Fractal dimension as a tool for detection of morphological changes caused by impact of mechanical waves on mushroom mycelium

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Received 8. 7. 2004

Key words: Influence of mechanical or acoustic waves on mycelium complexity, fractal dimension.

Abstract: The influence of low-power mechanical and acoustic waves produced by a tuning fork on the mycelium of *Psilocybe cubensis* is studied. The parameter for detection of an influence are complexity changes in the mycelium. Significant complexity changes were revealed by fractal dimension. Possible explanation by the mechanical or resonance model are discussed.

Zusammenfassung: Der Einfluß schwacher mechanischer und akustischer Wellen, hervorgerufen durch eine Stimmgabel, auf das Mycel von *Psilocybe cubensis* wird untersucht. Der Parameter für die Entdeckung eines Einflusses sind Veränderungen der Komplexität des Mycels. Signifikante Komplexitätsänderungen wurden mit fraktaler Dimension aufgezeigt. Mögliche Erklärungen durch das mechanische oder Resonanzmodell werden diskutiert.

In the past decade the morphology of mycelium colonies was used as parameter for detection of complexity changes that were caused by the variation of environmental factors (MATSURA & MIYAZIMA 1994). In this research we compare the colony morphology of treated vs. non-treated mycelium cultures of *Psilocybe cubensis* (EARLE) SINGER on the basis of their complexity feature – fractal dimension (FD). The colony treatment takes place through a tuning fork whose frequency has natural origin.

Material and methods

Cultivation

Mycelium of *Psilocybe cubensis* was cultivated in glass petri-dishes (8 cm diam.) on solid malt-yeast-agar (MYA). The quantity (25 ml/petri-dish) and the composition of the medium in petri-dishes were kept identical for each individual dish. In cooperation with SHU MATSURA (Tokai University, Japan) one standardized inoculation procedure was developed for this particular research, that can be used for similar investigations, where the comparison of exposed material with the control group is necessary. For that purpose one transfers the constant volume of inoculation substrate with a sterile inoculation tube and inserts it in a preprepared medium hole (Fig.1). The cross section of the tube and the hole should be identical (in our case 6 mm).

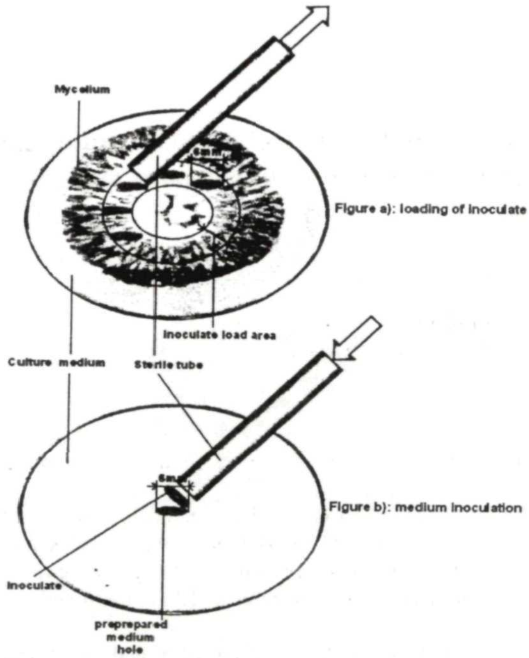

Fig. 1. Standardized inoculation procedure. *a* Loading of inoculate. Inoculate load area is chosen by the homogeneousness of the substrate. *b* Medium inoculation. Taken inoculate is pressed out of the sterile tube in the preprepared medium hole.

The inoculated petri-dishes were closed and put into two identical incubators (Heraeus) at 29°C. Humidity in petri-dishes was 90%. Specimens of one incubator were treated through a tuning fork (Wittner company, Germany, EU – standard design) with 194.71 Hz for 13 days. For that purpose the tuning fork was manually put against the resonance plate (made of styrol acryl nitrol copolymer – SAN) for 30 min daily (Fig. 2).

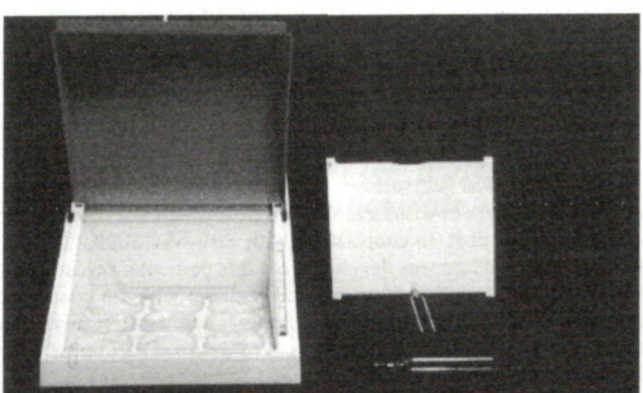

Fig. 2. Experimental set-up for research of the influences of the acoustic waves at mycelial tissue. Left: Incubator with inoculated petri-dishes. Right: Resonance plate with tuning forks.

The frequency used was calculated from the main chronobiological rhythm donor – day length (HILDEBRANDT 1998). One sidereal day has a total of 86 164, 09054 seconds. If we take a day as a period (T) than is the Frequency F = 1/T. This means that F = 0.000 011 605 763 Hz. If we octavate (multiply with 2*) that very low frequency for 24 times than we receive F = 194.712390 Hz what is approximately F = 194.71 Hz.

Evaluation

The petri-dishes with mycelium cultures (Fig. 3) were put in a digital image analysis device (Lemnatec, Germany**), where they were illuminated from the bottom side, scanned and transformed in black-and-white images using standardized colour-palette of the Lemnatec-device. In this way windows Bitmap digital images (600 x 600 dpi) were prepared for the determination of fractal dimension (box-counting). For that purpose we used "Fractal Dimension, Ver. 1.1." software from Bar Ilan University, Israel. During this inquiry we introduced and developed a new feature of this software that is dealing with determination of the fractal dimension of a biological tissue.

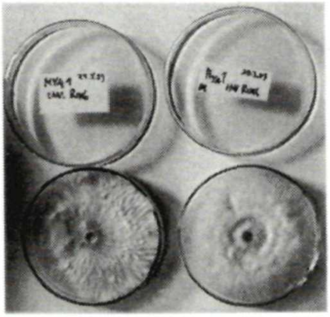

Fig. 3. Example of 13 days old mycelium cultures. *A* Exposed sample. *B* Control sample.

To analyse the univariant differences of the fractal dimension means between the exposed and control sample data for a variable, standard statistical evaluation analysis ANOVA (one-way variance-analysis) was used.

Results

The univariant ANOVA test showed the significant main effects and group differences between the independent variables (p = 0.02). By visual inspection of the colony images, an example of the differences between the fractal dimension of the samples is observable (Figs. 3, 4). In both cases the colonies are visibly different. The branching structures of the exposed sample A are more multifarious and rougher (Fig. 3). The control sample B became thick and compact without particular distinctive features. In the black-and-white bitmap images that were prepared for the determination of fractal dimension (Fig. 4) the morphological distinctions are also visible. The control sample (left) shows strong sub-colonies expansion, atypical bay-like colony growth and low level of branching. The exposed sample (right) shows high branching level, uniform growth and constant broadening in all directions starting from the place of inoculation.

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* After harmonical teachings and information theory we preserve the informational content of one frequency if we multiply it with 2 (BREITHAUPT 1979, COUSTO 1987, JOHANESSON 1992).

** The usage of the digital image analyses equipment was accomplished due to the obligingness of GERHARD SOJA (ARC – Seibersdorf research).

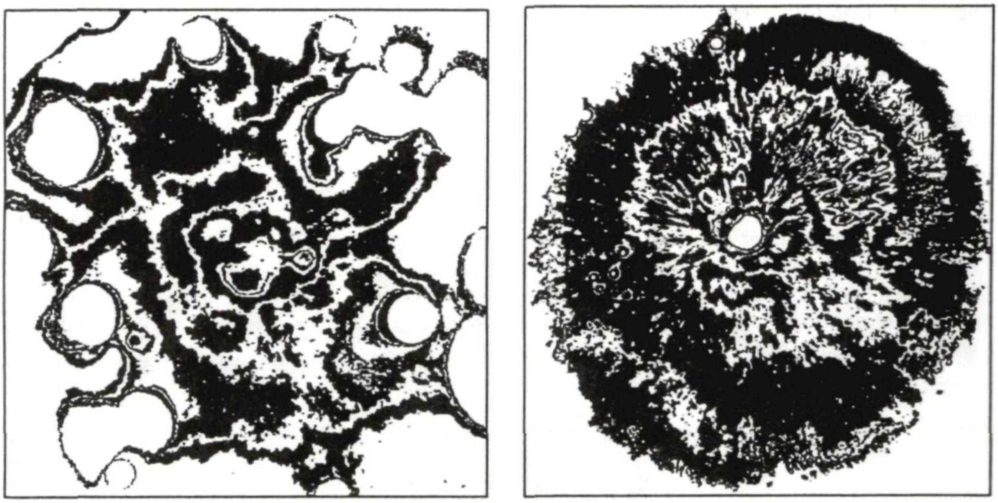

Fig. 4. Example of perceptible fractal dimension (FD) differences between mycelium cultures. Left – control sample (FD = 1.73). Right – exposed sample (FD = 1.87).

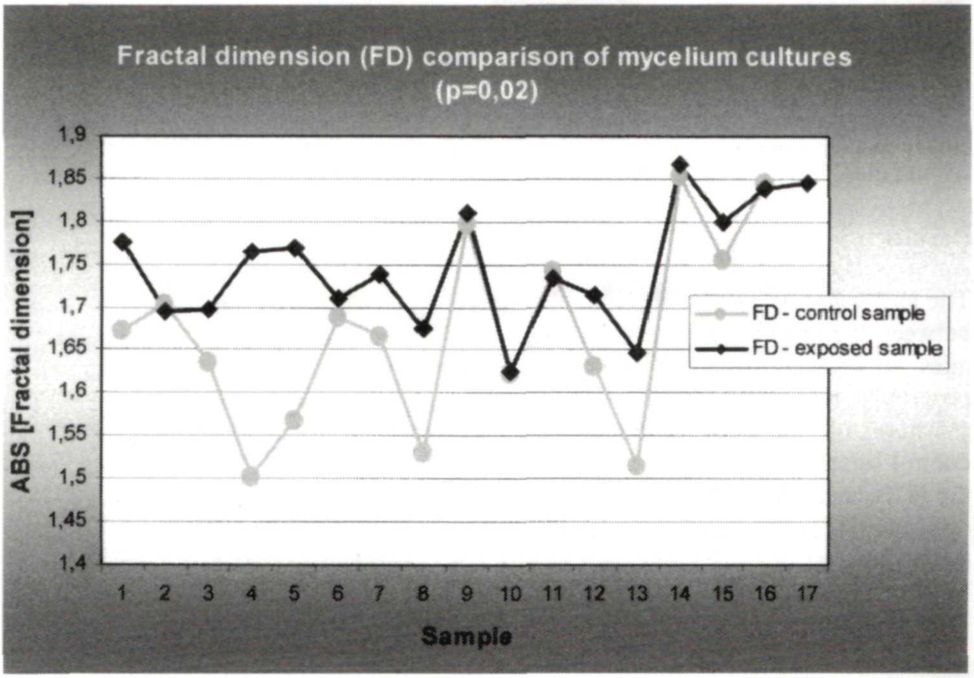

Fig. 5. Comparison of absolute (ABS) fractal dimension values of analysed mycelium cultures. Grey line with dots – control sample. Black line with rectangle – exposed sample. p probability factor.

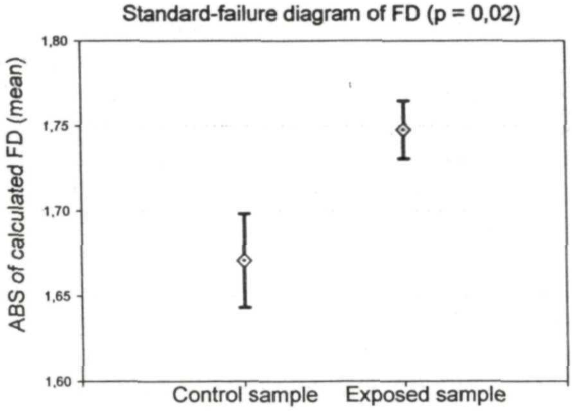

Fig. 6. Comparison of absolute (ABS) standard failure values in a scatterplot diagram with error bars for the calculated fractal dimension (FD) of analysed mycelium cultures. Left – control sample. Right – exposed sample. p probability factor.

Discussion

The univariant ANOVA test shows that there are significant ($p = 0.02$) differences between the samples examined. These statistically confirmed data were often evident and clearly perceptible (Figs. 3, 4). Although the control samples (Fig. 3 B, Fig. 4 left) look in their colony form also apparently different, their hyphal morphological characteristics, according to given fractal dimension, are not showing very high complexity level. From Fig. 3 we can see that the B-colony became homogeneous and remarkably smooth. Her visible branching is very low. Fig. 4 (left) is in contrast to the B-colony not smooth at all but the low level of determinate fractal dimension (although one of the highest among the control samples), compared to the exposed sample, suggests that the growth process of this colony does not show very high level of self-similarity. In addition there is a high affinity for singularity of growth probability that we can observe in apical hyphae-aggregation and irregular space-broadening. This quality might be an example of hyphal structures, where the strand hyphae are taking part, mainly, in the transport of nutrients and might affect a positive activity of exploring substrate for additional nutrients. As dispersal of nutrients in the medium is identical, we can interpret this unsteady broadening as a phenotypic variation whose cause is not yet known. Though the irregular display of cultures occurs, we can not take it as an attribute of the control samples, as a matter of fact we did note that some of the control mycelium cultures had remarkable uniform broadening (results). Thus the difference in fractal dimension, as a property of self-affinity and complexity of one system, remains.

Hyphae secrete various enzymes to decompose and absorb nutrient materials. As the nutrients are distributed uniformly in the substrates, the branching and growth celerity of the entire mycelium and hypha in particular must be one of the most important factors which determines its absorption. If one organism has better access and assimilation of nutrients then he has better survival expectations compared to his competitor. In this research we obtained results that show that the complexity of the branching structures is affected by the exposure to the mechanical waves.

To explain this fact we discuss here the mechanical and the resonance model. After the mechanical model the air and substantial oscillations and vibrations of frequencies used can affect the growth dynamics of a living structure. In order to cause some impacts at living matter mechanical waves need to have enough energy, that can disturb the biochemical and/or morphogenetical processes in organisms. The fact is that this energy quantum can not be obtained by a tuning fork (BUCHHOLZ 2001), which was used in this experiment for the acoustic irradiation. The resonance model indicates that structures in the living beings can resonate with the varying environmental oscillations (POPP & al. 1992, LIBOFF 1992). Using the resonance model it could be possible to explain some of the interactions between low-power abiotic environmental factors and living matter.

From Fig. 4 we can see that the exposed sample (black line) has a higher homogeneity than the control group. The same is confirmed at the standard-failure diagram (Fig. 6). This feature that we were able to observe at the preliminary inquiry may also be caused by the application of acoustic waves and needs further investigations.

Concluding remarks

The biological meaning of fractal dimension has not been entirely established, yet its application is spread world-wide. To assure our fractal dimension results and their relevance regarding morphology of the fungal colony, introduction of additional evaluation parameters, that are relevant to morphogenetic tasks, would be appropriate. As an increase of total biomass of individual structures at different organisational level the growth arises as the most valuable and integrative parameter.

Morphological appearance of the fungal colony is thought to be a direct indication of the flexible mycelium adaptations. Mycelial controls governing the complex hyphal growth may gain high efficiency if the forming of the whole colony is corresponding to the complex environmental influences (MATSURA & MIYAZIMA 1994). If we take the low-power mechanical waves as such the next question that appears is about their biophysical working mechanism. To ascertain if the mechanical or the resonance model (discussion) is valid further research with comparative-frequencies is needed.

For their cooperation we thank SHU MATSURA, Tokai University, Numazu, Shizuoka, Japan, ANAT VISHNE, Bar-Ilan University, Ramat-Gan, Israel, and SOJA GERHARD, ARC – Seibersdorf research, Seibersdorf, Austria.

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