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Cell morphology observations for discriminating between *Brettanomyces bruxellensis* strains among genetic groups

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It is essential to discriminate between *B. bruxellensis* isolates at the strain level, because stress resistance capacities are strain dependent and also related to the genetic groups (GG). In this work, we investigated further the correlation between genetic groups and cell polymorphism by analysing optical microscopy images *via* deep learning. A Convolutional Neural Network (CNN) was trained to discriminate between 74 different *B. bruxellensis* isolates belonging to 4 of the 6 genetic groups described. Compared to the microsatellite analysis, the CNN enabled the prediction of the genetic groups of *B. bruxellensis* isolates with 96.6 % accuracy in a faster and cheaper way and with the same genetic group affiliations. Based on these very promising results, further research is needed to validate this technique for all genetic groups.

The spoilage yeast *Brettanomyces bruxellensis* has many strain-dependent characteristics that explain its persistence in wine[5,6,7,8] and in cellars[9,10] : possessing specific nutritional requirements, resistance to low pH values, SO_2 tolerance and capacity to enter a Viable But Not Cultivable state[1,2,3,4]. Moreover, sensitivity or tolerance to SO_2 is related to genetic group. In a previous study, a correlation between the variability in yeast cell shape and the genetic group to which the cells belonged was observed in a few numbers of strains[8]. Therefore, this correlation was explored further using a wider range of individuals by analysing transmitted light optical microscopy images. This analysis was performed with deep learning, which is a method used in various biological fields for classifications according to cell morphology[11,12,13]. Here, a pre-trained CNN was fitted to predict the genetic groups of tested isolates.

Microscopic observations

A total of 74 isolates previously identified as *B. bruxellensis* and discriminated as belonging to 4 different genetic groups (GG1, GG2, GG3 and GG4) by microsatellite analysis were used[8] (Figure 2A). Each isolate was pre-cultured on a YPD medium plate at 28 °C, then a colony was inoculated in 15 mL YPD at 28 °C for 3 to 4 days. Then, optical microscopic transmitted light observations were made. Images were scanned at 1280 × 960 pixels, 8-bit grey scale, 40x magnification and 38 % brightness using the EVOS® FL Imaging System (Invitrogen, Bothell, WA, USA), and datasets were built. ImageJ software was used to measure cell morphology characteristics as described in a previous study[8]. Briefly, the length to width (l/w) ratio and the cell area were determined from the measurement of 100 single cells per isolate, that is 7400 cells. Several distinctive cell shapes appeared depending on the isolate: elongated cells (Figure 1A), small cells (Figure 1B), round cells (Figure 1C) and some isolates forming multicellular structures (independent cell aggregation or non-separation of daughter cells) (Figure 1D). The isolates were categorised according to genetic group, and the average length to width (l/w) ratio and average area were measured (Figure 2). Isolates belonging to GG1 and GG4 were characterised as having rounder cells. In addition, isolates belonging to the GG4 had the characteristic of forming multicellular structures, making it possible to rapidly discriminate between these two groups (Figure 2). GG2 and GG3 had cells that were more elongated, and it was possible to discriminate between these two groups by their average area: 11.5 µm² for GG2 (between 10.31 µm² and 12.78 µm²), corresponding to small cells, compared to 16.9 µm² for GG3 (between 14.5 µm² and 20.98 µm²) (Figure 2B). Depending on the GG, microscopic observations can be used to discriminate between isolates based on cell morphology, but is difficult to obtain reliable and reproducible predictions of the genetic group with the human eye. For this reason, a CNN was trained to develop a reproducible method that does not depend on carrying out laborious observations or on the human factor.

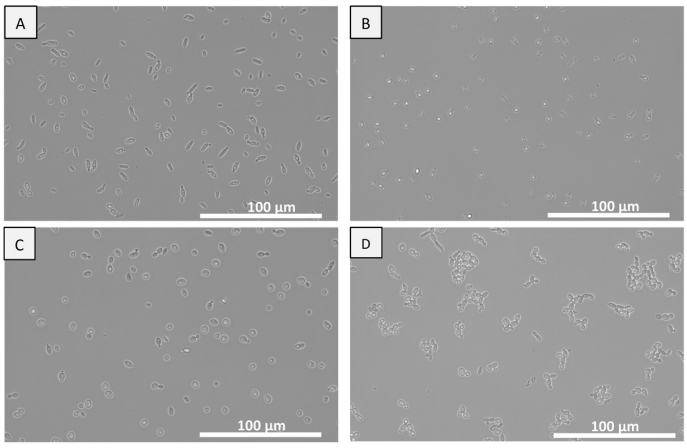

FIGURE 1. Polymorphism of yeast cells among the 74 *Brettanomyces bruxellensis* isolates: (A) elongated cells, (B) small cells, (C) round cells, and (D) presence of multicellular structures.

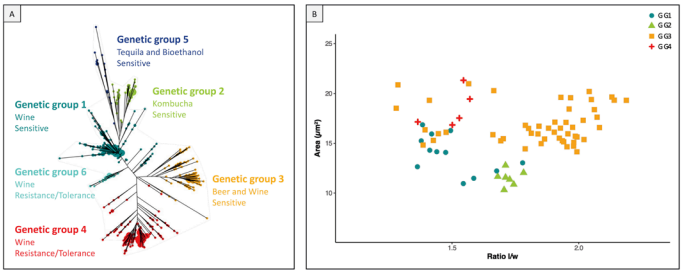

FIGURE 2. (A) Dendrogram of 1488 isolates of *Brettanomyces bruxellensis* divided into 6 genetic groups represented by different colours, based on Avramova *et al.* (2018)[2]. For each genetic group, the following is indicated: the main fermented substrate of isolation and the main behaviour in the presence of SO2 (B) Distribution of 74 studied isolates of B. bruxellensis according to average length to width (l/w) ratio and average area measurements. Each point corresponds to one isolate belonging to 4 of the 6 genetic groups described.

Deep learning

The GoogleNet Pre-trained Convolutional Neural Network (CNN) was chosen. This CNN was trained on over one million images classified into 1000 categories. This network thus learned a wide variety of features for a wide range of images. In order to exploit this network for the specific task of genetic group discrimination, it had to be adapted using learning transfer. Then, to increase the number of learning cases, some data augmentation techniques were used.
Thus, an image dataset of 12000 images was created to train the CNN. This dataset1 was randomly divided into two subsets: i) 75 % for CNN training (training dataset), and ii) 25 % for training performance validation (validation dataset). After 200 training iterations, the "real-world" performance of the CNN was evaluated vs unknown images and the accuracy calculated. For this, another image dataset (dataset 2) of 233 images was used.

From cell polymorphism to genetic groups

The GoogleNet CNN was used and trained to classify microscopic images into the four genetic groups. The validation step indicates that the model is able to predict the genetic group of an isolate from a simple microscopic observation more than 9 times out of 10.

To test the "real-world" performance of the model, a classification procedure was performed on dataset2. With these microscopic images, the model achieved an accuracy of 96.6 %, confirming its outstanding predictive power.
Thus, from a simple microscopic observation of a culture of *B. bruxellensis* belonging to GG1-2-3 or 4 (in our culture and image capture conditions), it is possible to predict the genetic group of the studied isolate with a high confidence level.

Conclusion

In this study, the polymorphism of yeast cells of the *B. bruxellensis* species clearly appears to be related to the genetic group of the isolates belonging to GG1-2-3 or 4. TheGoogleNet CNN was trained to provide the rapid and highly reliable screening of genetic groups of *B. bruxellensis* isolates; it was able to predict the genetic group of an isolate from a simple microscopic observation with 96.6 % accuracy. Furthermore, the discrimination could be extended to the genetic groups 5 and 6. Finally, this CNN provides is a rapid, simple and inexpensive tool for routine intraspecific discrimination between *B. bruxellensis* strains in order to predict problematic phenotypes in cellars. ■

Source: *Sourced from the research article: "Prediction of Genetic Groups within Brettanomyces bruxellensis through Cell Morphology Using a Deep Learning Tool" (Journal of Fungi, 2021).*

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