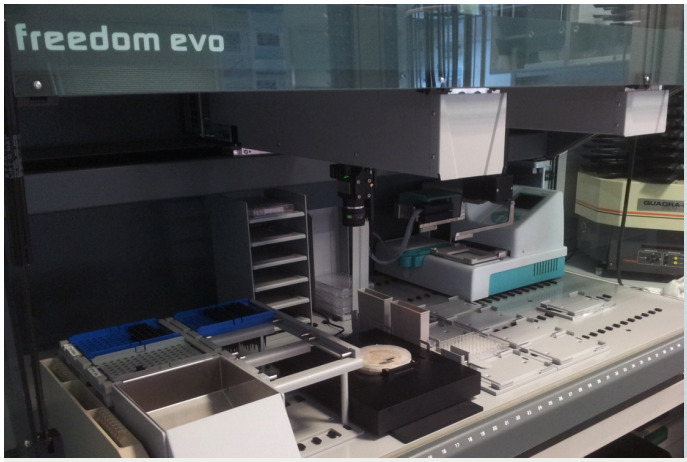

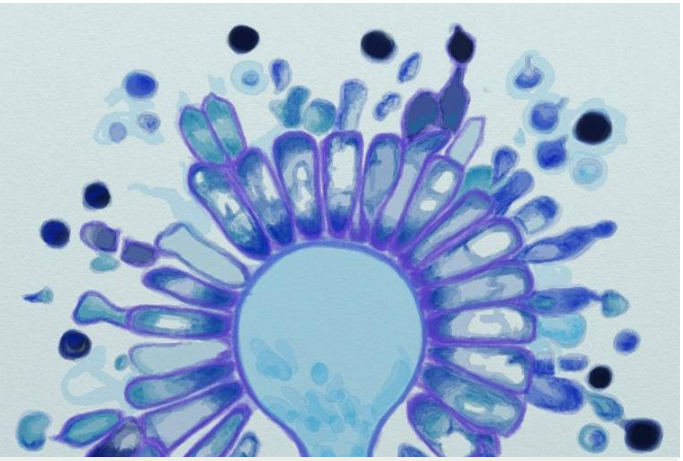

Colony picking – fully automated screening of fungi

Freedom EVO® platform with Pickolo™ colony-picker for biofuel production

Introduction

Abengoa Bioenergy New Technologies, based in Seville, Spain, is an international company applying innovative solutions for biofuel production. In this context, the Company was seeking to establish and automate a screening process to determine cellulase activity in mutant strains of cellulase-producing fungi.

Since manual screening processes are very costly and time consuming, automation was a prerequisite for a project of this magnitude. Abengoa established close collaborations with Biomar Microbial Technologies and Mejoran Lab Automation to create the optimal solution. Biomar, located in León, Spain, is a pioneer in research into micro¬biological applications to generate sustainable solutions in sectors such as healthcare, agriculture and renewable energy, and therefore has a solid background in generation, screening and bioactivity profiling of microbial collections. Mejoran, based in Madrid, Spain, is a laboratory automation solution provider.

The first step in the three-stage screening process was selection of colonies of different fungal mutants and their transfer to a 96-well plate to create microinocula for further fermentation. The second step involved fermentation of the different mutants in a 96-well plate format. Finally, the cellulase-producing capability of the different mutants was evaluated.

A Freedom EVO 150 workstation with SciRobotics' Pickolo colony-picker module proved the ideal solution for performing both the fungal selection and the subsequent screening of different culture conditions. The Freedom EVO workstation can easily identify, select and pick fungal colonies based on user-selected criteria. Additionally, Freedom EVOware® and the Pickolo software allowed Mejoran to define a unique picking procedure that achieved 100 % microinoculum growth for subsequent experiments. This was accomplished by picking from the perimeter of the fungal colonies, rather than from the center, in a sweeping motion which optimized the amount of biomass taken up by the tip.

Materials and Methods

Automation equipment

The Freedom EVO 150 workstation (Figure 1) is housed in a biological safety cabinet to ensure sterile conditions. It is equipped with an eight-channel Liquid Handling (LiHa) Arm using 200 µl disposable tips, a Robotic Manipulator (RoMa) Arm, a Multidrop® Combi nL (Thermo Scientific) and a SciRobotics' Pickolo module – consisting of the Pickolo software, a camera mounted to the LiHa, and a backlight carrier on the worktable – for colony picking.

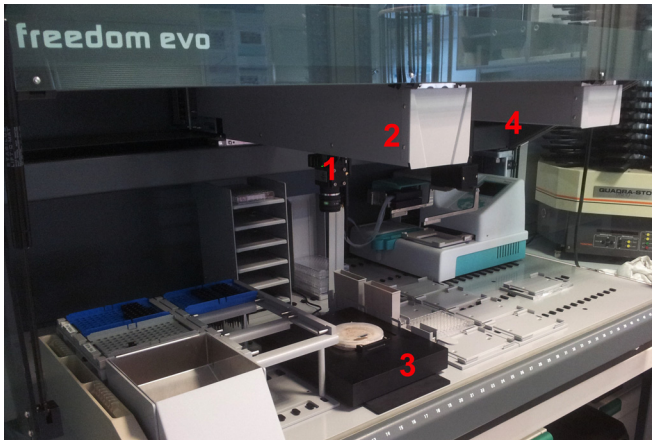

Figure 1 Freedom EVO with Pickolo module consisting of a camera (1), mounted on the LiHa (2) and the backlight carrier (3). Petri dishes are transported by the RoMa (4).

Procedure

Different cellulase-producing fungal mutants were grown from eight to 48 hours at 37 °C on a 1 % dextrose agar (Gibco) containing proprietary additives in a 90 mm Petri dish. Biomaterial was picked from the Petri dish and transferred to 96-well microplates to obtain microinocula for further downstream analysis. Figure 2 shows an overview of the automated workflow for selecting, picking and inoculating fungal colonies.

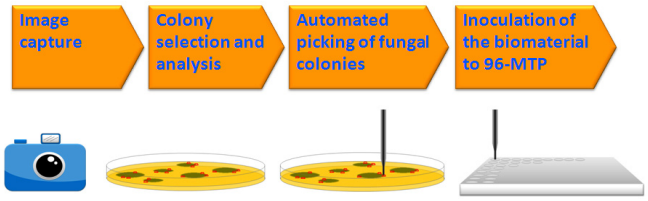

Figure 2 Schematic representation of the process workflow.

The picking criteria were defined in the Pickolo software which integrates with Freedom EVOware to perform the picking process on the Freedom EVO platform.

Results and discussion

Successful identification and selection of cellulase-producing fungi

The appearance of fungi is quite different from the classical bacterial colonies, as they develop into colonies of dark coloring with diameters larger than 1 cm. The strains investigated in this project showed typical filamentous growth of fungal mycelium. For this reason, a customized picking procedure was needed.

The Pickolo software offers a large number of different criteria to identify the best colonies for picking. Unlike other colony picking instruments, the system offers a unique feature that enables picking from the perimeter of larger colonies, rather than from the center. This is important, as the center of the colony usually consists of old cells which are beginning to undergo necrosis. New cells, which are more likely to be viable after picking, are located at the outer area of the colony. Figure 3 visualizes colony picking from the edge of fungal colonies.

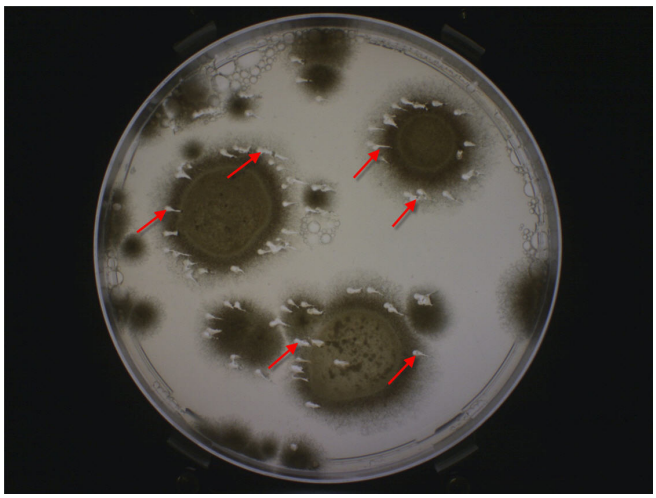

Figure 3 Picture taken with Pickolo software showing lignolytic fungal colonies with the scratches left after colony picking step (red arrows).

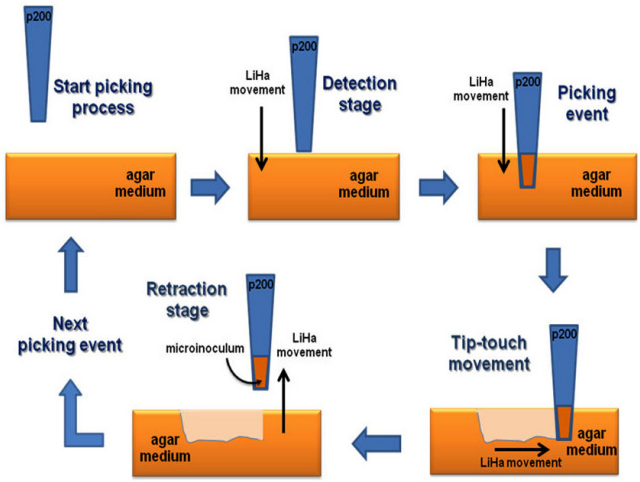

Figure 4 Graphical overview of the unique procedure for picking fungi from hard agar.

Collection of sufficient fungal biomass to ensure 100 % transfer efficiency for subsequent analysis

Cellulase-producing fungal strains are micro-organisms where parts of the fungal cells, the hyphae, grow 2-3 mm into the agar. To prepare material for further analysis it was important to collect all the biomaterial, both within and on the surface of the agar, to yield sufficient and viable inocula for further growth and expansion of the selected colonies.

A customized method was developed on the Freedom EVO workstation that combined insertion of the tip into the agar with a sideways movement, mimicking the typical sweeping movement of manual colony picking, while aspirating the biomass at the same time. This complete take up of the biological material cannot be performed on colony pickers that use pins or needles. The process is visualized in Figure 4 below.

Using this method, microinocula ranging from 60-100 µm in diameter were obtained with 100 % growth efficiency. All picked colonies were grown to microinocula in a 96-well microplate (Figure 5), a format which is ideal for further downstream testing. As all picked colonies were viable, this method also enabled a higher throughput than could be achieved manually.

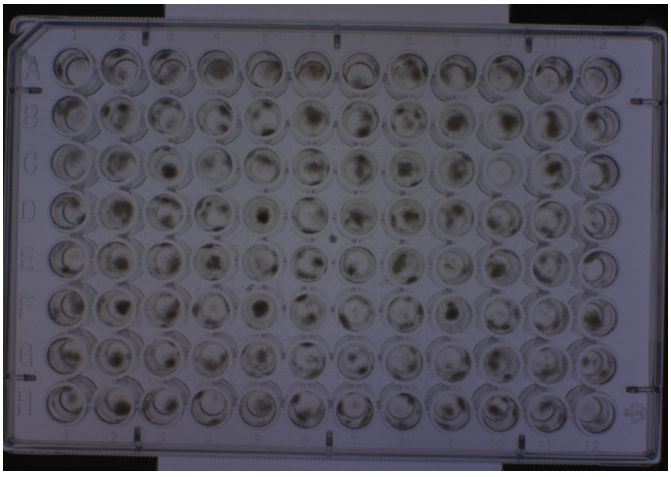

Figure 5 96-well plate showing growth of microinocula after 48 hrs of incubation.

Conclusion

This ambitious project for high throughput screening (HTS) of cellulase-producing fungal strains demonstrates the high capacity and unique features of the Freedom EVO colony picking workstation. The picking of fungi is more complex than for typical bacterial colonies, and requires advanced selection criteria to be combined with complex pipetting and a tip movement that mimics the manual picking process.

Using these capabilities, it was possible to identify and select large fungal colonies. Successfully picking sufficient viable biomaterial from the side of the colony created microinocula with 100 % further growth efficiency for subsequent investigations.

The automation solution shown here gave Abengoa the degree of reproducibility and efficacy required to perform their ambitious HTS program in the anticipated time frame.

Acknowledgements

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Australia +61 3 9647 4111 **Austria** +43 62 46 89 33 **Belgium** +32 15 42 13 19 **China** +86 21 2206 3206 **Denmark** +45 70 23 44 50 **France** +33 4 72 76 04 80
Germany +49 79 51 94 170 **Italy** +39 02 92 44 790 **Japan** +81 44 556 73 11 **Netherlands** +31 18 34 48 174 **Singapore** +65 644 41 886 **Spain** +34 93 490 01 74
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