Leveraging microalgae metabolism to produce natural food dyes

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Introduction

Microalgae & food industry

Microalgae, photosynthetic organisms in aquatic systems, transform atmospheric CO_2 into organic molecules like carbohydrates, lipids, and other bioactives. Consumer preference is shifting towards natural products, resulting in a rising demand for natural food pigments over synthetic additives[1]. The potential of microalgae to meet dietary needs has drawn the attention of the food industry: according to the European Food Safety Authority (EFSA), natural food pigment demand is increasing among consumers who prefer natural products over potentially toxic synthetic food additives[1]. However, natural pigments might be sensitive to light exposure and can change colour when exposed to different environmental conditions (pH and temperature); in addition, they are generally available in a limited colour range. All these factors motivate the R&D to investigate new natural pigments to be used as food dyes. To this aim, Fermentalg® laboratories have leveraged *Galdieria sulphuraria* metabolism to select microalgal strains producing a natural blue pigment rich in C-phycocyanin, extracted and commercialized as BLUE ORIGINS®[2].

Material & methods

Efficiently selecting microalgal strains demands high-throughput automation for swift screening. Fermentalg® scientists have developed innovative methods to screen and select *G. sulphuraria* strains using the QPix® 420 microbial colony picker (Molecular Devices®). (Fig. 1).

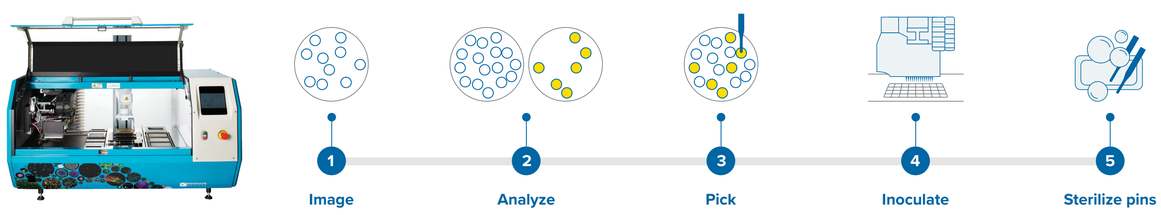

Figure 1. Colony screening workflow with QPix420 system.

Benefits of QPix 420 automated microbial screening

- High throughput colony picking with >3000 colonies picked/ hour and >98% picking efficiency
- Quantitative fluorescent screening for an efficient and objective selection of unique clones
- Maintain subsequent clonal integrity during bio-production
- A variety of fluorescent filter sets increases experimental flexibility
- Easy-to-use software ensures the right colony is picked every time

Results

A fluorescent approach to screen *G. sulphuraria* colonies

We employed a fluorescence-based method to select *G. sulphuraria* microalgae colonies. This organism is rich in C-phycocyanin, a water-soluble protein found in blue-green algae which gives them their characteristic bright blue colour. When excited with a red light, C-phycocyanin emits fluorescence with a peak at 642 nm[3]. Using the QPix 420 system, we captured images and screened *G. sulphuraria* colonies (2 weeks old) under transmitted light and fluorescence to identify and select colonies expressing high concentrations of C-phycocyanin. At first, a test image of the plate under transmitted light and the selected fluorescence was acquired (Fig. 2 A and B, respectively).

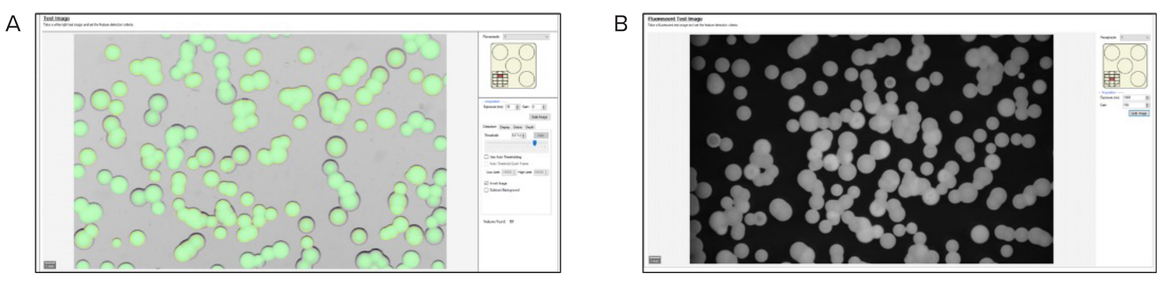

Figure 2. (A) Test image in transmitted light of the microalgae *G. sulphuraria*. (B) Test image of the same sample was acquired in fluorescence channel (Ex/Em filter: 628/692nm).

Results

Screening and detection of high-expressing C-phycocyanin colonies

Colonies were initially chosen based on morphological factors including size, compactness, axis ratio, diameter, and spacing. Selected colonies of *G. sulphuraria* were therefore included (Fig. 3 A, yellow) based on desired criteria. Quantitative, tuneable fluorescence intensity threshold specifies the desirable level of protein expression. To select cells containing the highest concentrations of C-phycocyanin, the range of fluorescence was adjusted to include colonies with a Mean Fluorescence Intensity (MFI) value between 36000 and 43000 (Fig. 3 B and C, respectively). Once selected based on user-defined criteria, colonies were accurately picked with QPix420 system.

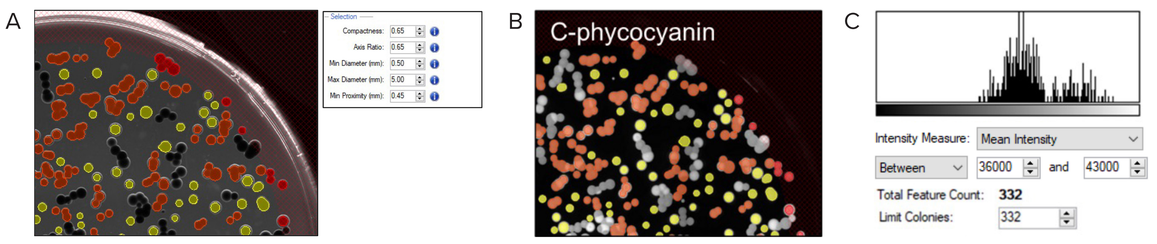

Figure 3. Fluorescent screening of *G. sulphuraria* colonies producing C-phycocyanin. (A) Representative image of *G. sulphuraria* colonies. Pickable colonies are displayed in yellow, colonies excluded according to selection criteria are depicted in orange. Top right panel: selection criteria defined for accurate colony picking. (B) Fluorescent imaging of *G. sulphuraria* colonies expressing different levels of C-phycocyanin. (C) Fluorescence histogram displayed as Mean Intensity.

Using the QPix 420, we analysed a diverse population of *G. sulphuraria* strains, each producing different C-phycocyanin concentrations. To test the ability of the system to discriminate and select the high-expressing C-phycocyanin colonies, which visually appear as dark green-coloured (Fig. 4 A).

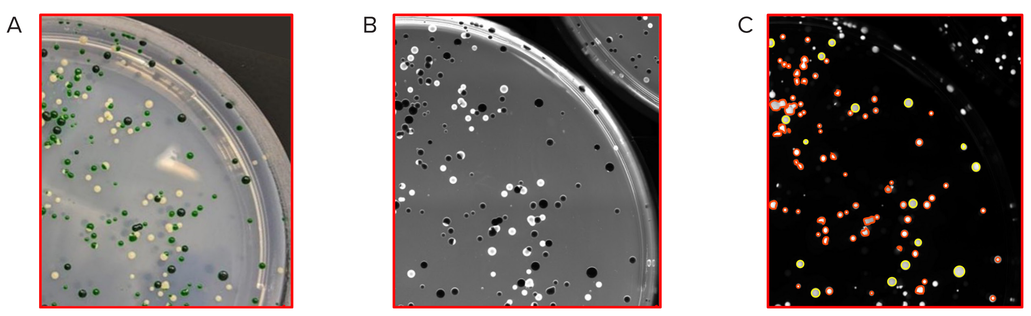

Figure 4. Selection of high-expressing C-phycocyanin colonies in an heterogenous strain population. Colonies with the lowest concentration of C-phycocyanin appeared white, while colonies expressing average to high levels of the pigment appeared light and dark green, respectively.

The different strains were therefore imaged in transmitted light with the QPix 420 and then screened based on C-phycocyanin fluorescent emission (Fig.4 B–C). A fluorescent signal for the dark green- and light green-coloured colonies was detected. Dark green colonies were successfully selected and picked based on the previously set MFI value ranging from 36000 to 43000.

Summary & conclusions

- Microalgae are gaining traction in the food industry because of the surging demand for natural and eco-friendly ingredients.
- Fermentalg® is leveraging microalgae metabolism to develop and screen multiple *G. sulphuraria* strains producing a low pH and high temperature-stable natural blue pigment as food ingredient (BLUE ORIGINS®)
- QPix 420 fluorescence capabilities were used to select clones producing the highest concentrations of C-phycocyanin, therefore accelerating the scale up of microalgae-based natural pigment production.

References

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