in electrospinning processes allow the trapped cells to exchange nutrients and metabolites while maintaining their metabolic activity.^{34,35} Electrospinning has been successfully used to encapsulate bifidobacterial strains, maintaining high microbial viability despite osmotic changes and electrostatic fields.

Electrospraying involves atomizing a liquid flow into droplets and has also been used to encapsulate probiotic strains onto protein-based or polysaccharide-based matrices. Microencapsulation through electrospraying with whey protein concentrate can prolong bifidobacterial cells survival, even under conditions of high humidity.^{34,36}

2.9. Nanoparticles to preserve microorganisms

Nanoparticles are tiny particles with diameters in the range of 1-100 nanometers. They have unique physicochemical properties, including a high surface area-to-volume ratio, which can make them effective at preserving microorganisms. Using nanoparticles for microbial preservation can involve coating the microorganisms with a layer of nanoparticles, which can protect them from environmental stresses such as high or low temperatures, radiation, or oxidative stress. The nanoparticles can also act as a slow-release vehicle for antimicrobial compounds or other chemicals that can help maintain the viability of the microorganisms during storage.^{36,37}

2.10. Supercooling

Supercooling is a method of cooling liquids or solutions below their freezing point without forming ice crystals.^{38,39} By avoiding the formation of ice crystals, supercooling can help preserve the viability of microorganisms that might otherwise be damaged by freezing. In one approach to using supercooling for microbial preservation, microorganisms are suspended in a solution containing cryoprotective agents (such as glycerol or DMSO) and then supercooled to temperatures below their freezing point. The supercooled suspension can then be stored at low temperatures, such as -80°C, without forming ice crystals, which can help maintain the microorganisms' viability. Long-term preservation methods with advantages and disadvantages are displayed in Table 3.

Table 4 compares short-term and long-term microbial and viral preservation methods in terms of microbial growth, viability, maintenance, and equipment.

3. Assessing the viability of preserved microbes, including viruses

Following the preservation of microbes and different viruses, their viability is assessed using different methods. Microscopic examination using vital stains: Cells are observed microscopically in a counting chamber after staining with vital stains. Vital stains can distinguish between live and dead cells based on specific staining patterns. Nowadays, molecular

techniques such as PCR (polymerase chain reaction) or QPCR (quantitative PCR/real-time PCR) can detect specific genetic markers to determine the presence or absence of viable microorganisms.^{24-27,35}

Discussion

Compared to traditional short-term microbial preservation methods, a microfluidic system has several advantages. It has high precision and control over the storage environment and is able to store small quantities efficiently, ideal for research and diagnostic purposes. Also, it offers a miniaturized, cost-effective alternative to large-scale storage. Limitations include the fact that the equipment would be expensive for large-scale applications, and the technique is still under development for widespread use.¹⁸⁻²¹

The long-term microbial preservation methods can be applied to preserve the microbes and viruses for short time periods as well. Compared to cryopreservation, the novel vitrification is an advanced form of cryopreservation that avoids ice formation by turning the cell suspension into a glass-like state. This method is increasingly used in preserving cells and microbial strains without ice damage. Further, ultra-low temperature storage, below -130°C, using liquid nitrogen or specialized freezers also represents a more refined approach to freezing, maintaining better long-term viability for bacteria.^{28-33,40}

Similarly, improved lyophilization techniques have emerged, such as adding specific stabilizers or optimizing the freeze-drying cycle, which can improve the preservation of microbial properties. The flash freezing followed by immediate freeze-drying in highly controlled conditions helps to reduce the damage and enhance the storage of sensitive microorganisms. A low maintenance cost and an easy transportation of lyophilized cultures are the main advantages.^{35,40}

Compared to traditional chemical preservation such as mineral oil or liquid paraffin storage, storage in silica gel and in sterile soil, encapsulation in biocompatible matrices such as alginate beads or silica gel has become a more refined alternative to simple chemical

Table 3. Long-term preservation methods with advantages and disadvantages

Preservation method	Method	Advantages	Disadvantages
Mineral oil or liquid paraffin storage	Preservation under room temperature or refrigeration temperature	Simple and inexpensive	Risk of contamination and not suitable for all microorganisms; requires significant space as the tubes must be stored in an upright position
Storage in distilled water	Preservation under the refrigeration temperature (4°C)	Simple and inexpensive	Microorganisms may lose viability over time
Storage in sterile soil	Preservation under the refrigeration temperature (4°C) or room temperature	Inexpensive; the natural environment for microorganisms	It may not be suitable for all microorganisms
Storage in silica gel	Preservation under room temperature	Long shelflife; easy to transport	It may not be suitable for all microorganisms; requires desiccation equipment
Lyophilization	Preservation under subzero temperatures (-20°C to -80°C)	Can preserve a wide range of microorganisms and enzymes	Expensive equipment; may require technical expertise
Cryopreservation	Preservation under ultra-low temperatures (-196°C or lower)	Long-term preservation; suitable for a wide range of microorganisms	Requires specialized equipment and expertise
Encapsulation	Preservation under room temperature or refrigeration temperature	Can protect microorganisms from harsh environments	Limited shelflife; may not be suitable for all microorganisms
Nanoparticles to preserve microorganisms	Preservation under room temperature or refrigeration temperature	Can protect microorganisms from environmental stress	Limited studies on its effectiveness for long-term preservation
Super cooling	Preservation under subzero temperatures without freezing	Simple and inexpensive	Limited applications and requires careful temperature control

Table 4. Comparison between long-term and short-term preservation methods

Aspects	Short-term preservation	Long-term preservation
Duration of preservation	Relatively shorter duration (days to months)	Longer duration (months to years)
Purpose	Temporary storage for immediate use or short-term studies	Extended storage for future use or long-term preservation
Storage conditions	Typically refrigeration (4°C) or room temperature	Specialized conditions such as freezing or desiccation
Microbial growth	Some microbial growth may occur over time	Minimal to no microbial growth
Viability	High initial viability, gradual decrease over time	High viability maintained throughout storage
Need for maintenance	Requires regular subculturing or transfers	Minimal maintenance required
Equipment/infrastructure	Simple and readily available lab equipment	Specialized equipment may be needed for storage

preservatives. This can protect microbes during storage and offer better control over release and activity.^{21,37}

The novel electrospinning and electrospraying techniques have several advantages over traditional preservation methods. They do not require temperature control, avoiding detrimental effects on microbial physiology. The polymer fibers provide a physical barrier, reducing exposure to environmental stressors such as drying, UV radiation, or osmotic stress. Electrospun fibers can be designed to gradually release the microorganisms when required, making them useful for controlled delivery applications, such as probiotics or bioremediation.³⁻⁶ Materials used in electrospinning, like polysaccharides, proteins, and biodegradable polymers (chitosan, alginate), are biocompatible, making them ideal for preserving microbial cultures. Potential uses include preservation of probiotics, bioremediation and vaccine development. However, the process of electrospinning can be technically complex and requires specialized equipment. And scaling the process for large quantities of microorganisms can be difficult. Some polymers used in electrospinning may not be suitable for all microorganisms or viruses, especially if they exhibit toxicity or inhibitory effects. Some microorganisms may lose viability during the drying or encapsulation process, especially if the polymers used are not optimized for preservation.^{24,25,35-37}

Supercooling has several advantages over traditional preservation methods. It prevents ice crystal formation and minimize the mechanical and osmotic stresses that often occur with freezing. And compared to cryopreservation, supercooling can be done at slightly lower temperatures, around 0°C making it potentially more energy-efficient. It is used to preserve viruses as well. However, maintaining the stable and precise temperature control is challenging. It can be used to preserve *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, *E. coli*, *Candida* spp., *Aspergillus* spp., and bacteriophages.^{16,23,29,31}

Conclusions

Microfluid systems, nanoparticles, electrospinning, electrospraying, and supercooling methods are available for novel and emerging microbial and viral preservation methods.

Each short- and long-term microbial and viral preservation method has advantages and disadvantages. Based on requirements, availability, and affordability, researchers and industry can select and use the appropriate preservation method.

Author contributions statement: JAASJ conceptualized the review. TS drafted the manuscript with an initial literature review. Both authors reviewed the search articles, and JAASJ critically reviewed them. Both authors read and approved the final version of the manuscript.

Conflicts of interest: All authors – none to declare.

Funding: None to declare.

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Please cite this article as:

Senaratne WMTN, Jayaweera JAAS. Comparison of microbial preservation methods: a narrative review. GERMS. 2024;14(4):375-386. doi: 10.18683/germs.2024.1447