Investigating mold fungi, nature's substances, to replace pesticides

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The entire collection of fungi isolates are grown in the laboratory at DTU Bioengineering as part of the big project. Credit: Thomas Steen Sørensen

DTU is helping a large international company find nature's own substances to combat fungal diseases in crops. The University's large collection of mold fungi and a minor collection of bacteria are part of the project—and one of them might hold the solution. Thirty-eight thousand, four-hundred mold fungi isolates take the lead role in the large research project "Smarter AgroBiological Screening" (SABS). In the project, DTU collaborates with the international company FMC, which produces plant protection such as pesticides for agriculture.

The elusive "lead actors" are stored in plastic tubes with red screw caps at exactly 9°C in a basement at DTU, and they form an internationally recognized collection of fungi that was started in 1988 by university researchers storing the first isolates.

Now, 35 years later, they will be studied carefully along with a minor collection of bacteria. Perhaps, these microorganisms can be used to produce biofungicides, i.e., natural substances that can fight fungal diseases in cereal crops. The expectations are particularly high for mold fungi.

"Biotechnologically speaking, the mold fungus is a really exciting organism, because each fungus has between 50 and 80 biosynthesis pathways. A biosynthesis pathway is a series of reactions inside the organism that enable the production of a bioactive substance. In comparison, a normal bacterium might have six to seven biosynthesis pathways while a yeast cell has none. It makes fungi really rich but also very complex to study. So exploring fungi has great potential, and perhaps we can find substances that can be used for disease control in agriculture," says Rasmus John Normand Frandsen, Associate Professor at DTU and coordinator of DTU's share of the project.

He elaborates, "For the vast majority of the substances—possibly up to 95% of them—we have no idea what they are used for or why the microorganisms produce them. But they are made in nature for a reason and perhaps with a purpose we can benefit from."

Pesticide use must be halved

Finding alternatives to chemical-based pesticides is urgent as the EU is proposing a halving of the member states' use by 2030 and a complete ban in sensitive areas.

But pesticides have—despite their bad reputation—ensured that crop yields are not destroyed by plant diseases and insects. According to a memo from Aarhus University, phasing out pesticides will result in significant production losses, and a total phase-out will cause an average decrease in grain yields of 23 percent as well as large losses—up to 50%—in sugar beet and potato production.

With increasing global food demand, we need to find other ways of securing good crop yields to realize a green transition of plant breeding that does not require including more land for growing crops and thus emitting more CO_2.

The collection of fungi is stored at exactly 9 °C in a basement at DTU. Credit:

Thomas Steen Sørensen

Preparing fungi collection for robots

So how do you examine 38,400 mold fungi isolates? Right now, there is only one, slow method; the handheld one, says Niels Bjerg Jensen, a project manager on the project and liaison to FMC. But as a key part of the SABS project, DTU's entire fungi collection will be "modernized," so we can avoid the handheld part in the future and use robots to screen the collection instead.

The modernization entails two laboratory technicians currently retrieving the isolates from the basement and unscrewing the red lid, one by one, to pipette spores from the fungus and transfer them to an agar plate where they can grow in the laboratory. After 8–10 days, the laboratory technicians can harvest the fresh spores and transfer them to a plastic tray with 24 holes (or wells, as they are actually called) where each well houses its own fungal isolate.

Then the robot takes over and eventually transfers the fungi to a plastic tray with 96 wells. Now the format of the fungi fits into the automated process where a robot can pipette spores from 96 fungi at once.

"In the future, this means we will be able to screen approx. 100 times more mold fungi at a time when we search for an organism to help us," says Niels Bjerg Jensen, who explains that the fungal collection in the new robot-friendly format will be stored at minus 80°C, so that the isolates can be retrieved again and again for future screenings.

High-throughput laboratories

The automated process, where the slow and handheld pipetting is skipped, means that both speed and amount of data increase tremendously. It is a trend that is seen worldwide called high-throughput laboratories.

"It is evident from the biotechnology research articles around the world that the data sets are getting bigger and bigger. Just a few years ago, it was normal for a data set to consist of perhaps a dozen microorganisms. Now it is possible to include hundreds of microorganisms," says Rasmus John Nordmand Frandsen.

Project Manager Niels Bjerg Jensen and laboratory technician Wiebke Marina Findeisen are controlling a batch of fungi. Credit: Thomas Steen Sørensen

It also places demands on the staffing of biotechnology laboratories, which now also have a need for profiles that can program robots and build data warehouses to structure the huge amounts of biological data.

As the screenings of the fungi at DTU generate data, it will also be possible to utilize artificial intelligence in the screenings of the fungi.

"Artificial intelligence can find connections and patterns on huge amounts of data that humans simply cannot survey, and it can facilitate faster identification of fungi that have the potential to help us," says Rasmus John Nordmand Frandsen.

Promising fungi discovered

In the SABS project, DTU has already screened and identified some promising fungi that were able to produce the coveted bioactive substances in the laboratory. The candidates have been delivered to FMC for further investigation. If the promising results continue, the next step is to test the substances under controlled conditions in field experiments where grain is grown using the natural fungicides.

For FMC, the project forms an opportunity to develop solutions that meet the needs of agriculture for completing a green transition.

"Biopesticides provide new methods for combating plant diseases and help prolonging the usefulness of existing chemistry. They are a sustainable tool that both meets the plant growers' need for new solutions and counteracts resistance, which helps extending the life of chemical active substances as well as protects the environment," says Burghard Liebmann, Plant Health Director R&D at FMC's European Innovation Center in Hørsholm, Denmark.

"FMC is excited about the collaboration with DTU in the SABS project. We benefit from DTU Bioengineering's large and diverse collection of microorganisms. DTU's expertise in microbiology, genomics, metabolism, automation, and artificial intelligence is valuable to the project."

Provided by Technical University of Denmark

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