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The MicroBioDiverSar Project: Exploring the Microbial Biodiversity in Ex Situ Collections of Sardinia

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Abstract: In the last decades, biodiversity preservation has gained growing attention and many strategies, laws and regulations have been enacted by governments with this purpose. The MicroBioDiverSar (MBDS) project, the first one regarding microbiological resources, funded by the Italian Minister of Agricultural, Food and Forestry Policies (Mipaaf) through the Law 194/2015, was aimed at surveying, cataloguing, and managing the microbial resources and the related information of three Sardinian collections (Agris BNSS, Uniss, and Unica). While microorganisms were reordered and inventoried, a federated database, accessible via the web, was designed by the bioinformatician of Ospedale Policlinico San Martino of Genova, according to both international standards and laboratory needs. The resulting MBDS collection boasts a great richness of microbial resources. Indeed, over 21,000 isolates, belonging to over 200 species of bacteria, yeasts, and filamentous fungi isolated from different matrices, mainly food, of animal and vegetable origin, collected in over 50 years, were included in the database. Currently, about 2000 isolates, belonging to 150 species, are available online for both the scientific community and agri-food producers. The huge work done allowed one to know the consistency and the composition of most of the patrimony of the Sardinian microbial collections. Furthermore, the MBDS database has been proposed as a model for other Italian collections that, as the MBDS partners, are part of the Joint Research Unit MIRRI-IT Italian collections network, with the aim of overcoming fragmentation, facing sustainability challenges, and improving the quality of the management of the collections.

Keywords: agri-food biodiversity monitoring and conservation; Sardinia microbial collections; database design and development; bacteria; yeasts; filamentous fungi

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1. Introduction

Agri-food biodiversity includes the variety and variability of animals, plants, and microorganisms that are important for food and agriculture and which are the result of the interactions between the environment, genetic resources, and management systems and practices used by humans [1]. According to what was recently reported by the Food and Agriculture Organization (FAO) in the report 'State of world biodiversity for food and agriculture' [2], the biodiversity at the base of our food systems is declining all over the world; and what is lost—species of plants, animals, and microorganisms—cannot be recovered. In particular, microorganisms play a fundamental functional role in agricultural systems; in fact, they are involved in the production of food (soil fertility, crop nutrition,

biocontrol, biofertilization), in the conservation of foodstuffs (toxins and pathogens), and in the production of processed foods (milk and cheese, wine, etc.); hence, their presence and biodiversity is functional to the sustenance of living organisms on earth [3]. The knowledge and conservation of microbial biodiversity of agricultural interest for the food sector therefore assumes a key role, as preserving microorganisms of food importance means protecting typical national products and the entire Italian food and wine tradition, contributing, at the same time, to the sustainability of production systems. There are a number of relevant examples that can be given: (i) protective microbial cultures, able to prevent the development of spoilage and pathogen microorganisms, are useful in avoiding products' deterioration and waste of resources, without using potentially dangerous chemical preservatives; (ii) starter cultures, adequately lowering the food matrix pH, particularly in the early phases of the product's manufacture, allow to use lower thermization temperatures, therefore saving energy; (iii) in addition, if the cultures are made of autochthonous microorganisms, they strengthen the link of the product with the territory, foster the consumption of local products, safeguarding local economies, encouraging us not to abandon rural territories, and also helping in ecosystems conservation [4].

Unfortunately, the sustainability of many microbial collections, among those hosted by Academic or other public Research Centres and established in support of specific productive sectors or research areas, is often at risk. Most of them depend on government support or research projects grants; some receive money from resources sales or services provision to their end-users (often, government-supported collections are not allowed to charge user fees). Therefore, many collections must constantly face sustainability problems linked to funding needs, which results in degradation of facilities, difficulties in maintaining adequately trained staff and applying innovative approaches to preserve, identify, characterise, and valorise their resources [5].

In the last decades, governments and international and regional organisations have adopted laws and regulations to promote biodiversity conservation and enhancement. Among these, there are the Sardinian Regional Law n. 16/2014, "Agricultural and rural development rules: agrobiodiversity, collective mark, districts", and the Italian Law 194/2015, "Provisions for the protection and enhancement of biodiversity of agricultural and food interest", both establishing instruments for the agrobiodiversity protection. In particular, the Italian law has implemented a national system (National Registry, Network, Portal, and Standing Committee), which aims to preserve, in situ and ex situ, local species (animals, plants, and microorganisms) of agri-food interest affected by the risk of extinction or genetic erosion. However, the definition "risk of extinction" not being applicable to the concept of microbial species, implementing decrees of both the laws do not refer to microbial biodiversity, except to say that, given the complexity of the subject matter, it will be included in further implementing decrees. Therefore, at present, only animal and plant resources are included in the National Registry and in Regional Repertoires, despite the great recognized importance of microbial biodiversity for the maintenance of natural ecosystems, research purposes, and biotechnological exploitation [6]. Moreover, the consistency of the microbial resources' patrimony preserved in ex situ collections by various Italian institutions is still unknown. In this context, MicroBioDiverSar (MBDS, Microbial Biodiversity of Sardinia) has been the first project concerning microbial resources funded by the Ministry of Agriculture, Food, and Forestry (Mipaaf) with the funds of Law 194/2015.

The aim of the project was surveying and cataloguing the microbial resources present in three Sardinian collections (BNSS, Uniss, and Unica, hosted by Agris Sardegna/Sassari, University of Sassari, and University of Cagliari, respectively), devising a federated database, to manage the information related to the microorganisms, and realizing a website to host the database, available at the link http://www.mbds.it/ (accessed on 24 June 2021). The database was designed and implemented according to international standards (i.e., OECD [7], CABRI [8,9], and MIRRI) by the bioinformatics laboratory of the Ospedale Policlinico San Martino (HSM) of Genova (Italy), one of the founding partner of the Joint

Research Unit (JRU) MIRRI-IT (http://www.mirri-it.it/ accessed on 24 June 2021) of which Agris, Uniss, and Unica have become associate members, during the development of the MBDS Project. The JRU MIRRI-IT is the Italian node of MIRRI, the pan-European Microbial Resource Research Infrastructure, born with the aim of overcoming the current fragmentation in the availability of resources and services, and enhancing the quality management system of collections, focusing on the needs of stakeholders interested in the biotechnological transfer of the microbial resources. Therefore, the MicroBioDiverSar project, by comparing the partner collections with other Italian ones, characterized by more advanced systems of management and conservation of microorganisms, was a notable growth opportunity for the Sardinian microbial collections.

At the beginning of the Project, within each partner institution, all the information regarding the accessions (e.g., species to which the microorganisms belonged to, their origin, year, and place of collection, and storage methods) were collected. This phase required in-depth documentary research into the paper archives of the host entities, since information on the isolates collected for several decades since the 1960s was not available in digital form, whereas data for the most recent isolates were reported in tabulated spreadsheets, often not homogeneous by structure. In addition, sometimes information on the same isolate was fragmented into different files. Thus, the next step was to create a unique spreadsheet containing information on all the isolates of each collection, following both international rules and the specific internal needs of each laboratory. At the same time, microbial resources have been physically rearranged, codified, and inventoried.

The work carried out highlighted the presence of a great wealth of microbial resources in the Sardinian collections' partners of the project. In fact, about 200 species of bacteria, yeast, and fungi (of which over 150 are already available online) have been included in the database, corresponding to over 21,000 isolates (of which over 2000 are already available online) from different matrices (milk and milk products from sheep, goat and cow; natural starter cultures; rennet paste; lamb digestive tract; table olives and their brines, olive oil; grapes, grape must, wine; beer, wheat, barley, malt; traditional pork sausages; fish intestines, mussels), entered in the collections in over 50 years.

The creation of the federated database is the fundamental tool that, through the web site, has made the list of MBDS collection resources and the related information accessible online, constituting important communication channels for data and microorganisms sharing with the business world that operates in the agri-food sector, and with the scientific community, for study and research purposes.

2. Database Design and Implementation

In this section, the architectural choices, the software tools, the schema, and the main features of the database mbds_db, implemented in the context of the MicroBioDiverSar project, are presented.

2.1. The Database Architecture

Since the involved collections did not yet implement their own database before the project, the first choices were to design one single database schema, to be adopted by all collections, and to implement three distinct databases sharing the schema as well as some reference tables, e.g., for species, geographic locations, growth media. These choices would have allowed all collections to manage their own data autonomously. Since one of the aims of the project was the creation of a common query interface for all catalogues, a centralized and integrated copy of the three databases had to be anyway created for public access.

The overall architecture of the system, then, includes three distinct databases, each implemented and located at the relative collection, and one server, which hosts the centralized integrated database and controls the homogeneities of the reference tables. This simple architecture is viable for the easy and efficient inclusion of further databases, in case more collections would join the project.

The key issue for the success of this common, but distributed, database architecture was then synchronization. Reference tables for common information must be synchronized so that all databases share their values at any time. Moreover, update of information on strains carried out by each collection on its database must be synchronized with the central repository to allow end users to query a constantly up-to-date integrated database.

Regarding the development and production environment, open software was privileged. This led to the adoption of the so-called LAMP environment, which includes Linux as operating system, Apache as web server, MySQL as database management system, and PHP as programming language (hence the acronym LAMP: Linux, Apache, MySQL, PHP). For users willing to implement their database in a Windows environment, we adopted the analogous environment WAMP (where W stays for Windows), and in particular, the Wampserver software (see http://www.wampserver.com/en/ accessed on 24 June 2021). This choice allowed us to have a simple and effective development environment, especially fit for the continuous development that is required in the scientific domain. Moreover, the simplicity of the programming language, joint with the widespread expertise available on both Apache and MySQL, allows users to make simple changes to the applications and the database.

2.2. The Database Information and Schema

The information to be included in the database and their format was decided by examining the newly established guidelines for the submission of catalogues to MIRRI-IS, the information system adopted by the Microbial Resource Research Infrastructure (MIRRI), and by comparing it with the needs of the collections participating in the project.

This analysis led to the selection of an original data set which incorporates almost all information defined by MIRRI guidelines, in a proper format for a future submission to the MIRRI-IS, along with many further data that are specific to the needs of the involved collections. It is noteworthy that, due to their different scientific interests (e.g., bacteria against yeasts), not all collections gather the same information for their strains, while the dataset is unique. The full dataset is available from the web site of the MicroBioDiverSar project (http://www.mbds.it/ accessed on 24 June 2021).

This dataset was then analysed from the entity–relationship point of view for its actual implementation in a relational database. The entities of the dataset are summarized in Table 1.

The schema of the database is shown in Figure 1. In this figure, section A includes all reference tables, that are shared by the local databases, while section B includes all local tables, whose contents are unique for each collection. Section C includes some additional local tables that are used for managing user access, backup/restore functions, and update of reference tables.

The database has been implemented in a relational environment based on the MySQL database management system.

2.3. The Features of the Applications: On-Line Integrated Query

Two sets of applications were developed: one for on-line access and query to the integrated database by end users, and one for the actual management of data by the collections.

Access to the integrated database is granted through the website of the MicroBioDiverSar project. End user authentication is not required. A simple form is available for querying the catalogues by resource type. The main query information is the species of the strain. Moreover, users may query the database by strain number and name, and by substrate and substrate category. An advanced query form is under development.

After the successful execution of a query, the user is faced with the list of retrieved strains that can be printed or exported as an MS Excel file or in PDF. By selecting one of the strain numbers of retrieved strains, the system returns the relative information, grouped in five distinct sections, one each for strain identification, origin, properties, literature, and notes. It is noteworthy that only a subset of strains is included in the query, according to

the decision of the involved collection, and that only a subset of available information is included in the output.

Table 1. Database entities. This table summarizes the entities identified for the database and their interrelations.

Entity	Contents	In Relation with
Strains	Basic information on strains, mainly related to the MIRRI-IS dataset	All, but Chemical compounds
Strain properties	Extended information on strains, mainly related to their chemo-physical properties	Strains, Chemical compounds
Strain management	Extended information related to the local management of strains, such as lab book notes and position of vials	Strains
Strain culture	Extended information on culture management, such as number of available vials in different storage conditions	Strains
Sequences	Information on known sequences of the strains	Strains
Species	Scientific species names, derived from authoritative sources (Mycobank, Prokaryotic Nomenclature Up-To-Date)	Strains
Literature	Information on bibliographic references for publications relatives to strains	Strains
People	Information on people who collected, isolated, identified, and deposited the strains	Strains
Projects	Information on projects for which strains were collected and identified	Strains
Geographic locations	Information on places where samples were collected	Strains
Habitats	Information on strain habitats	Strains
Substrates	Information on substrates	Strains
Substrate categories	Information on substrate categories (useful for queries)	Strains
Growth media	Information on available growth media	Strains
Chemical compounds	Information on chemicals and their interactions with strains as by-products, inhibitors, etc.	Strain properties

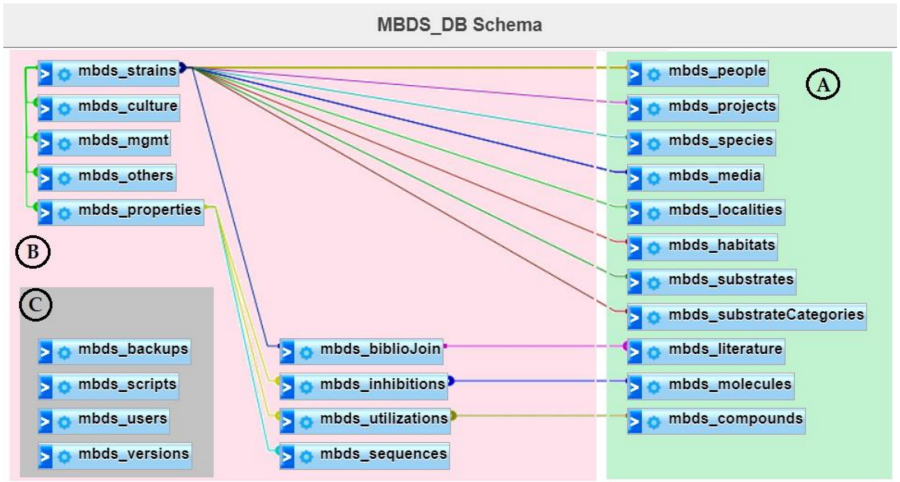

Figure 1. MBDS_DB schema. A: reference tables; B: local tables; C: control tables.

2.4. The Features of the Applications: Local Database Management

At the collections, access to applications for local database management requires user authentication. Three types of users, having different access rights, have been defined. The administrators can perform all actions, while the curators can manage data, and the other users can only query and see data. Administrators can create as many users as needed. See Table 2 for details on user types and related access rights.

Table 2. User types and access rights (√: Yes; X: No).

User Type	Query Strains	Insert Strain	Update Strain	Delete Strain	View Lists	Update Lists	Backup Restore	Manage Users	Upload Data
Admin	√	√	√	√	√	√	√	√	√
Curator	√	√	√	√	√	√	X	X	X
Basic	√	X	X	X	√	X	X	X	X

In the following paragraphs, we shortly describe all actions that become available after authentication through the main menu.

Query: Allows all users to query the local database. Two distinct query forms are available at present. The standard query form includes fields for strain number, strain name, project, species, substrate, and substrate category. The advanced query form includes fields for species, substrate, sampling location and date, as well as fields for compound utilization and inhibitors.

Insert: Allows admins and curators to insert new strains in the local database. With this action, only the following information is included in the database: strain number, strain name, project, resource type, species, and strain numbers of the same strain in other collections. Only the resource type and strain name are mandatory. The strain number can be automatically generated by the system, while the species can be specified later, which is useful when the identification has not yet been done. All newly inserted strains are marked as not publicly available.

Update: Allows admins and curators to complete the insertion of data on a strain or update it. The strain must be selected by strain number or strain name. For practical reasons, information is included in distinct sections for data respectively related to strain identity, identification, origin, isolation, properties, inhibitions, utilizations, literature, notes, culture, and storage. An additional section for bacteria properties is also available.

Figure 2 reports an example of the application for the management of strain data. It refers to the 'Origin' section of the 'Update' function. Information on strain origin is included in this section and can be inserted or updated according to the needs of the user. Update is supported by autocomplete functions for reference table values, dates, and enumerations.

Duplicate: Allows admins and curators to insert new strains by copying some essential data (e.g., species and origin) from one existing strain. It is useful when many new strains have been collected together, e.g., during a sampling campaign.

Delete: Allows admins and curators to delete strains from the database.

Reference lists: Allows admins and curators to see and manage reference lists, while other users may only see and query them. Lists are available for species, habitats, substrates, substrate categories, geographic locations, culture media, chemical compounds, inhibiting compounds, literature, people, and projects (see also Table 1). Figure 3 reports an example of the application for the management of reference tables. It refers to the 'Species' table that includes only those taxons for which at least one strain is present in the databases. The upper list reports existing values. The lower forms allow one to add new taxons from reference databases (namely, Prokaryotic Nomenclature Up-To-Date and Mycobank) and to update or delete one of the existing taxons (e.g., when information on a given species is updated at the reference databases).

Tools: Available to admins only. It allows one to create and restore database backups and to synchronize the local with the federated database by uploading an updated version

of the local database to the server. It also allows admin users to check if new versions of the applications are available and to update them.

Options: Allows admins to create and delete users and to define and change their passwords. Each user can autonomously update its password.

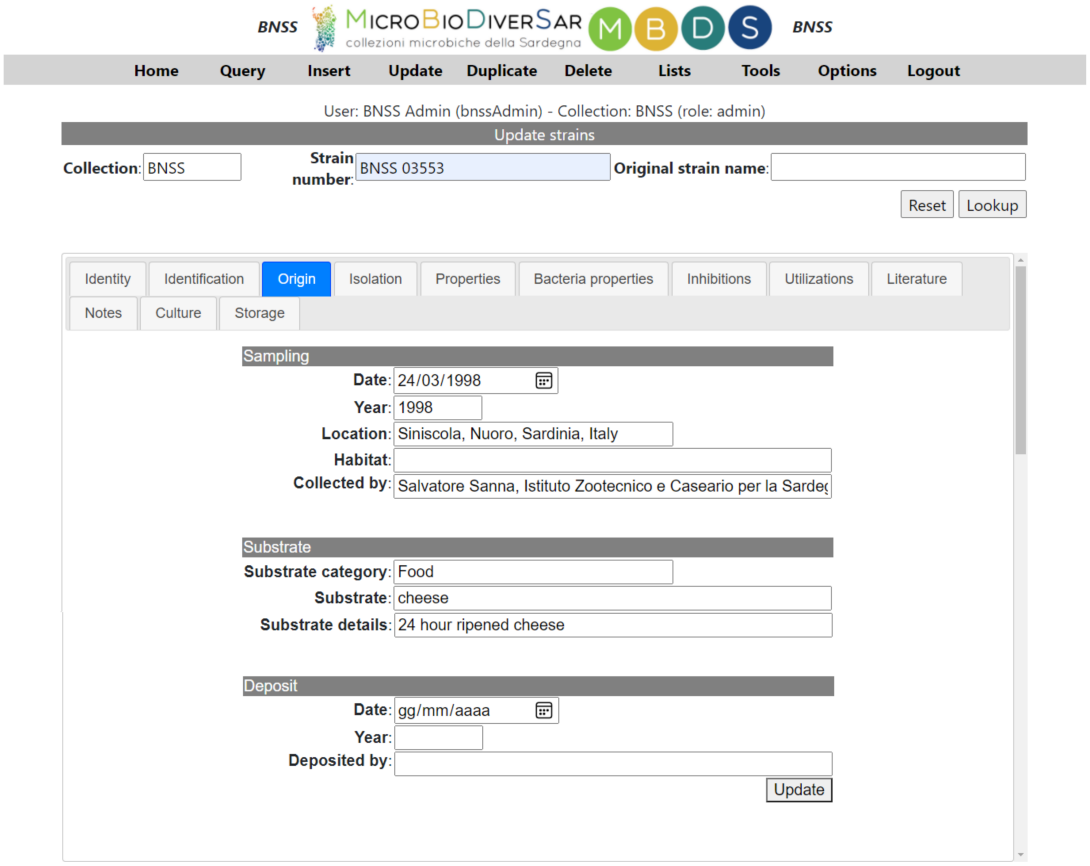

Figure 2. Example of the database management application related to the 'Origin' section of the 'Update' function.

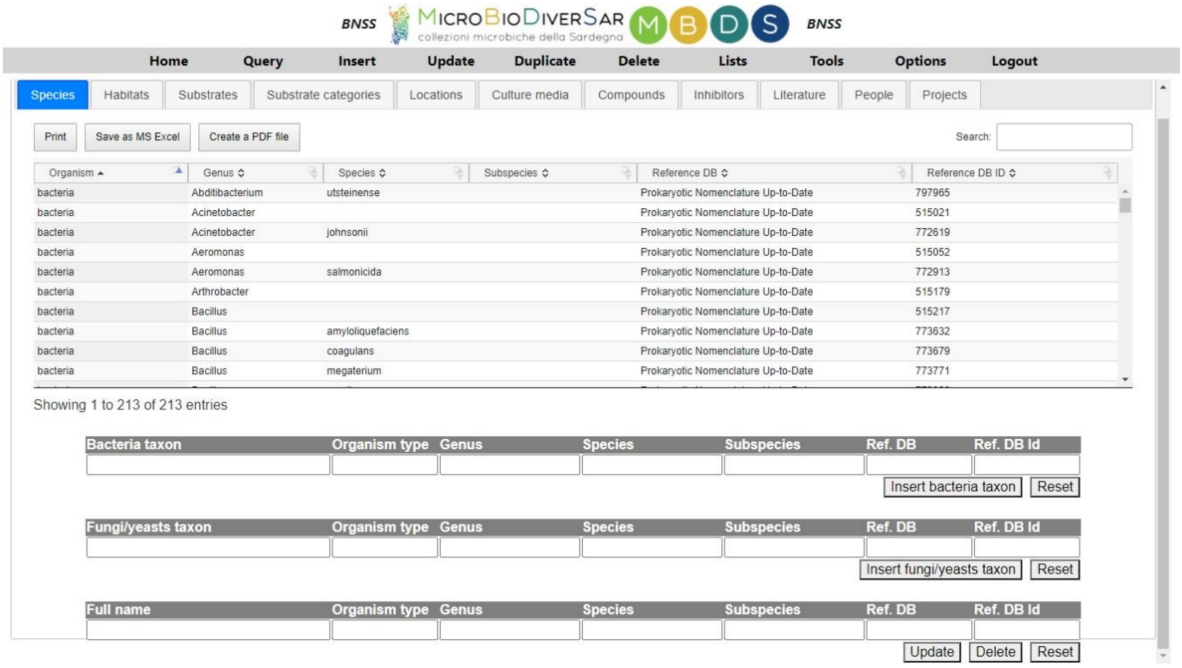

Figure 3. Application for the management of reference tables.

3. The MBDS Microbial Collection

In this section, a description of the three institutions constituting the MBDS Sardinian microbial collection, their history, the main issues, and the microorganisms studied is presented.

3.1. BNSS Agris Sardegna Collection

The bacterial collection BNSS was founded in 1967 by Dr. Severino Arrizza and Dr. Antonio Ledda, at the Istituto Zootecnico e Caseario per la Sardegna–Bonassai, Sassari (now merged within Agris Sardegna) [10]. Part of this collection is now included in MBDS, the Sardinian collection established with the project MicroBioDiverSar.

The acquisition of biological material has been done throughout several decades by the isolation of bacteria from different samples, mainly Sardinian PDO and traditional fermented foods. The first group of isolates originated by studies on the use of natural starter cultures (scotta-innesto) in Pecorino Romano cheese production for improving its quality and reducing production losses [11]. In those years, several scotta-innesto cultures were freeze-dried in toto, allowing the preservation of autochthonous strains and their balances within the microbiota before the wide use of selected starters. The collection has been enriched over the years thanks to several projects financed by the Autonomous Region of Sardinia, the Italian Ministry of Agriculture (Mipaaf) and the European Union.

At present, the BNSS collection includes about 21,000 isolates; among them, 8055 were isolated in pure culture and 72% of them (5762) have been identified at genus or species level with molecular biology techniques such as genus/species specific PCR [12–14], ARDRA [15], REP-$(GTG)_5$ [16], and partial sequencing of the 16S r-RNA gene. Many are bio-typed by PFGE [17–19] and/or REP-$(GTG)_5$ fingerprinting and profile analysis by BIONUMERICS® Software v. 6.6.11 (Applied Maths NV).

The most common isolation sources are represented by food of animal origin, but in the last years, isolates were taken also from vegetal food; a smaller number of isolates has animal origin (lamb digestive tract, ewe faeces, fish gut). Sheep and goat milk, fermented milk and cheeses, natural starter cultures, traditional Sardinian sausages, natural fermented olives are the main food from which isolates originate. Most of them are PDO or traditional products such as Pecorino Romano PDO cheese [20], Fiore Sardo PDO cheese [21–23], Pecorino Sardo PDO cheese [24,25], other ewe or goat milk products, i.e., Casu axedu, Fresa, Gioddu [26], homemade natural fermented sausage [27], table olives in brine [28,29].

The identified isolates are distributed in 34 genera and 59 species. The genus *Enterococcus* is the most represented (1,788), mainly isolated from raw or thermised milk cheeses. Other genera widely represented are *Lactiplantibacillus* (978) and *Lacticaseibacillus* (580), principally isolated from raw milk cheeses; bacteria constituting the starter microbiota of dairy production as *Lactobacillus* (746), *Streptococcus* (374), *Lactococcus* (428), *Latilactobacillus* (359), and *Staphylococcus* (257), among which are included species of natural fermented sausages' bacterial microbiota.

Moreover, the collection contains even lyophilised natural starter cultures not yet included in the online catalogue, although some of them have already been characterized for their genotypic (species and biotypes composition) [30] and phenotypic characteristics [31]. Particularly, their acidifying profile and safety (antibiotic resistance) were investigated, and they were successfully used in some dairy plants for experimental cheese making of Pecorino Romano within an innovation and technology transfer project.

Out of the 5762 identified isolates, 1756 are included in the catalogue available on the MicroBioDiverSar project website http//www.mbds.it/ (accessed on 24 June 2021) for the stakeholders of the productive sector or the scientific community.

The isolates of the BNSS collection included in the public catalogue belong to 45 different species. The main ones represented are: *Enterococcus faecium*, *Lactiplantibacillus plantarum*, *Lacticaseibacillus paracasei*, *Lactobacillus delbrueckii subsp. lactis*, *Streptococcus thermophilus*, *Lactococcus lactis*, *Latilactobacillus sakei*, *Enterococcus faecalis*, *Latilactobacillus curvatus*, and *Staphylococcus equorum*. *Enterococcus faecalis,* the most represented species (488), was isolated from several sources such as Fiore Sardo, homemade Pecorino Sardo, Pecorino

Romano, Casu axedu, scotta-innesto, and ovine faeces. The mesophilic no starter lactic acid bacteria (NSLAB) species, *Lacticaseibacillus paracasei*, and *Lactiplantibacillus plantarum* were largely isolated from Fiore Sardo, a raw sheep milk cheese; the thermophilic starter species *Lactobacillus delbrueckii* subsp. *lactis* was mostly isolated from Pecorino Romano PDO and the natural starter culture scotta-innesto; and *Streptococcus thermophilus*, a thermophilic starter species, was isolated, other than from PDO Pecorino Romano and scotta-innesto, from Casu axedu.

Lactiplantibacillus pentosus were collected from natural table olives, both as microbial communities and single isolates, and, after technological characterisation, were used as a starter in experimental and artisanal productions [29]. *Latilactobacillus sakei*, *Latilactobacillus curvatus*, and *Staphylococcus equorum* come from homemade Sardinian sausages, representing the characteristic bacterial microbiota of this kind of products. Isolates coming from fish (*Sparus aurata)* gut belong to *Pseudomonas* spp., *Sphingomonas paucimobilis, Aeromonas salmonicida, Erwinia persicina, Yersinia bercovieri, Psychrobacter maritimus:* some *Pseudomonas* spp. and *Sphingomonas paucimobilis* are biosurfatants producers [32] (Table 3).

Table 3. Species distribution and origin of isolates included in the Agris BNSS public catalogue.

Species	Isolates (No.)	Origin	
Enterococcus faecium (Orla-Jensen 1919) Schleifer and Kilpper-Bälz 1984	489	dairy, meat, and animal	Fiore Sardo PDO/home made Pecorino Sardo PDO/Natural starter culture/Pecorino Romano PDO/Casu axedu (fresh goat's cheese)/Traditional dry sausage/ovine feaces
Lactiplantibacillus plantarum (Orla-Jensen 1919) Zheng et al. 2020	301	dairy and animal	Fiore Sardo PDO/Casu axedu/Pecorino Romano PDO/ovine faeces
Lactococcus lactis (Lister 1873) Schleifer et al. 1986	139	dairy	Fiore Sardo PDO/homemade Pecorino Sardo PDO/Casu axedu (fresh goat's cheese)
Streptococcus thermophilus Orla-Jensen 1919 (Approved Lists 1980)	133	dairy	Natural starter culture (scotta-innesto)/Pecorino Romano PDO/Casu axedu/fermented milk (Gioddu)/Industrial Pecorino Sardo PDO
Lacticaseibacillus paracasei (Collins et al. 1989) Zheng et al. 2020	111	dairy and meat	Fiore Sardo PDO/Industrial Pecorino Sardo PDO/Pecorino Romano PDO/Traditional dry sausage
Lactobacillus delbrueckii subsp. *lactis* (Orla-Jensen 1919) Weiss et al. 1984	95	dairy	Natural starter culture (scotta-innesto)
Enterococcus faecalis (Andrewes and Horder 1906) Schleifer and Kilpper-Bälz 1984	92	dairy	Fiore Sardo PDO/ Pecorino Romano PDO
Lactobacillus delbrueckii (Leichmann 1896) Beijerinck 1901 (Approved Lists 1980)	12	dairy and animal	Natural starter culture (scotta-innesto)/Industrial Pecorino sardo PDO/Pecorino Romano PDO/ovine feaces
Streptococcus gallolyticus subsp. *macedonicus* (Tsakalidou et al. 1998) Schlegel et al. 2003	11	dairy	Pecorino Romano PDO

Table 3. *Cont.*

Species	Isolates (No.)	Origin	
Limosilactobacillus reuteri (Kandler et al. 1982) Zheng et al. 2020	10	dairy and meat	Natural starter culture (scotta-innesto)/Pecorino Romano PDO/Traditional dry sausage
Enterococcus durans (ex Sherman and Wing 1937) Collins et al. 1984	10	dairy	Fiore Sardo PDO
Enterococcus hirae Farrow and Collins 1985	9	dairy	Fiore Sardo PDO
Lacticaseibacillus rhamnosus (Hansen 1968) Zheng et al. 2020	8	dairy	Fiore Sardo PDO/Industrial Pecorino Sardo PDO
Levilactobacillus brevis (Orla-Jensen 1919) Zheng et al. 2020	8	dairy and meat	Fiore Sardo PDO/Traditional dry sausage
Lactobacillus helveticus (Orla-Jensen 1919) Bergey et al. 1925 (Approved Lists 1980)	5	dairy and animal	Pecorino Romano PDO/ovine faeces
Lactobacillus delbrueckii subsp. *bulgaricus* (Orla-Jensen 1919) Weiss et al. 1984	4	dairy	Fermented milk (Gioddu)/natural starter culture (scotta-innesto)
Pediococcus pentosaceus Mees 1934 (Approved Lists 1980)	4	dairy	Fiore Sardo PDO
Lacticaseibacillus casei (Orla-Jensen 1916) Zheng et al. 2020	2	dairy	Industrial Pecorino Sardo PDO
Loigolactobacillus coryniformis (Abo-Elnaga and Kandler 1965) Zheng et al. 2020	2	dairy	Fiore Sardo PDO
Lactiplantibacillus paraplantarum (Curk et al. 1996) Zheng et al. 2020	1	dairy	Fiore Sardo PDO
Lentilactobacillus hilgardii (Douglas and Cruess 1936) Zheng et al. 2020	1	dairy	Fiore Sardo PDO
Lentilactobacillus parabuchneri (Farrow et al. 1989) Zheng et al. 2020	1	dairy	Fiore Sardo PDO
Limosilactobacillus fermentum (Beijerinck 1901) Zheng et al. 2020	1	dairy	Pecorino Romano PDO
Enterococcus gallinarum (Bridge and Sneath 1982) Collins et al. 1984	1	dairy	Fiore Sardo PDO

Table 3. *Cont.*

Species	Isolates (No.)	Origin	
Latilactobacillus curvatus (Troili-Petersson 1903) Zheng et al. 2020	54	meat and dairy	Traditional dry sausage/Fiore Sardo PDO
Staphylococcus pasteuri Chesneau et al. 1993	10	meat and dairy	Traditional dry sausage/Pecorino Romano PDO
Staphylococcus warneri Kloos and Schleifer 1975 (Approved Lists 1980)	5	meat and dairy	Traditional dry sausage/Pecorino Romano PDO
Latilactobacillus sakei (Katagiri et al. 1934) Zheng et al. 2020	108	meat	Traditional dry sausage
Staphylococcus equorum Schleifer et al. 1985	40	meat	Traditional dry sausage
Staphylococcus xylosus Schleifer and Kloos 1975 (Approved Lists 1980)	17	meat	Traditional dry sausage
Staphylococcus succinus Lambert et al. 1998	10	meat	Traditional dry sausage
Staphylococcus epidermidis (Winslow and Winslow 1908) Evans 1916 (Approved Lists 1980)	7	meat	Traditional dry sausage
Leuconostoc mesenteroides (Tsenkovskii 1878) van Tieghem 1878 (Approved Lists 1980)	3	meat	Traditional dry sausage
Staphylococcus saprophyticus (Fairbrother 1940) Shaw et al. 1951 (Approved Lists 1980)	3	meat	Traditional dry sausage
Staphylococcus vitulinus corrig. Webster et al. 1994	2	meat	Traditional dry sausage
Leuconostoc carnosum Shaw and Harding 1989	1	meat	Traditional dry sausage
Leuconostoc citreum Farrow et al. 1989	1	meat	Traditional dry sausage
Sphingomonas paucimobilis (Holmes et al. 1977) Yabuuchi et al. 1990	6	fish	Fish gut (*Sparus aurata*)
Psychrobacter maritimus Romanenko et al. 2004	3	fish	Fish gut (*Sparus aurata*)
Pseudomonas brenneri Baïda et al. 2002	2	fish	Fish gut (*Sparus aurata*)

Table 3. *Cont.*

Species	Isolates (No.)	Origin	
Pseudomonas fluorescens Migula 1895 (Approved Lists 1980)	2	fish	Fish gut (*Sparus aurata*)
Aeromonas salmonicida (Lehmann and Neumann 1896) Griffin et al. 1953 (Approved Lists 1980)	1	fish	Fish gut (*Sparus aurata*)
Yersinia bercovieri Wauters et al. 1988	1	fish	Fish gut (*Sparus aurata*)
Erwinia persicina corrig. Hao et al. 1990	1	fish	Fish gut (*Sparus aurata*)
Lactiplantibacillus pentosus (Zanoni et al. 1987) Zheng et al. 2020	29	plant	Naturally fermented table olives (Tonda di Cagliari)

Some of the isolates of the collection have been tested for technological properties such as acidifying, lipolytic and proteolytic capacity, fermentative profile, production of flavours, and for some safety (antibiotic susceptibility, virulence genetic determinants, bacteriocin production) and probiotic (low pH and bile salt resistance) characteristics [11,21,33–35].

3.2. Uniss Collection

The pioneering work of Prof. Augusto Capriotti of the University of Sassari has laid the foundations, since the 1960s, for the creation of a microbial collection consisting of yeasts, bacteria, and fungi isolated from different local food and environmental matrices. This collection has been enriched over the course of 60 years thanks to studies conducted by researchers from the Institute of General and Applied Microbiology of the Department of Agriculture. This collection has been enriched over the course of 60 years thanks to studies conducted by researchers from the Institute of General and Applied Microbiology of the Department of Agriculture. Recently, this historical collection together with that of the Institute of Plant Pathology of the same department was formed in the UNISS collection and became part of the MIRRI-IT (Microbial Resource Research Infrastructure, Italy) collection network. Currently, the microbial isolates are stored at −80 °C and are going to be freeze-dried. The list of genera and species of microorganisms hosted in the UNISS collection is reported in Table 4.

Table 4. Genera and species distribution of UNISS microorganisms stored in the MBDS collection.

Bacteria			
Genera	**Species**		**Isolates (No.)**
Arthrobacter	spp.	Conn and Dimmick 1947	2
Bacillus	*amiloliquefaciens*	(ex Fukumoto 1943) Priest et al. 1987	10
	coagulans	Hammer 1915	1
	megaterium	de Bary 1884	1
	pumilus	Meyer and Gottheil 1901	4

Table 4. *Cont.*

	subtilis	(Ehrenberg 1835) Cohn 1872	5
	velezensis	Ruiz-García et al. 2005	1
Bifidobacterium	*pseudolongum*	Mitsuoka 1969	1
Brevibacillus	spp.	Shida et al. 1996	4
	agrii	(Nakamura 1993) Shida et al. 1996	9
	invocatus	Logan et al. 2002	6
	parabrevis	(Takagi et al. 1993) Shida et al. 1996	3
Cutibacterium	*acnes*	(Gilchrist 1900) Scholz and Kilian 2016	1
Enterococcus	*faecium*	(Orla-Jensen 1919) Schleifer and Kilpper-Bälz 1984	2
	hirae	Farrow and Collins 1985	2
Frigobacterium	spp.	Frigoribacterium Kämpfer et al. 2000	1
Kocuria	*rhizophila*	Kovács et al. 1999	4
Lacticaseibacillus	*rhamnosus*	(Hansen 1968) Zheng et al. 2020	1
Limosilactobacillus	*mucosae*	(Roos et al. 2000) Zheng et al. 2020	1
Leuconostoc	*citreum*	Farrow et al. 1989	1
Lysinibacillus	spp.	Ahmed et al. 2007	1
Microbacterium	*aerolatum*	Zlamala et al. 2002	4
Micrococcus	spp.	Cohn 1872	3
Pantoea	spp.	Gavini et al. 1989	3
Propionibacterium	*acnes*	(Gilchrist 1900) Douglas and Gunter 1946	1
Staphylococcus	*arlettae*	Schleifer et al. 1985	3
	capitis	Kloos and Schleifer 1975	1
	epidermidis	(Winslow and Winslow 1908) Evans 1916	2
	hominis	Kloos and Schleifer 1975	3
	pasteuri	Chesneau et al. 1993	3
	warneri	Kloos and Schleifer 1975	3
Streptococcus	*equinus*	Andrewes and Horder 1906	1
	spp.	Rosenbach 1884	1

Table 4. *Cont.*

Weissella	*cibaria*	Björkroth et al. 2002	1
Yeasts			
Genera	**Species**		**Isolates (No.)**
Aureobasidium	*pullulans*	(De Bary) G. Arnaud ex Cif., Ribaldi & Corte, 1957	3
Candida	*adriatrica*	Čadež, Cardinali, Ciafardini & G. Péter ,2012	6
	dendronema	Van der Walt, Klift & D.B. Scott	1
	diddensiae	(Phaff, Mrak, & O.B. Williams) Fell & S.A. Mey, 1967	1
	guilliermondii	(Castell.) Langeron & Guerra, 1938	1
	molendinolei	Čadež, Turchetti & G. Péter , 2012	8
	temnochilae	S.O. Suh, N.H. Nguyen & M. Blackw., 2005	2
	wickerhamii	S.A. Mey. & Yarrow, 1978	1
Clavispora	*lusitaniae*	Rodr. Mir. and Antonie van Leeuwenhoek, 1979	1
Cryptococcus	*carnescens*	(Verona & Luchetti) M. Takash, 2003	1
	magnus	(Lodder & Kreger) Baptist & Kurtzman, 1977	1
	victoriae	M.J. Montes, Belloch, Galiana, M.D. García, C. Andrés, S. Ferrer, Torr.-Rodr. & J. Guinea, 1999	1
Hanseniaspora	*uvarum*	(Niehaus) Shehata, Mrak & Phaff ex M.T. Sm., 1984	4
Hansenula	*anomala*	(E.C. Hansen) Syd. & P. Syd, 1919	5
	californica	(Lodder) Wick, 1951	1
	fabiani	Wick, 1965	1
Kluyveromyces	*africanus*	Van der Walt, Antonie van Leeuwenhoek, 1956	1
	bulgaricus	(Santa María) Van der Walt, 1971	2
	fragilis	(A. Jörg.) Van der Walt, 1971	1
	lactis	(Stell.-Dekk.) Van der Wal, 1965	1
	veronae	(Lodder & Kreger) Van der Walt, 1971	7
Lodderomyces	*elongisporus*	(Recca & Mrak) Van der Walt, 1971	1

Table 4. *Cont.*

Metschnikowia	*pulcherrima*	Pitt & Mill, 1968	13
Nakazawaea	*anatomiae*	Kurtzman & Robnett, 2014	2
Pichia	*farinosa*	(Lindner) Guillierm, 1904	2
	guilliermondii	Wick, 1966	1
	kudriavzevii	Boidin, Pignal & Besson, 1965	1
	manshurica	Saito, 1914	1
	membranifaciens	Hansen, 1904	2
	mexicana	M. Miranda, Holzschu, Phaff & Starmer, 1982	1
	nakazawae	Kodama, 1975	1
Pirula	*salina*	(P.A. Dangeard) Printz, 1927	1
Rhodosporidium	*babjevae*	Golubev, 1993 199	1
Rhodotorula	*glutinis*	(Fresen.) F.C. Harrison, 1928	2
Saccharomyces	*aceti*	Santa María, 1958	3
	bailii	Lindner, 1895	3
	bayanus	Sacc, 1895	14
	capensis	Van der Walt & Tscheuschner, 1956	1
	cerevisiae	Meyen ex E.C. Hansen, Medd. Carlsberg, 1883	16
	chevalieri	Reess, 1870	10
	ellipsoideus	Guillierm, 1914	36
	exiguus	Reess ex E.C. Hansen, 1888	1
	fructuum	Lodder & Kreger-van Rij, 1952	1
	italicus	Castelli, 1939	13
	kluyveri	Phaff, M.W. Mill. & Shifrine, 1956	4
	prostoserdovii	Kudryavtsev, 1960	1
Fungi			
Genera	**Species**		**Isolates (No.)**
Fusarium	*avenaceum*	Sacc, 1886	3
	cortaderiae	O'Donnell, T. Aoki, Kistler & Geiser, 2004	1

Table 4. *Cont.*

	crookwellense	L.W. Burgess, P.E. Nelson & Toussoun, 1982	2
	culmorum	Sacc, 1895	37
	fujikuroi	Nirenberg, 1976	1
	globosum	Rheeder, Marasas & P.E. Nelson, 1996	2
	graminearum	Schwabe, 1839	3
	meridionale	Aoki, 2004	1
	oxysporum	Schltdl, 1824	3
	phyllophilum	Nirenberg & O'Donnell, 1998	1
	pseudograminearum	O'Donnell & T. Aoki, 1999	1
	solani	Sacc, 1881	2
F. incarnatum	*equiseti complex*	Sacc, 1886	2

The microbial collection of the Institute of General and Applied Microbiology (MGA-UNISS) is strongly characterized by the presence of wine yeasts which, from 1967 until today, has been implemented thanks to extensive campaigns of isolation and physiological and technological characterization of the isolates. The main technological characteristics taken into consideration have been the fermentative vigour, resistance to SO_2 and pesticides, the ability to adhere to surfaces and form biofilms [36,37], the killer factor [38,39], the ability to produce or reduce malic acid, the synthesis of sulphur products and acetaldehyde. Among wine yeasts, the flor yeasts isolated over 50 years from wines aged under flor are of particular interest and constitute a heritage of microbial biodiversity unique in Sardinia. These yeasts, ascribed to the *Saccharomyces cerevisiae* species, come largely from musts and wines sampled in the cellars operating in the Vernaccia DOC area of Sardinia (Italy) and are of biotechnological interest in many fields [40,41]. In this aspect, the microbial collection of MGA-UNISS harbours one of the few collections of flor yeasts in the world. The researchers of the yeast group of MBDS are also part of the Italian Oenological Microbiology group which actively works within the International Organization of Vine and Wine, as well as being correspondents of the Academy of Italian Vine and Wine. Eighty-six yeasts and bacteria species of the MGA-UNISS collection, genetically and phenotypically characterized, are included in the MBDS catalogue, starting from the first isolations dating back to 1965 until today. These are mainly yeasts isolated from food and environmental matrices such as olives and olive oil, milk and cheeses, grapes, wine and must, fish, shellfish, meat, soil, and by-products of beer processing such as brewers' spent grain and wastewater [42–44]. As for bacteria, since 2012, 91 strains belonging to 36 species have been isolated, genetically characterized, and divided into 50 different biochemical profiles. The bacteria present in the collection are almost entirely of Sardinian origin and derive from five different substrates: olive oil, grape must, wheat, barley, and malt [45–47].

The microbial collection of the Institute of Plant Pathology regards specifically filamentous fungi and consists of 86 strains belonging to 15 different species of *Fusarium*, isolated from uncultivated lands in Sardinia and from plants of agricultural interest (wheat, rice, corn, tomato) between 2001 and 2018. The identification of the different species was carried out starting from monosporic cultures, through the observation of the morphological characteristics, and in many cases, also with the analysis of the Elongation Translation

Factor (EF-1-α). Thirty isolates are also deposited in the USDA Northern Regional Research Laboratory (NRRL) culture collection in Peoria, IL, USA.

Many of the microorganisms preserved in the UNISS collection have been selected on the basis their biotechnological potential and can therefore be used as selected starters. Selected starters consist of pure cultures of microorganisms, selected for their technological activities, to be inoculated for the fermentation of matrices that are not always the same as those from which they have been isolated [48]. Microbial isolates of the UNISS collection have been selected for the transformation of food matrices (beer, wine, dairy products, olive, etc.) and industrial by-products (brewers spent grains), as well as for the bioremediation and fertilization of soils. As for the different *Fusarium* spp. isolates, they can be used for studies on pathogenicity tests on plants of agricultural interest or for the evaluation of the effectiveness of chemical compounds, natural or biological microorganisms, in containing the damage caused by alterative microorganisms (Table 4). An important aspect to consider, strengthening the link between biodiversity preservation and production of local foods with geographical indication, is that microbial collections offer to local producers actions such as isolation, selection, storage, multiplication, production and sale of yeast, and bacterial starters. In the perspective of a circular economy, the UNISS microbial collection will also offer different services for the agri-food and agro-environmental sector, from the production and optimization of microbial starters to waste management and assessment of the quality and safety of fermented products. In addition, the UNISS collection will provide applicative protocols for: (i) bioremediation (bioprotectors, biostimulants, and biofertilizers); (ii) monitoring of microbial processes in the food, environmental, and agricultural fields; (iii) product quality and safety certification. To do that, UNISS will promote the constitution of a spin-off within the framework of a systemic approach to agri-food R&D, as an effective missing link in the technological transfer of food biotechnologies. This is an opportunity for small and medium-sized companies to combine tradition and technologies that can help make fermentation processes manageable without the negative standardization often associated with large-scale industrial production.

3.3. Unica Collection

The UNICA microbial collection, started in 1985 by Prof. Francesca Palmas, head of the Hygiene section of the Department of Experimental Biology, at present, belongs to the Department of Medical Sciences and Public Health (DSMSP) of the University of Cagliari. Over the course of the years, several UNICA researchers have isolated and preserved more than 2000 microorganisms with considerable application potential in the agri-food, health, and environmental fields. Among the identified isolates, the most represented genera are the former *Lactobacillus* and *Enterococcus* for bacteria, *Candida* and *Debaryomyces* for yeasts, and *Penicillium* and *Aspergillus* for moulds.

Part of the UNICA-DSMSP microbial collection is now included in MBDS and presently consists of 655 strains comprising bacteria, mainly represented by lactic acid bacteria (belonging to 12 genera and 22 species) and yeasts (15 genera and 31 species), as indicated in Table 5. The most represented species are *Lactococcus lactis* (53 strains), *Enterococcus faecium* (41 strains), *Lactiplantibacillus plantarum* (36 strains), and *Lacticaseibacillus casei* (26 strains) among bacteria, and *Debaryomyces hansenii* (36 strains), *Yarrowia lipolytica* (27 strains), and *Candida parapsilosis* (14 strains) among yeasts. Of these strains, 90 (57 bacteria and 33 yeasts) were characterized both phenotypically and genotypically and are available in the MBDS catalogue for external users (food industry or scientific community).

The strains of the UNICA-DSMSP collection have been isolated from different food matrices, mainly Sardinian dairy products (sheep and goat milk, typical products such as Fiore Sardo, Casu axedu, Pecorino Sardo, Ricotta) but also from honey, mussels, fresh pasta, and fermented meat products [49–58]. The UNICA-DSMSP microbial collection represents a precious source of resources capable of fostering innovation in the regional agri-food industry and contributing to the qualitative and hygienic–sanitary improvement of the various products, as well as being a tool to protect and enhance microbial biodiversity.

Table 5. Genera and species distribution of UNICA-DSMSP microorganisms stored in the MBDS collection.

	Bacteria	
Genera	**Species**	**Isolates (No.)**
Enterococcus	spp.	120
	durans (ex Sherman and Wing 1937) Collins et al. 1984	11
	faecalis (Andrewes and Horder 1906) Schleifer and Kilpper-Balz 1984	5
	faecium (Orla-Jensen 1919) Schleifer and Kilpper-Balz 1984	41
Lactobacillus	spp.	110
	helveticus (Orla-Jensen 1919) Bergey et al. 1925	1
Lentilactobacillus	*buchneri* (Henneberg 1903) Zheng et al. 2020	2
Lacticaseibacillus	*casei* (Orla-Jensen 1916) Zheng et al. 2020	4
	rhamnosus (Hansen 1968) Zheng et al. 2020	1
	paracasei (Collins et al. 1989) Zheng et al. 2020	26
Limosilactobacillus	*fermentum* (Beijerinck 1901) Zheng et al. 2020	3
Levilactobacillus	*brevis* (Beijerinck 1901) Zheng et al. 2020	11
Lactiplantibacillus	*paraplantarum* (Curk et al. 1996) Zheng et al. 2020	1
	plantarum (Orla-Jensen 1919) Zheng et al. 2020	36
Latilactobacillus	*sakei* (Katagiri et al. 1934) Zheng et al. 2020	9
Lactococcus	spp.	25
	lactis (Lister 1873) Schleifer et al. 1986	53
	cremoris (Orla-Jensen 1919) Li et al. 2021	2
	raffinolactis (Orla-Jensen and Hansen 1932) Schleifer et al. 1988	1
	lactis subsp. lactis bv. diacetylactis (NCBI:txid44688)	1
Leuconostoc	spp.	15
	mesenteroides (Tsenkovskii 1878) van Tieghem 1878	5

Table 5. *Cont.*

	Bacteria	
Genera	**Species**	**Isolates (No.)**
Pediococcus	spp.	4
	acidilactici Lindner 1887 (App. Lists 1980) emend. Jud. Commission 1996	4
	pentosaceus Mees 1934	2
Streptococcus	*thermophilus* (Orla-Jensen 1919) Schleifer et al. 1995	7
	macedonicus Tsakalidou et al. 1998	1
	Yeasts	
Genera	**Species**	**Isolates (No.)**
Candida	*albicans* (C.P. Robin) Berkhout, De schimmelgeslachten Monilia, Oidium, Oospora en Torula: 44 (1923)	2
	atlantica (Siepmann) S.A. Mey. & Simione, Mycotaxon 66: 100 (1998)	1
	catenulata Diddens & Lodder, Die anaskosporogenen Hefen, II Hälfte: 486 (1942)	2
	inconspicua (Lodder & Kreger) S.A. Mey. & Yarrow, International Journal of Systematic Bacteriology 28 (4): 612 (1978)	2
	krusei (Castell.) Berkhout, De schimmelgeslachten Monilia, Oidium, Oospora en Torula: 44 (1923)	2
	parapsilosis (Ashford) Langeron & Talice, Annales de Parasitologie Humaine Comparée 10: 54 (1932)	14
	sake (Saito & Oda) van Uden & H.R. Buckley, Mycotaxon 17: 298 (1983)	1
	utilis (Henneberg) Lodder & Kreger, The Yeasts: a taxonomic study: 546 (1952)	2
	zeylanoides (Castell.) Langeron & Guerra, Annales de Parasitologie Humaine Comparée 16 (5): 501 (1938)	10
Cryptococcus	*curvatus* (Diddens & Lodder) Golubev, Mikologiya i Fitopatologiya 15 (6): 467 (1981)	2
	uniguttulatus (Zach) Phaff & Fell, The Yeasts: a taxonomic study: 1140 (1970)	3
Cutaneotrichosporon	*guehoae* (Middelhoven, Scorzetti & Fell) Yurkov, Xin Zhan Liu, F.Y. Bai, M. Groenew. & Boekhout, Studies in Mycology 81: 140 (2015)	3

Table 5. *Cont.*

	Yeasts	
Genera	**Species**	**Isolates (No.)**
Cystobasidium	*slooffiae* (E.K. Novák & Vörös-Felkai) Yurkov, Kachalkin, H.M. Daniel, M. Groenew., Libkind, V. de Garcia, Zalar, Gouliam., Boekhout & Begerow, Antonie van Leeuwenhoek 107 (1): 180 (2014)	3
Debaryomyces	*hansenii* (Zopf) Lodder & Kreger, The Yeasts: a taxonomic study: 280 (1952)	36
Filobasidium	*uniguttulatum* Kwon-Chung, International Journal of Systematic Bacteriology 27: 293 (1977)	1
Geotrichum	*candidum* Link, Magazin der Gesellschaft Naturforschenden Freunde Berlin 3 (1): 17, t. 1:26 (1809)	3
Hannaella	*oryzae* (Nakase & M. Suzuki) F.Y. Bai & Q.M. Wang, FEMS Yeast Research 8 (5): 805 (2008)	1
Kluyveromyces	*lactis* (Stell.-Dekk.) Van der Walt, Antonie van Leeuwenhoek 31: 347 (1965)	4
	marxianus (E.C. Hansen) Van der Walt, Antonie van Leeuwenhoek 31: 347 (1965)	3
Pichia	*kudriavzevii* Boidin, Pignal & Besson, Bulletin de la Société Mycologique de France 81: 589 (1965)	1
Rhodotorula	*mucilaginosa* (A. Jörg.) F.C. Harrison, Transactions of the Royal Society of Canada. Section 5, Biological Sciences 22: 191 (1928)	6
Saccharomyces	*cerevisiae* (Desm.) Meyen, Archiv für Naturgeschichte 4 (2): 100 (1838)	1
Trichosporon	*aquatile* L.R. Hedrick & P.D. Dupont, Antonie van Leeuwenhoek 34: 474-482 (1968)	3
	cutaneum (Beurm., Gougerot & Vaucher bis) M. Ota, Annales de Parasitologie Humaine Comparée 4: 12 (1926)	3
	gracile (Weigmann & A. Wolff) E. Guého & M.T. Sm., Antonie van Leeuwenhoek 61 (4): 307 (1992)	1
	jirovecii Frágner, Ceská Mykologie 23 (3): 160 (1969)	1
	lactis Lopandic, Sugita, Middelhoven, Herzberg & Prillinger, Trichosporon caseorum sp. nov. and Trichosporon lactis sp. nov., two basidiomycetous yeasts isolated from cheeses: 99-116 (2004)	6
	mucoides E. Guého & M.T. Sm., Antonie van Leeuwenhoek 61 (4): 312 (1992)	2

Table 5. *Cont.*

	Yeasts	
Genera	**Species**	**Isolates (No.)**
Wickerhamiella	*pararugosa* (Nakase, Komag. & Fukaz.) C. Vega & Lachance, FEMS Yeast Research 17 (5): fox054, 8 (2017)	1
Yarrowia	*deformans* (Zach) M. Groenew. & M.T. Sm., Antonie van Leeuwenhoek 103 (5): 1025 (2013)	7
	lipolytica (Wick., Kurtzman & Herman) Van der Walt & Arx, Antonie van Leeuwenhoek 46 (6): 519 (1980)	27

Many strains of autochthonous lactic acid bacteria and yeasts of the UNICA-DSMSP collection have been characterized at a taxonomic, biochemical, molecular, technological, and functional level for their potential use in the food industry.

As summarized in Table 6, the strains have been phenotypically identified based on several taxonomic and biochemical features, and the identification was confirmed by species specific PCR and or by sequencing [59–62]. The isolates have been also subjected to technological tests relevant for the production of a wide range of fermented foods (production of aromatic substances, acidifying, proteolytic, and lipolytic activity). Several strains were molecularly characterized with the PCR-RAPD (Random Amplified Polymorphic DNA) or PCR-RFLP (Restriction Fragment Length Polymorphism) techniques. It is well known that autochthonous microorganisms, beside conferring typicality and quality to foods, can also be endowed with functional properties of probiotic interest and therefore useful for improving consumer's health [63]. To this end, important traits such as the ability to survive the passage in the digestive tract (with the SSDP: Simulated Stomach–Duodenum Passage test), the stability at extreme pH values and high concentrations of bile salts (essential for the probiotic to reach the intestine in an optimal condition), the ability to adhere to intestinal cells (which represents both the first step in the colonization process and one of the mechanisms by which probiotics exert their action), the ability to antagonize pathogenic and/or spoilage microorganisms through the production of different substances with antimicrobial action (organic acids, hydrogen peroxide, carbon dioxide, bacteriocins) were evaluated [64–71].

Table 6. Tests used for the characterization of the strains of the UNICA-DSMSP collection.

Characterization	**Bacteria**	**Yeasts**
Taxonomic-	Morphology	Morphology
Biochemical	Gram positive	Fermentation of GLU-GAL-LAC
	Catalase negative	Assimilation of LAC-LAT-CIT
	Gas production from glucose	Urease activity
	NH_3 production from arginine	Growth at 10°, 15°, 45 °C
	Esculin hydrolysis	
	Growth at 10°, 15°, 40°, 45 °C	
	Tolerance to 6,5/10% NaCl	

Table 6. *Cont.*

Characterization	Bacteria	Yeasts
	Growth at pH 3,9/9,6	
	Carbohydrates fermentation profile	
Molecular	Species-specific PCR	Species-specific PCR
	PCR-RAPD	PCR-RFLP
	Sequencing of 16S rDNA	Sequencing of D1-D2 26S rDNA
Technological	Acidifying activity	Proteolytic activity
	Proteolytic activity	Lipolytic activity
	Lipolytic activity	Growth atl 6, 10, 15, 20% NaCl
	Fermentation of citrate	
	Milk coagulation	
	API ZYM	
Functional	Survival at pH 2.0/2.5	Survival at pH 2.0/2.5
	Simulated stomach-duodenum passage	Simulated stomach-duodenum passage
	Autoaggregation ability	Autoaggregation abillity
	Hydrophobicity	Hydrophobicity
	Adhesion properties	Adhesion properties
	Antimicrobial activity in vitro e in situ	Killer activity
	Bile salts deconjugation	Bile salts deconjugation
	Antibiotics resistance	
	Cholesterol assimilation	

The technological potential and biodiversity of the microorganisms present in the UNICA-DSMSP microbial collection can be exploited for different purposes. Over the years, numerous strains have been used in research projects aimed at: (i) their use as fermentation agents (autochthonous starter and/or integrative cultures) with the purpose of obtaining foods with unique qualitative characteristics. The isolated strains were characterized, selected, and used in pilot experiments with subsequent organoleptic, chemical-physical, and microbiological evaluation of the finished products [72–74]; (ii) their use as bioprotective culture, based on the ability of some strains to inhibit the development of pathogenic and alterative microorganisms, in order to increase the safety and shelf-life of the products and ensure greater sustainability of the production process [75–77]; (iii) the development of innovative/healthy products for new market segments. In recent years, the production of innovative functional foods that represent a driving force for the territory, through the enhancement of typical local products, has emerged among the research priorities in the agri-food field [78]. Our studies have highlighted the presence, in typical artisanal

products, of lactic bacteria and yeasts with functional properties of probiotic interest, and some of these have been used successfully in the production of cheeses [65,68,73].

4. Conclusions

The need to improve the sustainability of crop cultivation and food production is becoming a prominent factor for the next generation industries. Particularly, the use of microbial inoculants with specific metabolic activities represents a feasible and eco-friendly approach to increase crop yields and quality, restore soil fertility, control pathogenic or spoilage organisms, and recycle and dispose of wastes. In this context, microbial collections play a fundamental role as they promote the isolation, characterization, and preservation of such microorganisms, thus enlarging the spectra of potentially useful biotechnological applications for the safeguard of the environment and the sustainability of food production. The work carried in the MicroBioDiverSar Project allowed one to reach some important goals, such as having a fairly precise overview of the consistence of the resources hosted in the ex-situ Sardinian microbial collection (MBDS), create a federated database, based on international standards, for the microorganisms' information storage and management, that is now being used as a template for other Italian collection belonging to the JRU MIRRI-IT, and to build a dedicated website. For the results of the project to continue to have a strategic significance and a concrete value over time, additional implementing decrees of regional and national laws must be issued, and structural funds should be specifically dedicated to microbial resources, thereby ensuring the sustainability of microbial collections and the survival, maintaining, and managing of microorganisms in accordance with internationally recognised quality standards. That is how the collections can aspire to attain biobank status and play a key role for the development of local and global biotechnology, bioeconomy, and social welfare in a sustainable way.

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