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Preparatory action on EU plant and animal genetic resources

Institute of Sciences of Food Production National Research Council (ISPA)

Overview

Microorganisms are essential components of biological diversity, fundamental elements which guarantee the existence of sustainable ecosystems. About 50% of the living biomass on the planet is microbial and microorganisms provide an important source of genetic information for molecular biology and biotechnology which is being used in several business applications. Therefore it is of key economic importance to collect, store and characterise these microorganisms which can be used for several purposes.

The role of the first microbial culture collection (CBS in the NL), apart from research purposes, was related to agriculture, brewing and medicine. In 1930, with the discovery of penicillin, the importance of these collections strongly raised together with the awareness that fungi are great sources of biological activities. The industrial and biotechnological applications of fungi include brewing and wine making, baking food processing, enzyme productions, antibiotics, organic acid and vitamin production, genetic engineering, pesticide and insecticide development.

The increasing number of culture collections worldwide, private or institutional, mirrors the need to preserve these cultures protecting the microbial gene pool for biological research, industrial applications and the preservation of biodiversity.

Microbials and microorganisms group several types of organisms, including fungi. Fungi that are moulding agents of agri-food products include species from the genera *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria* and *Claviceps* whose ability to form mycotoxins - substances responsible for repeated episodes of food poisoning both in livestock and in humans - is the cause for the justified apprehension for food safety. This toxigenicity is unevenly distributed among species within a given fungal genus, and within the same species only some strains will have it, each usually having its own toxigenic profile.

The need to assess this biodiversity, which is a requirement for the safety and protection of foodstuffs, led to the foundation of specific collections (or fungal reference material) and to research into new molecular diagnostic methods. These are the main objectives of the ITEM collection in Bari.

1. Objectives

In that context, the objective of this case study is to describe and identify added values and bottlenecks being faced by the ITEM Microbial Culture Collection in Bari, Italy which is recognised as an EU key factor in the conservation and sustainable use of agro-food toxigenic fungi. Additionally, the study analyses how this large collection has been used to develop new products and supported the development of business applications in the food sector, particularly in the seasoning of salami in Italy, as well as in yeast and bacteria of winery fermentation of Apulia region.

2. Description of the case

Since 1997, ITEM microbial culture collection is based in Bari at the Institute of Toxins and Mycotoxins from Plant Parasites (ITEM - CNR). Successively in 2001, ITEM was merged with other four Institutes of CNR to fund the Institute of Sciences of Food Production at CNR. This new Institute (ISPA-CNR) was set up in 2001 is located in Bari and involves four sections distributed throughout the country, namely in Lecce, Sassari, Turin and Milan.

The ISPA main objectives include the production, storage, preservation and processing of food, with improvement of quality and safety; selection, characterisation and preservation of microorganisms important for the agro-food sector, developing useful tools for quantifiable and significant reductions in toxin contamination for food and feed and development of innovative techniques for the improvement of production processes.

Since 2001, the ITEM Microbial Culture Collection belongs to ISPA. The ITEM-ISPA collection includes more than 13,000 strains belonging to various agro-food microorganisms with phytopathological and toxicological significance; about 6,500 are available as public resources to the scientific community. Thousands strains belonging to toxigenic genera of *Aspergillus*, *Alternaria*, *Fusarium*, and *Penicillium*, represent a great biodiversity in the ITEM collection to deepen the knowledge on fungal biology and strategies development for reducing mycotoxin contamination. In addition, about 300 yeasts and 400 lactic bacteria strains with peculiar properties has been also preserved and characterised for autochthonous industrial fermentation of typical Apulian wines, table olive and dairy products.

In 1998, ITEM joined the European Culture Collection Organisation (ECCO). The catalogue of the Collection was first published in 1997 and it is available online¹. For each fungal strain the following information is provided: a) name of the species, author/s, ITEM accession number; b) geographical origin, substrate/host, isolation, year, depositor, accession numbers of other Collections; c) biological, molecular, chemical and toxicological information; d) specific references.

The Institute of Sciences of Food Production (ISPA-CNR) is a centre of excellence, worldwide renowned, acting in the fields of scientific research, innovation and technology transfer aimed to improve safety and quality of agro-food products. By creating synergistic

¹ www.ispa.cnr.it/Collection

actions between scientific research and production sectors with the transfer of scientific knowledge, ISPA-CNR approaches and objectives are to foster technological innovation paths of small, medium and large national and foreign agro food companies. Among the activities of the Institute, the collection and preservation of microorganisms important for the agro-food sector is a relevant issue. This work is not only intended to serve the purpose of conservation as significant is dedicated to the creation and management of projects between scientific researchers and production sectors.

The achievements of these objectives go through the development of partnerships, development of cooperation at EU level as well as outside Europe. To sustain this activity ITEM hope to offer new services dedicated to private sector; like screening and counselling. One of these available services is e.g. the confidential safe deposit ITEM offers for those valuable cultures for which patent protection has not been sought. These cultures are stored frozen (below -150°C) and/or on agar (see www.server.ispa.cnr.it).

Main of the researches activities of ISPA are connected also with the aims of ITEM Culture Collection as follows:

- Research on biodiversity, phylogenetics and population genetics of filamentous fungi, mainly on toxigenic fungi;
- Identification of toxigenic fungi and evaluation of mycotoxigenic risks;
- Development of new molecular probes to detect microbial contamination;
- Development of new diagnostic methods for agro-food important microorganisms;

In recent years at ISPA, particular efforts have been made to analyse the etiology, epidemiology and ecophysiology of toxigenic fungi as well as their ability to produce and accumulate mycotoxin in pre- and post-harvest of the most important crops and agro-food chain, especially in the Mediterranean area.

One of the recent concrete outputs of the research activities is the identification of a new *Penicillium* species (*Penicillium salamii*), by a group of researchers led by Giancarlo Perrone of ITEM, which is used during the seasoning of dry-cured meat. The research and its application have been summarised as follows by the research group²: “Both industrially and handmade salami are quickly colonised by a composite mycobiota during seasoning, often with a strong predominance of *Penicillium* species. These species are involved in the improvement of the characteristics and taste, and in the prevention of the growth of pathogenic, toxigenic or spoilage fungi. During the survey of fungal species occurring on the salami surface and in the air of the seasoning and storage areas of a salami plant (Calabria, Italy), two *Penicillium* species were predominantly present. One species was identified as *Penicillium nalgiovense*, and the other was related to, but distinct from, *Penicillium olsonii*. Further molecular and biochemical analyses showed that this strain has high homology with

² Peronne G. and al. 2015, “*Penicillium salamii*, a new species occurring during seasoning of dry-cured meat”. [Int J Food Microbiol](#). 2015 Jan 16;193:91-8. doi: 10.1016/j.ijfoodmicro.2014.10.023. Epub 2014 Oct 23

the not yet described species named "Penicillium milanense" isolated in Denmark and Slovenia on cured meats. The taxonomic position of these strains in Penicillium was investigated using calmodulin, β tubulin and ITS sequences, phenotypic characters and extrolite patterns, and resulted in the discovery of a new Penicillium species, described here as *P. salamii*. A literature search showed that this species occurs on (cured) meat products worldwide. In our study, *P. salamii* predominated the salami and capocollo surface in levels similar to the commonly known starter culture *P. nalgiovense*, irrespective of the room or age of seasoning. Preliminary inoculation trials with *P. salamii* showed that it was able to colonise salami during seasoning, indicating that this species could be used as a fungal starter for dry-cured meat”.

This new strain could be in futue of commercial use in seasoning plans, technological properties and preliminary seasoning studies need to be conducted. Business actors are required to develop a marketing and commercial plan for this new species which has been deposited in Culture Collection at international level. However, it seems that to date no clear candidates have emerged in order to develop commercial products from this new strain.

Analysis

3. Funding and support

Funding and policy support, is an ongoing issue. In 1997 they received funds to start the structuration of the catalogue. Most of the time they did not have sufficient support to maintain the collection. In general, some funds come from research projects of ISPA in which collection and characterization of microorganisms of agro-food importance is a priority issue.

Regarding EU funding, ISPA has been widely involved in the participation in EU projects in the last 20 years, mainly in research activities related to toxigenic fungi and mycotoxins: identification, detection, characterisation and control such as: CONTROL MYCOTOXIN FOOD QLK1-1999-00996; ALTERNARIA QLK1-1999-0098; OCHRA WINE – Risk QLK1-CT-2001-01761; MYCO-GLOBE 007174; RAFBCA - QLK1-CT-2001-01391; RAMFIC-QLK5-CT2000-01517.

The Institute of Sciences of Food Production (ISPA) has been a beneficiary of the EU MYCORED FP7 project (KBBE-2007-222690 2009-2013) ("*Design and development of novel integrated strategies for worldwide mycotoxin reduction in food and feed chains*") which aimed at improving network synergy in mycotoxin research. In this project a big increase in the collection worldwide of mycotoxigenic fungi from different food matrices has been achieved by ITEM Collection,

However direct participation of ITEM Collection to funds is limited; they are not many opportunities in the domain of microorganisms.

The main part of the budget used for ITEM Collection, comes from overheads of ministerial or EU projects. No direct funds are devoted from CNR or Ministry to subsistence of ITEM Culture Collection. There is no clear budget dedicated to the collection itself. Resources are found in the context of research activities but the conservation work is not recognised from a financial point of view.

Some fund comes from the commercialisation of the strains (around .600-1000 euros/year) (ITEM receives, prepares and sends about 20 requests of material all around the world per year). This activity is very limited even if potential to increase this activity exist. However this “commercialisation” of strains is not the business of researchers; they are not prepared for such type of activities and ITEM does not have a specific and dedicated structure to develop the commercialisation of such new strains.

Recently, in 2014 within a Ministerial Project “BIOforIU” multidisciplinary infrastructure for the study and appreciation of marine and terrestrial biodiversity in the context of the "Innovation Union", the ITEM Culture Collection has received two new Cryogenic Storage Chest Freezers equipped with software for barcode cataloging of the strains.

Finally, in the next year it is foresee the transfer of the crio-preservation equipment and the microbiological lab for the Culture Collection in a new dedicated space of the ISPA-CNR building. The idea is to gain the ISO 9001 certification after in the next future.

4. Positioning at local, regional level or international level, partnerships and networking

The ITEM operates in the Italian context which is rather rich in terms of the number of microbial domain biological resources centres (mBRC). A recent inventory that has been carried out in the context of the Microbial Resource Research Infrastructure (MIRRI) project has highlighted the important role of the ITEM and other structures, in particular:

- The University of Turin (MUT) and Azienda Ospedaliera Universitaria San Martino-Instituto Nazionale per la Ricerca sul Cancro (USMI) which are both partners with the MIRRI consortium;
- The University of Perugia (DBVPG) which a collaborative party of MIRRI (as well as the ITEM);
- 19 additional mBRCs have replied to a survey that was launched to perform this inventory; and
- Many other research collections have been identified which belong to universities, CNR, CRA, IZS and hospitals.

This Italian MIRRI network presents several added values and in particular it guarantees the security of microbial biodiversity according to best practices for the future use and protection

of public funding investment, legal certainty on genetic resources use becoming an important tool to comply with national and international obligations (CBD, Nagoya Protocol, etc.), data inter-operability, broader access to material for exploitation of microbial diversity, access to new technologies, services and expertise to meet researchers' and stakeholders' needs. This network also facilitates the development of clusters of expertise and innovative services for the benefit of the whole communities.

At European level, the ITEM is a member of the European Culture Collections' Organisation (ECCO) (joined in 1998) which was established in 1981. The aim of this long standing, voluntary and scientific-technical network is to promote collaboration, as well as the exchange of ideas and information about all the aspects of culture collection activity. Corporate membership is open to the representatives of any microbial resource centres that provides a professional public service on demand and without restriction that accepts cultures for deposit, provides catalogues and is housed in countries with microbiological societies affiliated to the Federation of the European Microbiological Societies (FEMS). In addition, the members' collections must be registered with the World Federation for Culture Collections (WFCC). The organisation welcomes sustaining and individual members.

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At international level, ITEM has various collaborations established worldwide, among which the main important are: Kansas State University, ARS USDA Culture Collection – Peoria Illinois, CBS Fungal Biodiversity Center, IBT Culture Collection of Fungi of DTU – Denmark, Micoteca da Universidade do Minho, Braga – Portugal, and Biodiversity-Mycology Group of Agriculture and Agri-Food Canada. Projects objectives with these research organisations are genetic analyses and descriptions of specific strains; as well as preservation of specific material. These relationships are stable over time.

5. Communication

ITEM's communication is mainly based on being active in the different networks that have been described above. As researchers, the main communication targets are other researchers in Italy, in Europe or at international level.

6. Outputs, added value and sustainability

The conditions for the sustainability of the collection and for its development are that maintenance and production stay linked to the food safety activity. This link is necessary to secure the long term sustainability and funding of the collection. The added value of the collection should be demonstrated via the development of projects and/or products that have added value for food safety policies and strategies. The example of the new *Penicillium salamii* species that has been developed in collaboration with several researchers is a key example of what the ITEM should produce on a regular basis to remain sustainable.

However, the added value of such research has to be demonstrated to non-research stakeholders through the supply chain. This is why it is important to build pathways for the valorisation of such research in the food chain. Without such type of valorisation the research activities may be at risk in the medium term. It is only by demonstrating the added value of the research at market level that research activities will continue to be funded.

The second source of income would be to increase the selling of strains to commercial actors. Here again this activity is not the responsibility of researchers and therefore the bridge between researchers and users of genetic resources is not established correctly. This leads to an underuse of the strains in the collection.

Conclusions

STRENGTHS	WEAKNESSES
<p>Strong scientific cooperation at international level;</p> <p>Delivery of concrete research results in the form of a new <i>Penicillium</i> strain.</p> <p>Maintaining important microorganisms biodiversity</p> <p>Barcoded cryoconservation system – database</p> <p>Specificity of the collection at International level</p>	<p>Lack of funding and a dedicated budget to maintain the collection;</p> <p>Lack of recognition and visibility at research level;</p> <p>Lack of priority by national government.</p> <p>Few service activities</p> <p>No ISO certification</p>
OPPORTUNITIES	THREATS
<p>Develop commercial products (additives for seasoning) to sell them in Northern EU countries;</p> <p>Raising awareness of targeted seasoning in EU southern countries.</p> <p>Improve services and network of culture collection at Italian and European levels</p> <p>Improve collaboration with industries involved on food and agriculture</p>	<p>Lack of business development expertise;</p> <p>Low usage of seasoning products in Italy.</p>

The ITEM-ISPA case study is a key example of research activities that led to concrete results as here a new *Penicillium* strain adapted for the seasoning of salami was identified. This research led to the registration of the strain. This work has been conducted by a group of researchers at international level. Therefore, the work has been carried out by mutualising resources from different centres of expertise.

This research brought on significant outcomes despite the lack of funding and resources dedicated to the project, and especially dedicated to the *ex situ* conservation activities. The *ex situ* collection is not recognised as a specific activity as there is no budget specifically allocated to it. Funding takes mainly the form of in-kind support by the researchers themselves. The tasks of curators are done by researchers and there is no specific curator

job within the unit. Regarding the maintenance of the IT system, the work is also done by the researchers and some IT tasks are performed by external IT support.

While the research work has been recognised within the research community and the new strain has been registered at international level (World Federation for Culture Collections), there is a lack of possibility to make this new strain a commercial product. In Italy, neither additives nor flavourings are used for the seasoning of salami, as microorganisms are naturally present in seasoning rooms. However, the ITEM-ISPA researchers interviewed consider that using microorganisms to improve seasoning would help reduce production loss. Marketing expertise is required to commercially develop this product. This is not the role of the researchers, who lack knowledge on how to bridge this gap between the research and business development of their strains.

Figures: Visit of the laboratory and the collection together with Dr. Giancarlo Peronne





Annex 1 – List of interviewees

- Dr. Giancarlo Perrone – Reseracher ITEM – ISPA
- Dr. Antonio Logrieco – Scientific responsible ITEM – ISPA
- Vincenzo Ricci – Technical responsible ITEM – ISPA

Annex 2 – List of references

Project MYCoRed: European project part of the seventh framework program
<http://www.mycored.eu/>

WFCC; The WFCC is concerned with the collection, authentication, maintenance and distribution of cultures of microorganisms and cultured cells
<http://www.wfcc.info/about/>

Official websites of the institute
<http://www.cnr.it/>
<http://server.ispa.cnr.it/ITEM/Collection/>

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