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(RESEARCH ARTICLE)

Colony forming units quantification in mycoplasma cultures by artificial vision system

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Abstract

This research proposes the application of an automatic system for the recognition and counting of colony forming units (CFU) for mycoplasmas, applying a digital image treatment technique. This initiative will facilitate the quantification of colonies, in order to accelerate the diagnostic processes in research where these microorganisms of relevance in public health are involved.

Keywords: Mycoplasmas; Recognition; Counting; CFU; Artificial vision system

1. Introduction

Software is a set of computer programs, instructions and rules to perform tasks on a computer, allowing specific tasks to be carried out. Software engineering is made up of processes, methods (practices) and an arrangement of tools that allow professionals to develop high-quality computer software, with the aim of establishing meaningful categories for different software applications. According to the usefulness of the systems, the following classification is known: real-time systems, embedded systems, management systems, artificial intelligence systems, personal computer systems, engineering and scientific systems, the latter being the ones that are of interest in the present research [1].

The computer programs that allow the development of workflows in microscopy (capture, visualization, processing, image analysis and data treatment) can be of two types in relation to their license: proprietary software, free software or open source. The proprietary microscopy software is based on collaboration between universities and research centers that are responsible for creating prototypes and are responsible for algorithm research, and commercial companies, these are responsible for developing and marketing the software. Free software and open-source software have a common origin. Although they mean almost the same thing, their definitions have an ideological character and are influenced by economic interests [2,3].

Leica, Zeiss, Nikon and Olympus specialize in microscopy software, as well as Hardware for them, and all these microscopes have image analysis software that helps and facilitates research and allows them to be used as analytical stations for high performance. These complex systems allow regular flows to be followed during image acquisition, filtering, measurement, documentation, and storage [4,5].

Mycoplasmas are the smallest bacteria (300-800 nm) described today, and they lack a cell wall, their cell membrane contains sterols obtained from the tissues or media where they grow. Colony forming units (CFU) are small (50-600 nm

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in diameter), compared to other bacteria. Therefore, they cannot be detected with the naked eye, and therefore their quantification implies time and experience in their identification through the microscope [6,7].

In the field of microbiology, an example of image processing is the detection and quantification of colony forming microorganisms or biofilms, which is a process that is traditionally done manually, time-consuming and highly dependent on human interpretation. [8]. Traditional methods of counting and evaluating images have as a common factor that they consume a large amount of resources such as time and human personnel. The objective of the present investigation was the application of a software coupled to stereoscopic microscopy that facilitates the quantification of mycoplasmas CFU.

2. Material and methods

2.1. Preparation of liquid and solid phase cultures of mycoplasmas

Mycoplasma fermentans PG-18 was thawed at room temperature, resuspending 1 ml of sample in 1 ml of Eaton broth, incubating at 37°C/72 hours, then 5 µl of the broths with growth of *Mycoplasma fermentans* PG-18, incubating at 37°C/72 hours, the entire microbiological process was carried out in triplicate.

2.2. Quantification of colony forming units through software

For the quantification of CFU stereoscopic microscopy (Zeiss) connected to a *hp* computer was used, using image detection and segmentation techniques, and using Eaton solid medium mycoplasma cultures to evaluate the detection of CFU.

The OpenCV library was used because it presented most of the functions that were required, with the exception of those that are needed for the erosion matrix (a mathematical morphology operation, which is required to identify the space delimited by an edge.

The image segmentation application allowed subdivision to isolate the region or object of interest. The segmentation algorithm was based on one of the two essential properties of gray values, the discontinuity or similarity between the gray levels of the adjacent pixels. The image was divided in relation to the change of the gray scale (outlier detection, line detection and edge detection).

3. Results and discussion

3.1. Mycoplasma cultures preparation

During the incubation times for the cultures of *Mycoplasma fermentans* PG-18, in Eaton broth and Eaton agar, optimal growths were obtained (Figure 1), these samples were used to perform the CFU quantification test using software.

3.2. Colony forming units quantification

The detection of CFU was based on the calculation of local derivation operators, since if the gray level between a pixel and its neighbors changes, the pixel belongs to the edge. During the detection of CFU in cultures, smoothing the image was established to reduce unwanted effects, detect possible candidates to become vantage points and choose among candidates that are marginal. The vision system takes images with a resolution of 5 megapixels in order to be able to properly identify the colonies despite their small size. It is important to note that there are investigations where this initiative has been implemented, but the count is applied for other bacteria and not for mycoplasmas [9-12].

Subsequently, a thresholding of the image was carried out in order to identify the pixels corresponding to the Petri dish, taking several images of the bottom and the Petri dish under different light conditions and their histograms were analyzed. It was obtained that the trend of the H channel values of those corresponding to the Petri dish are within a range of 0.5 to 0.65, considering these values to perform the image thresholding [9].

The software code presented the following sequence: "original image read" - "convert image to grayscale image" - "corrosion expansion" - "expansion" - "corrosion" - "binary method threshold processing" - "Find the connected domain" - "compare the area of the connected domain" - "create an empty matrix, place the area of connected domains" - "create an empty matrix, assign the number minus the smallest area" - "calculate the area and remove the connected

domains with small areas ”-“ calculate the number of connected domains and draw the number at the center of gravity coordinate point ”-“ draw numbers at the center coordinate point ”-“ show images ”.

When executing the program, the grayscale images were obtained with the identified structures (Figure 2A) and the original image with the identified contours superimposed (Figure 2B), in addition to the identifying number of each contour. Figure 2A shows the image resulting from the thresholding and the respective binarization. It can be seen that several pixels corresponding to the background passed within the threshold. The threshold cannot be made so restrictive because the pixel values under different light conditions tend to change and if the threshold is very restrictive there is a risk of not detecting parts of the Petri dish. It was processed on the console, printing on the terminal the number of quantified CFU=16 (Figure 2C).

In order to eliminate the pixels corresponding to the background, the sets of adjacent pixels smaller than 100 (reduced groups) are eliminated, in the same way the morphological operations of filling in objects and closening were carried out, as has been reported in different investigations [13,14].

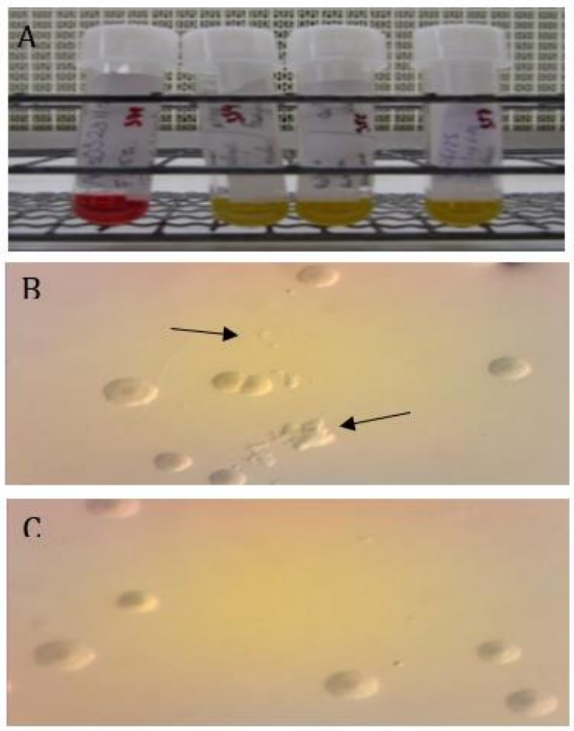

Fig. 1. Liquid culture shows the change of the pH indicator, red indicates no growth and yellow optimal growth (A). Solid phase culture showing the characteristic growth of mycoplasma colonies in "fried egg“ form, the arrows indicate the presence of particles that are not colonies and can be confusing in their quantification (B). Solid phase growth showing optimal growth in particles absence (C), stereoscopic microscopy 4X.

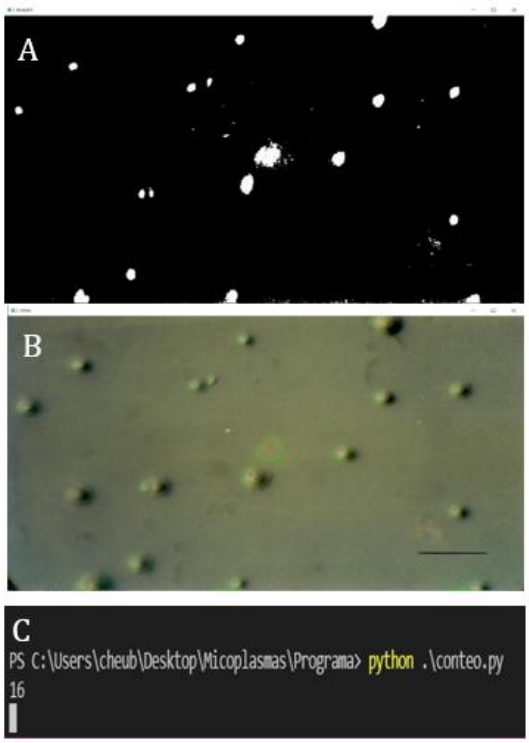

Fig. 2. When the program run, the grayscale image with the detected contours obtained on the screen (A). The original image with the detected contours superimposed and the identifier number of said contour (B). The number of counted CFU=16 is terminal printed (C).

4. Conclusion

Taking into account the pathogenic role of mycoplasmas, which are indisputably present in a large number of infectious diseases, and can be found colonizing the respiratory and urogenital tracts of both man and animals, in addition to infecting plants, cell cultures and biotechnological products for biomedical. The application is of relevance to this research, since this application will make it possible to add to the improvement of diagnoses.

The automation of the colony count significantly reduces the margin of error in this procedure and avoids the fatigue of the people who perform it, because it is a routine work that requires users to pay close attention. The development of this algorithm allows reducing costs in laboratories, because those that are in the market to develop these tasks are costs despite their limited benefits.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that they have no competing interests.

Authors contributions

UTJA, BRJJ and AGD carried out the software design and executed the work. RA was the general coordinator and wrote the manuscript with input from all authors.

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