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Fungal volatile fingerprints: discrimination between dermatophyte species and strains by means of an electronic nose

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Abstract

The potential of an electronic nose consisting of a hybrid gas sensor array system has been examined for species discrimination and strain identification of dermatophytes which are causative pathogens for human and animal infection. Temporal volatile production patterns have been studied at a species level for a *Microsporum* species, two *Trichophyton* species and at a strain level for the two *Trichophyton* species. After about 120 hours principal component analysis (PCA) and cluster analysis showed possible discrimination between the species from controls. Data analysis also indicated probable differentiation between the strains of *T. rubrum*. The same could not however be achieved for the strains of *T. mentagrophytes* during preliminary experiments for the same time period, signifying a good similarity between the strains of this particular species based on volatile fingerprints. This study suggests that volatile production patterns shows promise for species and strain identification of these dermatophytic fungi thereby facilitating early diagnosis and early management of patients.

Keywords

Dermatophytes, electronic nose, volatile fingerprints, strain and species differentiation, metal oxide sensors

1. Introduction

Dermatophytes are responsible for causing superficial infections in keratinised tissues of humans and animals. The predominant pathogens in humans being *Trichophyton rubrum* and *T. mentagrophytes,* while *Microsporun canis, T. verrucosum* are examples of animal pathogens [1]. Clinically it is important to identify the species causing infections in order to enable appropriate treatment. However, conventional techniques comprising microscopy, culture and biochemical tests are tedious, time consuming and require skilled personel.

Moreover, conventional strain typing of species is also difficult because of the lack of stable characteristics such as colony morphology or microscopic appearance. These characteristics are not easily noticeable when subcultured, or might be artifacts due to growth conditions or contaminating bacteria [2]. Thus hampering the identification of strain-related differences, which could serve to be useful for answering certain epidemiological questions such as sources of infection or strains belonging to a specific geographical area. It would also help in identifying the types of infection caused (i.e. skin or nail) in addition to determining the cause of relapse (i.e. either being a failure in treatment or infection by a new strain) [3].

Molecular approaches for species identification and strain typing have been studied such as PCR, random amplification of polymorphic DNA (RAPD), restriction fragment length polymorphisim (RFLP) and restriction enzyme analysis [2,3,4]. These have shown promise, although tend not to be feasible for routine clinical purposes apart from being complex, laborious and expensive with respect to certain equipment and associated materials.

Sahgal *et. al.* [5] have previously reported that volatile fingerprint patterns by means of an electronic nose (e-nose), enabled differentiation between certain *Trichophyton* species. The objective of the current study was to evaluate the potential of using volatile production patterns for (a) strain differentiation of the two common *Trichophyton* species and for (b) identifying/discriminating between species infecting humans and animals using e-nose technology.

2. Materials and Methods

2.1 Fungal species and media

In order to investigate the discrimination between the dermatophyte species infecting humans and animals, two *Trichophyton* type cultures (*T. mentagrophytes*, NCPF:224; *T. rubrum,* D12) and an isolated *Microsporum* culture from dogs (*M. canis,* M177) were used.

For investigating the differentiation between strains of two individual species five strains of *T. mentagrophytes* (including strains M61, M62, M63 and M64) and four strains of *T. rubrum* (including strains R55, R57 and R59) were studied. The type cultures were obtained from the National Collection of Pathogenic Fungi (NCPF), Bristol, U.K. and other cultures were kindly provided by Prof. F. J. Cabañes, Autonomous University of Barcelona, Catalonia, Spain.

Cultures were grown on Sabouraud Dextrose Agar (SDA), prepared in house by mixing 10 g L^{-1} mycological peptone (Amersham, Little Chalfont, U.K.), 40 g L^{-1} glucose (Acros Chemicals) and 15 g L^{-1} agar Technical No. 3 (Oxoid) with a small amount of chloramphenicol (Sigma); and incubated at 25 °C.

2.2 Sample preparation

Spore suspensions were prepared by harvesting spores from three week old cultures with a loop and suspending them in 10 ml sterile RO water and Tween 80 (Acros Chemicals). At least 25 replicate agar plates for each species and/or strains were inoculated with 250 µl of 10^6 spores ml^{-1} suspensions to make a spore lawn over the agar surface. The inoculum range for inter-species discrimination was ≈ 4.6 x 10^6 spores ml^{-1}, while the inoculum for the strains discrimination ranged from ≈ 8.5 x 10^6 and ≈ 2.9 x 10^6 spores ml^{-1} for the two *Trichophyton* species, *T. mentagrophytes* and *T. rubrum* respectively. Blank agar plates served as controls for the experiments. The treatments were incubated at room temperature i.e. 25 °C for a period of 24 to 120 hours.

2.3 E-nose and data analysis

Every 24 hours five replicates for each fungal treatment were destructively sampled. Four 2 cm diameter discs of the treatments were placed in 25 ml glass vials and sealed with septa and screw caps. These were then set aside for one hour to equilibrate at room temperature for headspace generation above the sample. Samples were then randomly placed in the NST 3320 Lab Emission Analyser (Applied Sensors, Linköping, Sweden), a 24 sensor hybrid e-nose comprising 12 MOS sensors, 10 MOSFET sensors, a capacitance based humidity (relative humidity) sensor and an IR-based CO_2 sensor, for headspace analysis. The data generated were analysed using the built-in software package - NST Senstool and MATLAB 7.3 (The Mathworks Inc., Natick, MA). Data interpretation was carried out by applying multivariate statistical techniques such as principal component analysis (PCA) and heirarchical cluster analysis (HCA) on the obtained sensor responses.

3. Results

3.1 Inter-species discrimination

T. mentagrophytes, *T. rubrum* and *M. canis* were studied in order to investigate the potential of discriminating between dermatophytes causing human and animal infections using their volatile fingerprint production patterns. Initial experiments indicate possible differentiation between the fungal species. Figure 1 shows a PCA scores plot of the separation between the samples after 120 hours incubation at 25 °C. The first two principal components accounted for approximately 94% of the variance in the data.

Cluster analysis performed on the same data set using just the first two principal components also showed a similar result. The dendrogram (Figure 2) was constructed using Euclidean distance and Ward's linkage measure. Although, four distinct groups can be visualised, one sample from C was clustered as belonging to R possibly an outlier.

3.2 Differentiation between strains of T. mentagrophytes

Five strains of *T. mentagrophytes* were examined to study the effects of their VOCs produced to facilitate discrimination/similarity amongst strains. Results suggest that the controls can be separated from the strains/treatments after about 48 hours, but this was more clearly evident over 72 hours. However, even after 96-120 hours strains could not be differentiated from each other (Figure 3).

3.3 Discrimination between T. rubrum strains

To investigate the potential of discriminating between strains of *T. rubrum* based on their volatile profiles, four strains were examined. Initial experiments indicated that within 120 hours it was possible to differentiate between controls and three of the strains of this fungal species – R, R55 and R57. The PCA scores plot in Figure 4 depicts the separation between the samples after 120 hours incubation. The data accounted for approximately 86% of the variance in the first two principal components.

Subsequent cluster analysis on the data also showed that strains R, R55 and R57 could be clustered while the controls and R59 could not be effectively discriminated (Figure 5). However, a sample from R55 was shown to be clustered with R. The dendrogram was constructed as described previously.

4. Discussion

The use of an electronic nose for identifying *Trichophyton* species has been previously reported by Sahgal *et. al.(2006),* whereby successful discrimination was obtained within 96-120 hours [5]. Here a similar approach has been extended to include other dermatophyte species to broaden the detection and discriminating potential for both human and animal infecting species. Almost clear discrimination between the sample groups was observed after 120 hours, suggesting potential of distinguishing human from animal pathogens. This could prove to be significant from the clinician's perspective, in terms of prescribing drugs and starting the correct treatment. Nevertheless, further studies are required to draw definite conclusions.

If species identification is important for administration of appropriate drugs, then strain differentiation can serve to be essential for monitoring drug resistance, especially in the event of treatment failure, in the strains of these species. A variety of

molecular typing methods have been employed for strain recognition of dermatophytes. For example, previous studies have reported either the inability to distinguish between strains of *T. rubrum*, or some minor differences being shown for a small number of strains [6, 7] using these techniques. However, Jackson *et. al.* [4] showed that RFLP analysis of the nontranscribed spacer regions of the ribosomal-DNA enabled *T. rubrum* strain differentiation. Furthermore, no correlation was observed for strain typing of *T. mentagrophytes* by restriction enzyme analysis on internal transcribed spacer regions of DNA [2]. These techniques although useful, are not suited for a clinical setting.

Volatile production patterns of these two species have also indicated positive outcomes in terms of differentiation between strains. In case of *T. rubrum* slower growth rate of some the strains could explain the inability to distinguish it from the controls, suggesting greater strain variation in this species. This could also imply that the volatile patterns of the strains pertaining to these species might be slightly different from each other. On the other hand, no distinction between strains of *T. mentagrophytes* could be due to a great deal of similarity between the strains indicating that their volatile fingerprints hardly differ. However, further studies are needed to fine-tune the use of the e-nose for such an application.

Previous reports have also shown the use of different kinds of e-noses for strain differentiation of fungi, especially those detrimental to the food industry owing to toxin production, which then adversely affects consumers. In one study [8] it was possible to differentiate between mycotoxigenic and non-mycotoxigenic strains of food spoilage fungi *Fusarium moniliforme* and *F. proliferatum* respectively, within 48 hours using a conducting polymer sensor array. Falasconi *et. al.* [9] also demonstrated that a MOS e-nose could classify the strains of *F. verticillioides* based on their

toxigenic behaviour on agar as well as on grain. Furthermore, Needham and Magan [10] examined *Penicillium verrucosum* strains for production of ochratoxin as opposed to those that did not produce the toxin using conducting polymer sensors.

5. Conclusion

This study had demonstrated the ability of the electronic nose to efficiently decreasing the time needed for identification of dermatophytes causing human and animal infections including strains of certain species in a quick and reproducible manner. We have also shown that there is little variation between strains of some dermatophyte species, while for others there are intra-strain differences. This study shows the capability of developing neural networks based on the data now available to examine and classify unknown clinical samples.

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References

[1] F. J. Cabañes, Dermatophytes in domestic animals, in: R.K.S Kushwaha and J. Guarro, Biology of dermatophytes and other keratinophilic fungi, Revista Iberoamericana de Micologia Bilbao, 2000, pp. 104-108.

[2] J.-H. Shin, J.-H. Sung, S.-J. Park, J. A. Kim, J.-H. Lee, D.-Y. Lee, E.-S. Lee, J.-M. Yang, Species identification and strain differentiation of dermatophyte fungi using polymerase chain reaction amplification and restriction enzyme analysis, Journal of the American Academy of Dermatology 48 (2003) 857-865.

[3] L. C. Baeza, M. J. S. M. Giannini, Strain differentiation of *Trichophyton rubrum* by random amplification of polymorphic DNA (RAPD), Revista do Instituto de Medicina Tropical de São Paulo 46 (2004) 339-341.

[4] C. J. Jackson, R. C. Barton, E. G. V. Evans, Species identification and strain differentiation of dermatophyte fungi by analysis of ribosomal DNA intergenic spacer regions, J. Clin. Microbiol. 37 (1999) 931-936.

[5] N. Sahgal, B. Monk, M. Wasil, N. Magan, *Trichophyton* species: use of volatile fingerprints for rapid identification and discrimination, B. Jr. Dermatol. 155 (2006) 1209-1216.

[6] D. Liu, S. Coloe, R. Baird, J. Pedersen, Application of PCR to the identification of dermatophytic fungi, J. Med. Microbiol. 49 (2000) 496-497.

[7] Z. Zhong, R. Li, D. Li, D. Wang, Typing of common dermatophytes using random amplification of polymorphic DNA, Jap. J. Med. Mycol. 38 (1997) 239-246.

[8] G. Keshri, N. Magan, Detection and differentiation between mycotoxigenic and non-mycotoxigenic strains of *Fusarium* spp. using volatile production profiles and hydrolytic enzymes, J. Appl. Microbiol. 89 (2000) 825-833.

[9] M. Falasconi, E. Gobbi, M. Pardo, M. Della Torre, A. Bresciani, G. Sberveglieri, Detection of toxigenic strains of *Fusarium verticillioides* in corn by electronic olfactory system, Sensors and Actuators B: Chemical 108, (2005) 250-257.

[10] R. Needham, N. Magan, Detection and differentiation of toxigenic and non-toxigenic *Penicillium verrucosum* strains on bakery products using an electronic nose, Asp. Appl. Biol. 68 (2003) 217-222.

Figure 1**:** PCA scores plot based on headspace analysis after 120 hours for the fungal species. Key: M – *T. mentagrophytes*, R – *T. rubrum*, C – *M. canis* and B – controls.

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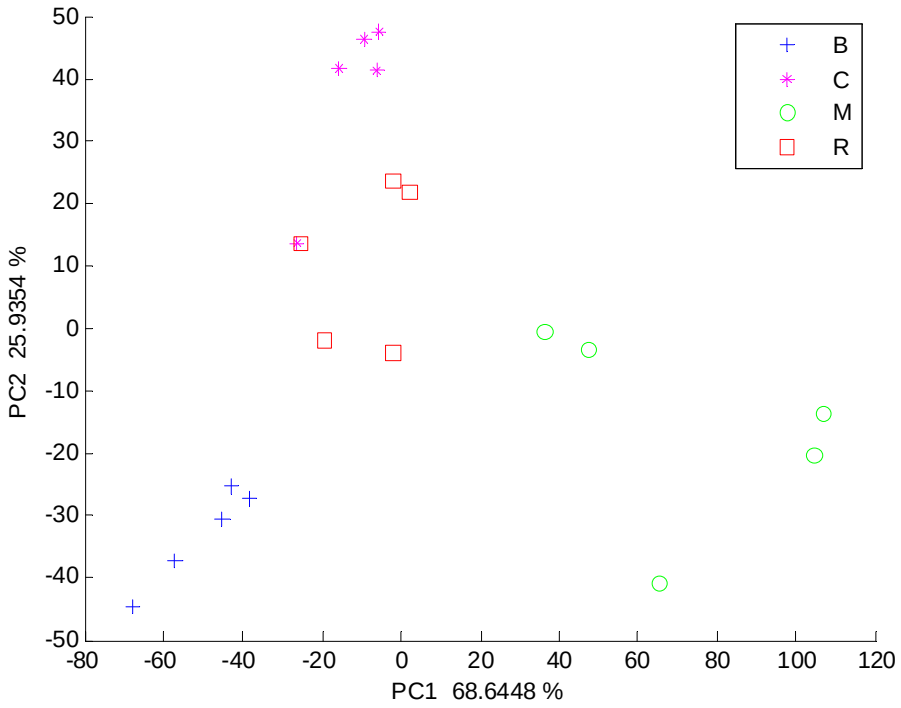

Figure 2**:** Tree diagram based on the PCA results of the fungal species using the first two PCs. Key: M – *T. mentagrophytes*, R – *T. rubrum*, C – *M. canis* and B – controls.

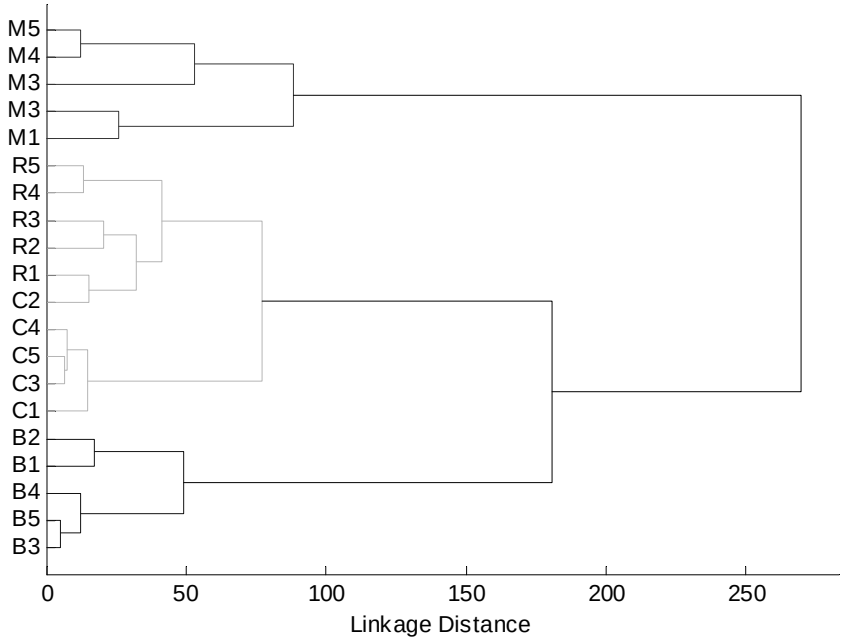

Figure 3: PCA scores plot after 96 hours showing discrimination between controls (B) and *T. mentagrophytes* (M) strains.

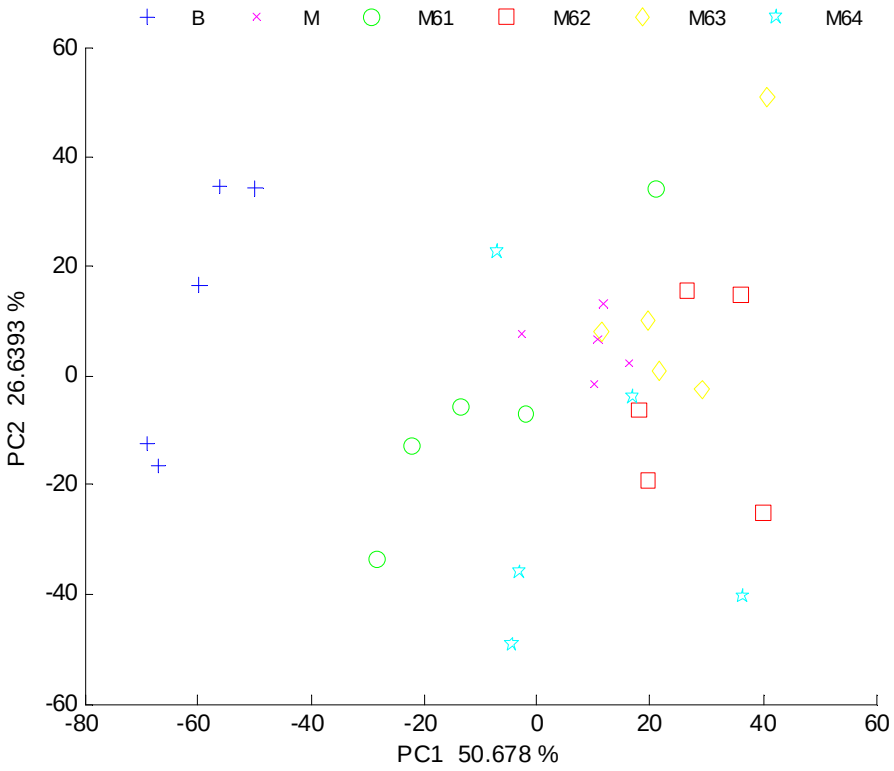

Figure 4: PCA scores plot depicting possible discrimination between 3 strains of *T. rubrum* (R) after 120 hours. [B-controls]

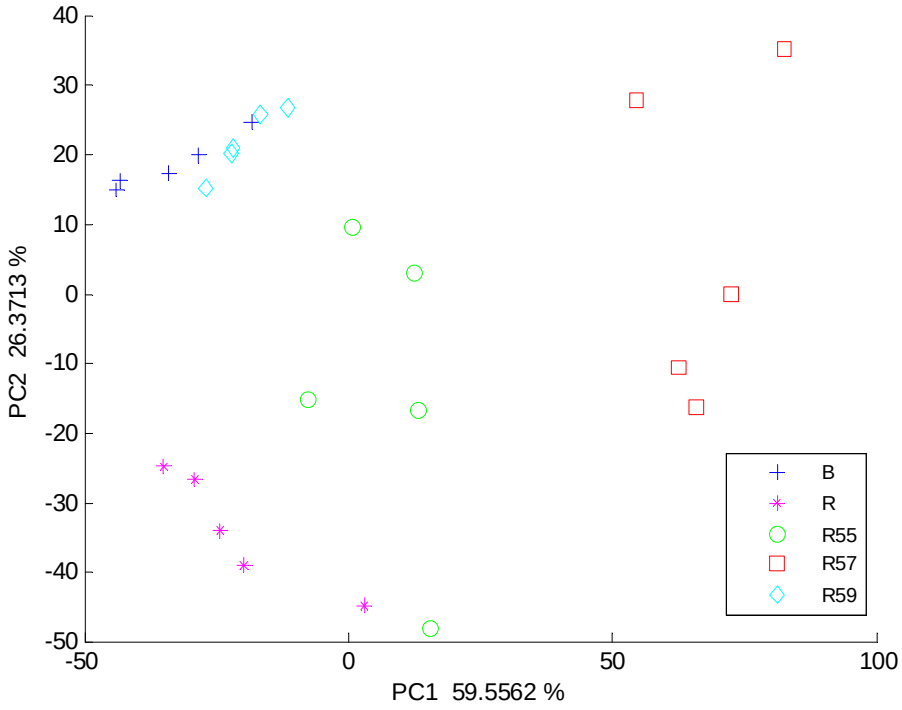

Figure 5: Dendrogram based on the first 2 PCs of fungal strains after 120 hours. Key: B - Controls and R - *T. rubrum.*