DYNAMIC SPECKLE STUDY OF MICROBIAL GROWTH

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ABSTRACT

In this work we present a characterization of yeast dynamic speckle activity during growth in an isolated agar culture medium. We found that it is possible to detect the growth of the microorganisms even before they turn out to be visible. By observing the time evolution of the speckle activity at different regions of the culture medium we could extract a map of the growth process, which served to analyze how the yeast develops and spreads over the agar's medium. An interesting point of this study concerns with the influence of the laser light on the yeast growth rate. We have found that yeast finds hard to develop at regions with higher laser light illumination, although we used a synchronous system to capture the speckle pattern. The results obtained in this work would serve us as a starting point to fabricate a detector of growing microorganism colonies, with obvious interesting applications in diverse areas.

Keywords: Dynamic speckle, microorganisms, sensing, biospeckle, biosecurity.

1. INTRODUCTION

Recently, keeping track of biosecurity conditions have become an increasingly important aspect in a wide variety of areas [1]. In this context, the presence of microorganism populations is a relevant parameter to assess in different environments, like an operating room or a poultry farm. In general, microbial analysis requires the use of specific culture media and a suitable laboratory possessing, at least, an optical microscope. However, the dynamic speckle technique is being studied as an alternative method to evaluate microbial activity [2,3].

Dynamic speckle patterns are known to look like "boiling" patterns whose degree of activity is called speckle mobility or speckle activity (SA). If the object under study is of a biological kind, then the dynamic speckle pattern observed receives the name of biospeckle. In this case, the SA is termed biospeckle activity (BA) due to its relation to the biological activity of the specimen under investigation [4]. Quantification of BA is being increasingly investigated as, for example, a quality-control parameter for fruits and vegetables [5]. Besides this, several works have been also devoted to analyzing microbiological or fungal activity [3,6,7].

There are several approaches to quantify and analyze the SA of different specimens. Among them, one finds the analysis of speckle pattern's time history (THSP) by means of different descriptors like the inertia moment (IM) [4], the generalized differences (GD) method [8], the modified time correlation method [9], the time evolution inspection of the speckle pattern's texture [10], the contrast imaging method [11], the method of empirical mode decomposition [12], the Fujii difference method [13], the analysis of the speckle pattern's phase evolution [14], and so on. In a recent work we proposed a simple and straightforward algorithm [15] for calculating a single SA index from a sequence of images, aiming to simplify calculations and overcome some disadvantages of the mentioned methods.

In this work we present a characterization of SA during growth of a reference yeast culture in an agarose growth medium. This technique allows near real-time and *in-situ* assessment of the growth evolution of microorganisms, which is advantageous from the viewpoint of simplicity and efficiency during evaluation of biological contamination. By observing the time evolution of SA of the culture medium we could observe the growth process of microbial colonies.

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The results presented in this work serve as a starting point to develop a detector of growing microbial colonies, with obvious interesting applications in diverse areas.

2. METHODOLOGY

2.1 Experimental details

We have analyzed SA through a recently proposed method which is described in detail in Ref. [15]. Here we limit to show the basic equation to determine the SA from a sequence of images, which reads

$$SA = \frac{1}{m \times n} \sum_{k=1}^{N-1} \frac{1}{N-1} \sum_{i=1}^{m} \sum_{j=1}^{n} |I_{k+1}(i,j) - I_k(i,j)| \qquad (1)$$

where N is the number of images from which SA should be determined, m and n are the image sizes (height and width) and $I_k(i, j)$ is the gray scale intensity value at each pixel. Therefore, Equation (1) allows calculating the SA from a sequence of N images at a certain instant t_i, i.e. obtaining SA(t_i). There are basically three important parameters that must be taken into account for the analysis, namely: (i) the number of frames or images, N ($\geq$2), used to calculate a single SA value, (ii) the period of time between those N frames, τ, whose lower limit is given by the highest attainable acquisition rate of the camera and (iii) the period of time, t, between subsequent calculations of SA. Hence, each calculated value, SA(t_i), involves the averaged activity over a finite temporal extension, between the first and the last frame, given by $\Delta\tau = (N-1)\tau$. The experimental setup is schematized in Fig. 1(a) and the different parameters involved are shown in Fig. 1(b).

An expanded 200 mW violet (λ = 480 nm) semiconductor laser was used as a coherent light source. The bottom of culture medium was directly illuminated with the laser and observed through a magnifier in order to avoid the influence on the measurements of humidity condensation on the top surface of the Petri capsule. We have operated the laser in two different modes to analyze the effect of laser light illumination in the growth of microorganisms, namely: (i) a continuous mode, in which the laser continuously illuminated the sample during the whole experience and (ii) a synchronous mode, in which the laser was just turned on during image acquisition.

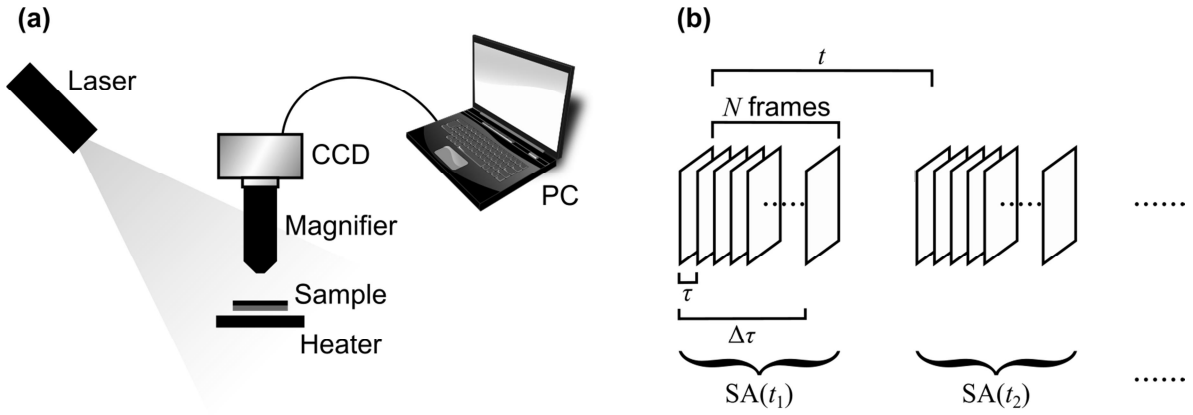

Figure 1. Schematic representation of (a) the experimental setup and (b) different parameters involved in the acquisition and calculation of speckle activity evolution.

2.2 Preparation of the culture medium

To evaluate the growth of microorganism we used Petri capsules with an agarose culture medium in which we inoculated spoors of yeast, penicilium or molds. First, the agarose medium was prepared in liquid solution and it was subsequently introduced in the Petri dishes. All elements were sterilized on an autoclave. Once the capsule temperature reached an optimal temperature, the selected spores were inoculated in the culture medium. To obtain a randomly contaminated sample we just opened one of the Petri dishes and exposed it during 30 minutes to the environment.

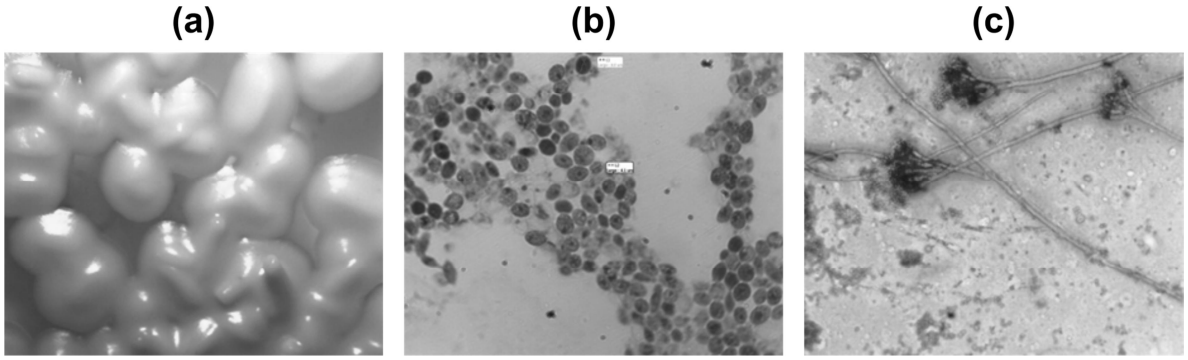

Figure 2. Reference microorganisms: (a) yeast under a 22x microscope view; (b) yeast under a 100x microscope view; (c) peniciluim under a 100x microscope view.

3. RESULTS

In the following subsections we describe the results obtained from different experiences. The first of them consisted in analyzing the dynamic speckle pattern and SA of the culture medium without microorganisms, in order to determine reference values for SA and calibrate the system. In a second experience we studied the effect of continuous laser illumination in a yeast sample. Subsequently we observed the evolution of SA during growth of yeast, which was inoculated in the culture medium and incubated at a temperature of 30 °C. After incubation succeeded, the samples were exposed to laser light in synchronous mode during the observation period. Finally, two Petri dishes with culture medium were exposed to different environments, namely: (i) a chemical lab and (ii) a public bath during 30 min. Both samples were subsequently analyzed by the method described to observe the evolution of SA and compare them with the SA of known inoculated yeasts. For all experiences, observation periods ranged between 4 to 8 days and image acquisition was performed at 30 or 60 min time intervals.

3.1 Reference experience for calibration

Results of the first test yielded SA values less than 0.8, with an average value of 0.54, as is shown in Fig. 3(a). These values are quite low for this particular method of calculation [10], which was expected for the absence of microorganisms. On the other hand, reference values obtained from yeast at the initial stages of observation were greater than 2, as presented in Fig. 3(b), evidencing a difference with respect to the "clean" or sterile culture medium.

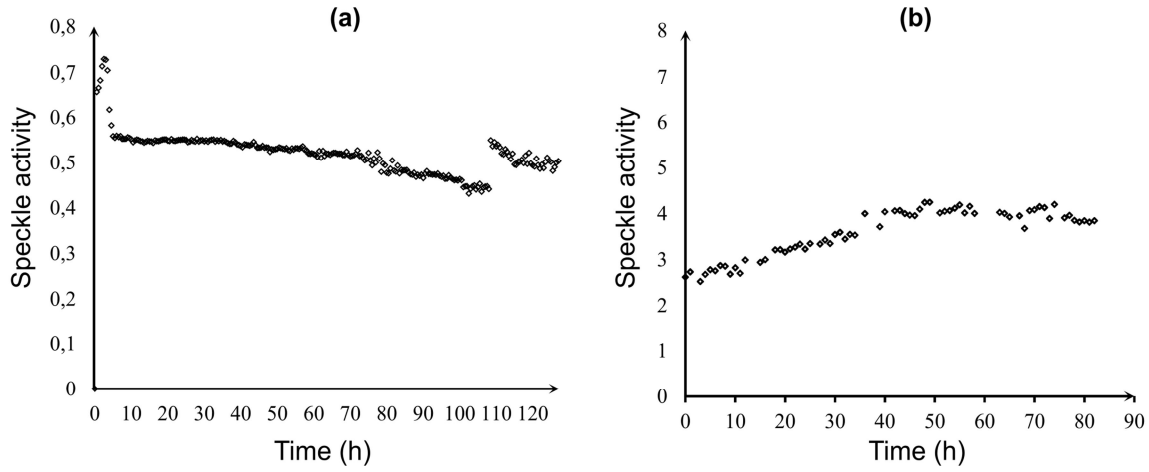

Figure 3. (a) Reference SA curve obtained from the "clean" culture medium (agarose), i.e. without any contamination; (b) SA curve obtained from the initial stages of a yeast culture growth (using synchronous mode).

3.2 Yeast growth inhibition with laser in continuous mode

Using the laser in continuous mode, the yeast culture was observed during a total time of 240 hours. At the end of the experience it was noticed that the yeast grew only outside the zone of laser incidence, as shown in Fig. 4(a). The obtained SA (normalized) curve at this zone for the last 80 hours of observation is shown in Fig. 4(b). The SA values at the last stages (not shown) were coincident with those obtained in the reference curve of Fig. 3(a).

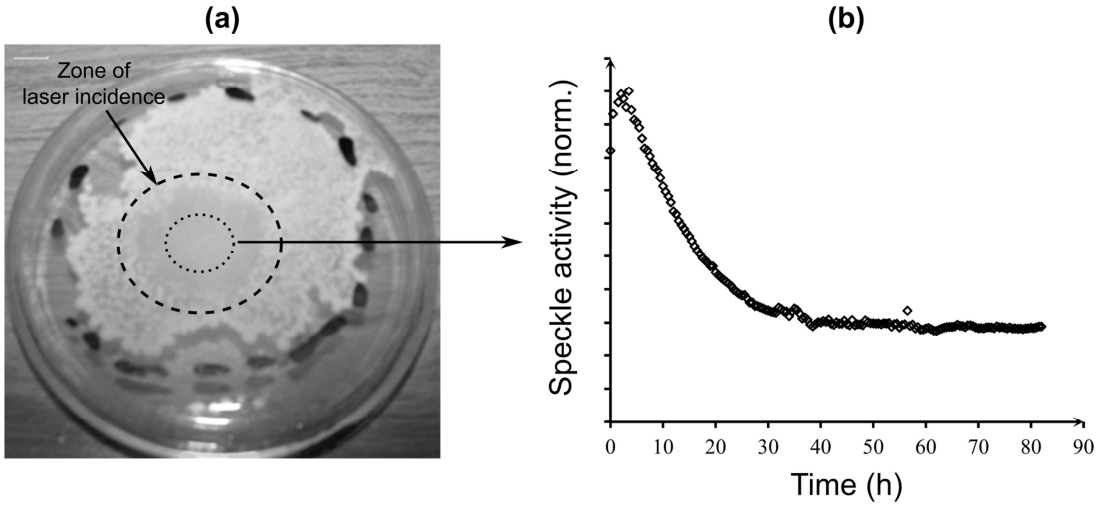

Figure 4. (a) Yeast culture developed on a Petri capsule. The area of laser incidence is marked with the external circle and the growth inhibition is clearly visible inside it; (b) SA curve obtained approximately at the inner circle.

3.3 Yeast growth with laser in synchronous mode

In this case, we synchronized the laser and the camera for image acquisition during yeast growth. The main goal for this experience was to analyze the time evolution of SA during growth without affecting it with laser light. In all tests performed, SA increased from nearly the reference value of Fig. 3(b) to greater values evidencing growth. It is worth noting that the shape of the curve follows approximately an exponential function, which is characteristic of growing microorganism colonies. Moreover, the first stage of the curve with an almost constant (and low) level (above the reference value) of SA is due to what is called the latency stage of the inoculated microorganisms. After that period the microorganisms started to grow almost exponentially.

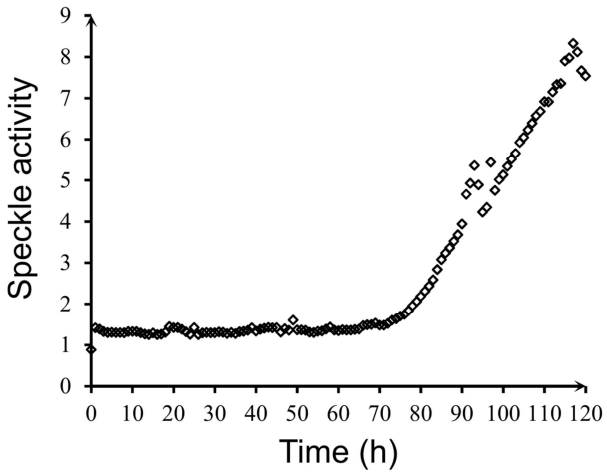

Figure 5. Evolution of SA for a yeast culture obtained with synchronous laser illumination. A nearly exponential trend is observed, which is characteristic of growing microorganism populations.

3.4 Environmental culture growth

The curves obtained from the Petri dishes that were opened and exposed to different environments, a chemical lab and a public bathroom, are shown in Figs. 6(a) and (b), respectively. Microbiological analysis indicated the presence of more than 3 different types of fungi (two different yeast colonies and a mold colony) for the culture exposed to the chemistry lab, whereas for the public bathroom there were more than 10 different fungi colonies. Both curves show characteristic population growth behaviors, an exponential growth trend is observed in Fig. 6(a) while growth, stabilization and subsequent diminution of population is seen in Fig. 6(b).

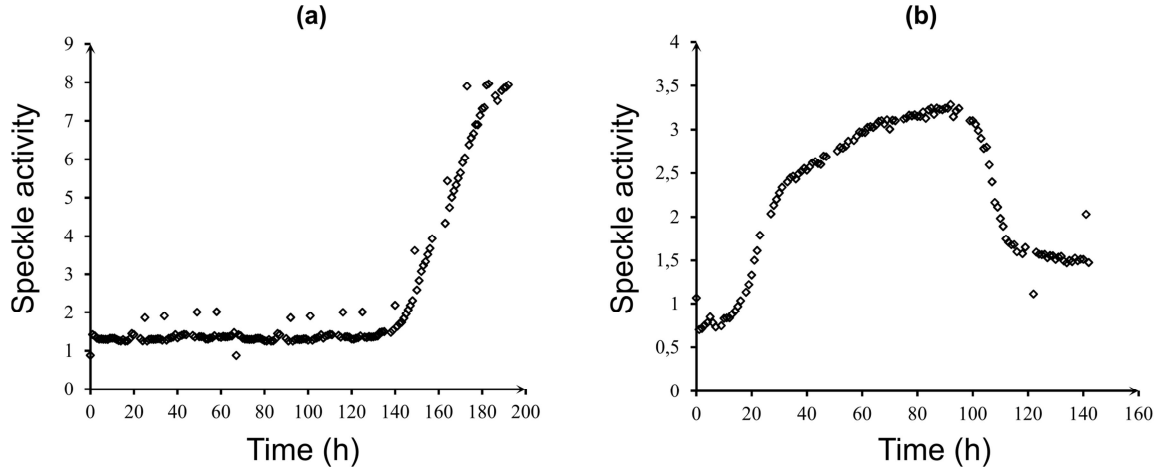

Figure 6. SA time evolution of Petri capsules exposed to (a) chemistry lab and (b) public bathroom environments.

4. CONCLUSION

Summarizing, three different experiences were carried out in order to analyze the SA evolution of different culture media. First we performed a reference test, to analyze typical SA values of a "clean" culture medium, i.e. in the absence of microorganisms. Subsequently we analyzed SA activity of yeast cultures inoculated *ad-hoc* and finally we exposed the culture medium to different environments to observe the SA evolution of randomly inoculated microorganisms. For laser illumination we used to modes, continuous and synchronous, to have a clue about laser damage or growth inhibition. In the calibration stage we established the reference SA value of the culture medium without microorganisms to be below 0.8 for the particular algorithm used. For culture media with inoculated yeasts, this value was slightly higher and about 2. The obtained curves showed a clear increase of SA when microorganisms developed and grew in the culture medium. Yeast samples exposed to the continuous use of an expanded 200 mW (λ = 480 nm) semiconductor laser showed clear signs of inhibited growth. However, the synchronous mode of laser illumination proved to be better and least invasive for carrying out the observations. These results conform a starting point to develop a controlled method for real time and *in-situ* monitoring biosecurity conditions.

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