Diversification of edible wild mushroom cultivation with new native species

Summary

The project was led by Bolet Ben Fet (TEB Verd SCCL). The operational group consists of: Bolets de Soca (Tresseras Multimèdia SL), the Catalan Mycology Society, the Wood and Furniture Guild and the Institute of Agrifood Research and Technology (IRTA). IRTA acted as a technology and research centre, and two lecturers from the University of Barcelona (UB) joined the team of researchers.
A total of 120 strains belonging to 14 fungal species were isolated from the specimens collected in the field. A mixture of wheat, rye and sorghum grain in equal parts was designed with a water content adjusted to 50-60% and sterilised in the autoclave. It was tested successfully with 87 different strains of 11 fungal species for the production of the inoculum (seed). The small-scale trials were carried out using a substrate based on hardwood sawdust adapted to 60-65% humidity levels.
An incubation temperature of 20-25°C was suitable for all the species.
The project has made it possible to develop methods and protocols for the cultivation of eight edible fungal species from native strains. The cultivation protocols can be considered developed for: *Agrocybe aegerita*, *Fistulina hepatica*, *Lypphyllum decastes*, *Meripilus giganteus*, *Pleurotus eryngii* and *Polyporus squamosus*. They were also developed for better known species including: *Ganoderma lucidum* and *Grifola frondosa*, but using native strains.

Objectives

Catalonia is a country with a strong mycological presence and tradition, but the cultivation of wild forest mushrooms focuses on a few species that are mostly of Asian origin; this is a pioneering initiative in this type of cultivation. Diversification into other species with closer links to local tradition would increase the diversity and current range of edible fungi. These new products would give local producers a competitive advantage and open up new opportunities for export.
The main objective of this project was to incorporate new species of fungi which are mostly lignicolous and native to Catalonia in the cultivation of edible mushrooms in order to diversify production and improve the commercial range of our country's producers.

Description of the actions carried out in the project

Field surveys in various ecosystems and collection of specimens of various species of lignicolous fungi
During the project, 43 consignments were sent to the IRTA laboratories containing specimens collected in the field of 18 different fungal species. Field prospecting took place in the autumn months of the two-year project, and the specimens of the following species were collected: *Agrocybe aegerita*, *Albatrellus pes-caprae*, *Auricularia* sp., *Calocybe gambosa*, *Fistulina hepática*, *Grifola frondosa*, *Laetiporus sulphureus*, *Lyophyllum decastes*, *Meripilus giganteus*, *Pleurotus eryngii*, *Polyporus squamosus*, *Sparassis crispa and Volvariella* sp., *as well as Ganoderma lucidum*, *G. resinaceum*, *Infundibulicybe geotropa*, *Lentinellus ursinus* and *Pleurotus dryinus*, which were not on the initial list of candidate species, but which were considered interesting from other points of view or for other applications due to their characteristics.

Isolation in pure culture and maintenance of a collection of strains of the fungal species gathered
More than a hundred fungal strains belonging to 14 edible species were isolated from the specimens collected: *Agrocybe aegerita*, *Calocybe gambosa*, *Fistulina hepatica*, *Ganoderma lucidum*, *Ganoderma resinaceum*, *Grifola frondosa*, *Infundibulicybe geotropa*, *Laetiporus sulphureus*, *Lyophyllum decastes*, *Meripilus giganteu*, *Pleurotus eryngii*, *Polyporus squamosus*, *Sparassis crispa and Volvariella*

caesiotincta. This bank of fungal strains obtained and maintained at IRTA was used to produce inocula and to perform all the tests in the project, and remains available for future research and development work.

Development of seed production methods (fungal inoculum)

The results led to the design of a mixture of wheat grain, rye and sorghum in equal parts (1:1:1 in volume) with a water content adjusted to 50-60% and sterilised in the autoclave (120°C, 60-90 minutes depending on the total volume) as a robust matrix for inoculum production, which is ideal for growing virtually all the strains tested, and provides the ease of handling required for use in inoculation (or sowing) of the cultivation substrates. The optimum incubation temperature was set at 23°C (with a range of ± 2°C). The seed production method developed was successfully tested with 87 different strains of 11 fungal species. Seed incubation times ranged from 4 to 16 weeks depending on the growth rate of the fungal species. Most were between 8 and 10 weeks.

Determination of the optimal cultivation conditions for each fungus

Small-scale trials were performed with all the strains with which a viable seed production was achieved, in order to ascertain whether it was possible to cultivate these fungi under standard conditions. The small-scale trials were carried out using a substrate based on hardwood sawdust (52% chestnut, 12% holm oak, 12% beech, 23% cereal and 1% gypsum) adjusted to 60-65% humidity levels. An incubation temperature of 20-25°C was suitable for all the species.

Six of the fungal species tested gave positive results to varying degrees under standard growing conditions: *Agrocybe aegerita*, *Fistulina hepatica*, *Grifola frondosa*, *Meripilus giganteus*, *Pleurotus eryngii and Polyporus squamosus*.

The cultivation substrate had to be modified for *Lyophyllum decastes* for it to grow normally and obtain consistent mushroom production levels. The modification consisted of the replacement of a large proportion of the sawdust from deciduous trees with compost from heather (*Erica* spp.).

Laetiporus sulphuresus developed rapidly under standard substrate and growing conditions, but no fruit formation took place. New substrates composed of conifer sawdust need to be tested for other species such as *Sparassis crispa*.

Pilot production tests under industrial conditions.

After the seed production systems and cultivation conditions were established, the mushroom production phase was adjusted to conditions similar to an industrial scale.

According to the results obtained, the cultivation protocols can be considered fully developed for: *Agrocybe aegerita*, *Ganoderma lucidum*, *Grifola frondosa*, *Meripilus giganteus*, *Pleurotus eryngii and Polyporus squamosus*. Cultivated mushroom production was also achieved for *Fistulina hepatica*, which improved with incubation under dark conditions, and *Lyophyllum decastes*, when the composition of the cultivation substrate was modified with the addition of composted heather.

Methods and protocols for the cultivation of eight edible fungal species from native strains were developed during the project. Some of these species, such as *Fistulina hepatica*, *Lyophyllum decastes* and *Polyporus squamosus* have never been cultivated before.

Evaluation of the nutritional and organoleptic characteristics of the new fungi cultivated.

In general, the values obtained in the nutrient composition studies were as expected for these types of fungi. For example, the water content is slightly lower in *Lyophyllum decastes* compared to the other species, the total protein content is higher in *Agrocybe aegerita* and *Polyporus squamosus*, the levels of extractable polyphenols are relatively high, and the total lipids content is low. These profiles have high levels of unsaturated fatty acids, with a ratio between saturated and unsaturated which is much lower than 1, indicating that the fats in these species are good candidates for use in human and/or animal food.

Another interesting question to be answered was whether the chemical composition of cultivated mushrooms is similar to that found in wild specimens collected in natural ecosystems. These comparisons have to date been made for *Fistulina hepatica*, as it was one of the few species of which we had enough wild specimens to be able to perform the studies. There were no significant differences in total protein or fat content between wild and cultivated *Fistulina hepatica*. There were also no

differences in the vitamin C content or in the relative proportions of the various fatty acids. A slightly higher polyphenol content was observed in the cultivated mushrooms.

Description of the contribution to the AEI's goal of boosting productivity and sustainable resource management.

From the outset, this project aimed to incorporate new fungal species native to Catalonia into the range of cultivated edible mushrooms. The results obtained achieved the project's objective, which was to increase and diversify the current range available on the market towards new products with more local roots.

The project also provided new fungal species: *Agrocybe aegerita*, *Fistulina hepatica*, *Ganoderma lucidum*, *Grifola frondosa*, *Lyophyllum decastes*, *Meripilus giganteus*, *Pleurotus eryngii and Polyporus squamosus*, which are recognised as edible or as having interesting medicinal properties in various parts of Catalonia. They had not previously been cultivated and were obtained only by gathering in the forest. In the case of *A. aegerita*, *G. frondosa* and *P. eryngii*, the cultivated strains came from other countries. These new native species and strains will boost the productivity of local farmers.

In addition to these species, of which three are already being produced, there are others, such as *Laetiporus sulphureus* and *Sparassis crispa*, for which the modifications that need to be made to the cultivation protocols in order to achieve the formation of mushrooms have yet to be determined, despite having undergone the initial stages of cultivation. Research and development should continue given the interest in both species.

The inclusion of these fungal species which produce edible mushrooms in an agronomic cultivation system will reduce the pressure from pickers on their natural populations, thereby reducing the environmental impact of the collection, and contributing to the sustainable management of these resources.

This will help preserve biodiversity and the role that these fungi play in the biogeochemical cycles of natural ecosystems.

The substrates used to cultivate these mushrooms are obtained from non-food waste produced by forestry and agriculture, enabling its use and recycling. Most species are grown on substrates in which residues from deciduous wood (chestnut, holm oak and oak) is used as the main component. Some of the species we are adding will require changes to these substrates, involving the use of residues from other woods, such as conifer sawdust for *Sparassis crispa*, or heather compost for the cultivation of *Lyophyllum decastes*. The use and recycling of this waste can be extended to other materials and wood sources that have been used less extensively to date.

Final results and practical recommendations

- A crop bank of 120 strains belonging to 14 edible fungal species native to Catalonia has been established. This collection of pure strains is available for future research and development work.
- A viable seed production method has been established for 11 of the fungal species mentioned above.
- The project has made it possible to develop methods and protocols for the cultivation of eight edible fungal species from native strains: *Agrocybe aegerita*, *Fistulina hepatica*, *Ganoderma lucidum*, *Grifola frondosa*, *Lyophyllum decastes*, *Meripilus giganteus*, *Pleurotus eryngii and Polyporus squamosus*. Some of these species, such as: *F. hepatica*, *L. decastes* and *P. squamosus* have never been cultivated before.
- Nutrient and cytotoxicity analyses for all the newly cultivated species are being developed.
- The complete cultivation cycle has not yet been reached for *Laetiporus sulphureus*. No mushrooms have been obtained. Interest in the species has led to the start of a bachelor's degree final project at the University of Barcelona to continue the research.

Conclusions

Eight species of fungi were added to mushroom cultivation. Some of them, such as Fistulina *hepatica*, *Lyophyllum decastes*, *Meripilus giganteus and Polyporus squamosus* have never been cultivated

before. Other more well-known strains, including: *Agrocybe aegerita*, *Ganoderma lucidum*, *Grifola frondosa and Pleurotus eryngii*, cultivated from native strains.

The diversification of edible mushroom cultivation should continue in the long term in order to incorporate new products into the market. Cooperation between companies in the sector and research centres with support from government institutions proved to be an effective way of achieving these results.

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Subject area(s) of application

☒ Agricultural production system

☒ Biodiversity and environmental management

☒ Competitiveness and agricultural and forestry diversification

Geographical area(s) of application

PROVINCE(S): Barcelona, Tarragona, Lleida and Girona

REGION(S): All

Dissemination of the project: publications, seminars, multimedia, etc. (State links)

https://www.teb.org/carles-diaz-participa-a-la-xerrada-telematica-diversificacio-del-cultiu-de-bolets/

https://www.irta.cat/ca/el-cultiu-dels-bolets-de-soca-autoctons-cami-de-ser-una-realitat/

Dissemination activities as part of the DACC's Annual Technology Transfer Plan (ATTP).

- Diversification of edible wild mushroom cultivation with new native species. Online technical seminar. Thursday 8 April 2021.
- New native species in the cultivation of edible mushrooms. Online technical seminar. Thursday 11 November 2021
  - https://youtu.be/H-WxV6-A89c
  - https://ruralcat.gencat.cat/c/document_library/get_file?uuid=791f762d-95a8-4031-ac28-8c444ccae453&groupId=20181
  - https://ruralcat.gencat.cat/c/document_library/get_file?uuid=6c3789b8-1f92-4ad3-a157-ec1ed86a0119&groupId=20181

Social media.

Project website

https://www.bolets.net/

More information on the project

PROJECT DATES	TOTAL BUDGET
Start date (month-year): July 2019	Total budget: €199,850.00
Completion date (month-year): September 2021	DARP funding: €79,740.15
Current status: Executed	EU funding: €60,154.85
	Own funding: €59,955.00

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Order ARP/133/2017 of 21 June, approving the regulatory bases for grants for cooperation for innovation by promoting the creation of European Association for Innovation operational groups in the areas of agricultural productivity and sustainability and the execution of innovative pilot projects by those groups, and Resolution ARP/1282/2018, of 8 June, announcing the call for the grant.

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