dentification of basidiomycetes using image nalysis of colony morphology and pigmentation

hael Edberg Hansen[1], Per Waaben Hansen[2], Sara Landvik[3], Mikako Sasa[3], Kristian Fog Nielsen[4], and Jens Micha stensen[1]

ormatics and Mathematical Modelling DTU, [2]Videometer A/S, [3]Novozymes A/S, [4]BioCentrum-DTU.

roduction

with fungal cultures is a great challenge. One strain can demonstrate a wide range of morphologies depending on the he colonies, the medium, and external factors such as temperature and light. On the other hand, colonies of different nay have similar morphologies, and it can be difficult to distinguish between them.

nology to capture color images reproducibly has evolved greatly in recent years. This new technology has prior to this own promising results for the identification of terverticillate species of *Penicillium* [1, 2]. The establishment of public es of images captured under standardized conditions would allow scientists using this technology to document and data mining and improve the understanding of fungal growth and development.

pose of this study, as a fundamental step, was to test the system's ability to recognize and distinguish between selected of Basidiomycota. Two closely related species and a more distantly related one were selected from each of the two *Pholiota* and *Polyporus*. The applicability of growth rate and/or pigmentation of the colonies as parameters was d.

terials and methods

ies chosen for this experiment were *Polyporus ciliatus* (isolates 1-4), *P. brumalis* (isolates 5-7), *P. tuberaster* (isolates *holiota aurivella* (Fig. 1a, isolates 19-21), *Ph. squarrosa* (isolates 15-18), and *Ph. gummosa* (Fig. 1b, isolates 11-14), first mentioned species of both genera presumed to be the closest related. All the strains originated from the nes A/S culture collection.

ins were transferred from growing colonies onto three media, PDA (Difco Laboratories, Detroit, USA), YES [3] and ALK g a 5 mm bore. Three plugs were placed onto each plate using a template indicating the exact positions. Two plates epared of each combination, and the plates were incubated at 26°C. Images of the plates were taken at days 8 and 15.

were captured in a reproducible way using a VideometerLab instrument (Videometer A/S). Prior to image analysis each as calibrated with respect to color, geometry and self-illumination, thereby gaining a set of directly comparable images.

d for automatic region extraction of the colonies was applied [1, 2]. From these regions colony size was estimated. Figs. d show the results after image acquisition and region extraction. Colors from the surface of the colonies were captured defined regions. From all colors captured, the mean and skewed mean (70-95 % brightest pixels) were chosen for nalysis.

Component Analysis (PCA) [5] was used to reveal underlying structures in data which may not be obvious at a first Applying PCA to sizes and colors produces a new set of variables, so-called scores, which describe the main variation d in the features. PCA ranks the score according to importance, the first being the most significant.

sults and discussion

A models were calculated based on:

e information only (radius – 6 variables in total), and

or information only (mean and skewed mean of three colors: red, green, and blue – 36 variables in al).

lting scores are plotted in Figs. 2 and 3. Each isolate number appears twice in the plots corresponding to the two s of each isolate. The replicates are close to each other indicating that the calculated scores do not contain much error.

, three distinct groups can be seen corresponding to *Polyporus ciliatus*, *P. brumalis*, and *Pholiota gummosa*, showing representatives of these three species may be separated solely by the use of size information. The remaining isolates e separated by size information only, as these strains had not grown sufficiently. However, the color information rized in scores 2 and 3 based on the color variables) can be used to separate these isolates (*Polyporus tuberaster*, *squarrosa*, and *Ph. aurivella*) (see Fig. 3). The grouping is not as clear as in Fig. 2 which is due to the fact that the are very small, resulting in noisy color estimates.

ze (scores 1 and 3) and color (scores 2 and 3) information are combined, it is possible to separate the 21 isolates into ps corresponding to the six species which they represent. This is shown in the classification tree presented in Fig. 4.

nclusions

e use of either size or color alone was not sufficient to separate the 21 isolates. Three of the species could be separated ed on size alone. It was possible to obtain a distinct grouping of 21 different isolates of six strains belonging to two erent species using size and color information extracted from images of the cultures.

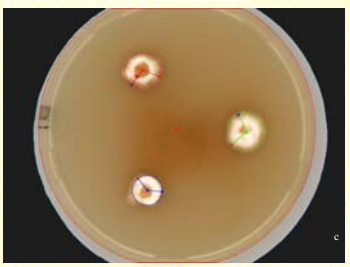
s study has confirmed that different species can be separated using image analysis. In future, the system's ustness/limitations, i.e. to what extent will a strain still be recognized as the same strain in response to morphological nges caused by changes in incubation time, different media, etc. will be tested.

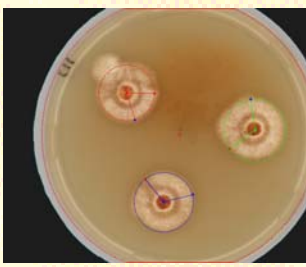
knowledgements

ject was supported by the Danish Technical Research Council under the project "Programme for predictive ology: Functional biodiversity in *Penicillium* and *Aspergillus*" (grant no. 9901295). The authors wish to thank Jens H. for providing the photographs in Figs. 1a and 1b.

ferences

M.E. Hansen, F. Lund and J.M. Carstensen, "Visual clone identification of *Penicillium commune* isolates" submitted to Journal of Microbiological Methods).
T. Dörge, J.M. Carstensen and J.C. Frisvad, "Direct identification of pure *Penicillium* species using image analysis", Journal of Microbiological Methods (2000), 41:121-133.
R.A. Samson, E.S. Hoekstra, J.C. Frisvad, and O. Filtenborg, "Introduction to food and airborne fungi", Centraalbureau voor Schimmelcultures, Utrecht, 6th edition, 2000.
T.A. Reshetilova, T.F. Solov'eva, B.P. Baskunov, & A.G. Kozlovskii (1992) "Investigation of alkaloid formation by certain species of fungi of the *Penicillium* genus". Mikrobiologiya 61: 873-879. (In Russian).
H. Martens and T. Næs, "Multivariate calibration", 2nd ed., Wiley (1992).





Figs. 1a-1d. *Pholiota aurivella* (left column) and *Ph. gummosa* (right column) as they appear in nature (upper row) an when grown on ALK medium (bottom row). Figs. 1c and 1d show the results after digitization, region extraction, and localization of the colonies. Only the part of the colony that is free of direct influence from neighboring colonies is used analysis.

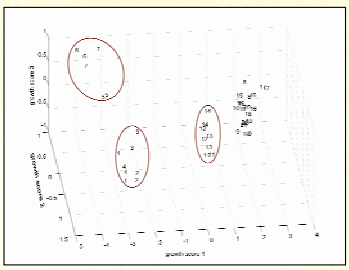


Fig. 2. Three-dimensional plot of the three growth scores obtained by PCA. The scores account for 87.2 % (score 1), 6.4 % (score 2), and 3.6 % (score 3) of the total variance in the original data. *Polyporus ciliatus* (1-4), *P. brumalis* (5-7), and *Pholiota gummosa* (11-14) form distinct groups.

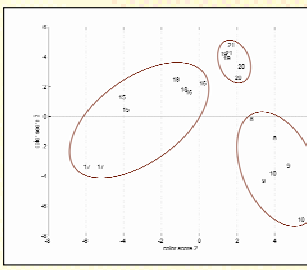


Fig. 3. Plot of two color scores obtained by PCA only (*Polyporus tuberaster* (8-10), *Pholiota squarrosa* (15- and *Ph. aurivella* (19-21)). The scores account for 28 (score 2) and 21.3 % (score 3) of the total variance i original data. The three species form distinct groups.

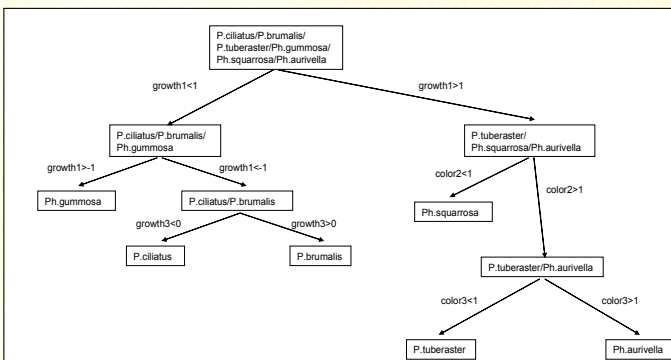


Fig. 4. Classification tree of the 21 isolates falling into 6 groups corresponding to the species to which they belong. Gro and growth3 are growth scores 1 and 3 from Fig. 2. Color2 and color3 are color scores from Fig. 3. It can be seen that *Polyporus ciliatus* and *P. brumalis* are more similar to each other than to *P. tuberaster*, by the fact that they show up in different branches of the tree. The same applies to the relationship between *Pholiota aurivella*, *Ph. squarrosa*, and *Ph. gummosa*, of which the first two are most similar. These groupings coincide with the presumed phylogenetic relationsh these genera.