ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 11 (2): 91-94, 2012
ISSN 1682-296X / DOI: 10.3923/biotech.2012.91.94

A Simple Platform for Real-time and Dynamic Assay of Single Microbial Cell

[1]Yi-Ran Yang and [1,2]Hui-Hui Liu
[1]Department of Gastroenterology, The Third Hospital of Wuhan, Wuhan, Hubei, People's Republic of China
[2]Genomic Medicine Institute, Cleveland Clinic Foundation, Cleveland, OH, USA

Abstract: A simple and rapid agarose immobilization method of microbial cells was developed to study the behavior of a single living cell in the changeable environment. Using this technique, process of apotptosis of a single microbial cell and subcellular particles' movements were recorded real-timely. The proliferation process of *Pseudomonas aeruginosa* was also pursued.

Key words: Immobilization, dynamic process, real-time, single cell

INTRODUCTION

Single microbial cell assays were applied extensively in various researches (Pogliano *et al*., 1995; Deich *et al*., 2004; Gato and Means, 2011; Nasseri *et al*., 2011; El-Ahmady *et al*., 2011), such as the subcellular location and cell behaviors, in which cell immobilization played an important role. Though cells had been fixed by using many methods such as with amino-silanes and flagellin antibodies (Cluzel *et al*., 2000) or on a poly L-lysine-treated coverslip (Pogliano *et al*., 1999), long-range real-time record of single microbial cell's behaviors and the movements of subcellular structures in the adjustable environment was still challenging. Moreover, due to the small size and quick proliferation of microbial cells, many techniques performed in cases of a single animal or plant cell (Rahmat *et al*., 2006; Huang, 2006; Rajendran *et al*., 2008; Khorshid *et al*., 2011) were not good to the living single microbial cell (Kehr, 2003).

METHODS AND RESULTS

To realize those characteristics including long-range observations, real-time records, adjustable environment around microbial cells, we designed a square well in the center of a glass slide and immobilized the microbial cell in agarose gel at a low concentrations (Fig. 1a,b). The behaviors of cell were monitored and recorded by inverted Olympus microscope (FM, Olympus IX-70) with an oil immersion objective (Olympus, 100×, N.A.=1.35) and a U-MWB filter set

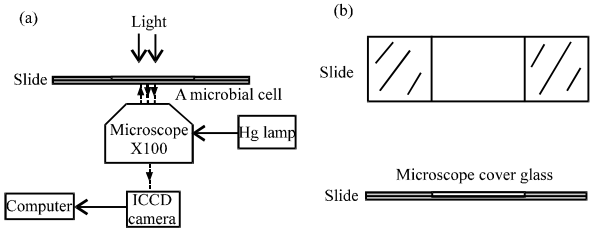

Fig. 1(a-b): Schematic view of the experimental apparatus. (a) We employed an inverted Olympus microscope to perform the research on single microbial cell. The microbial cell was immobilized in the agarose gel on a platform. An ICCD camera was used to record the movement of a bacterium immobilized in agarose gel and (b) The platform, on which the cell was immobilized with agarose gel

(Olympus, 450-480/500/515 nm) and recorded by an ICCD (intensive charge-coupled device) camera simultaneously.

In initiation of an experiment, *Saccharomyces cerevisiae* cells were pre-cultured in YPD (1% yeast extract; 2% tryptone; 2% glucose, Sigma) at 28°C and 220 rpm overnight. 50 μL of the resulting cell suspension was inoculated into 5 mL of YPD and cultivated for an additional 1 h. One milliliter of 1% agarose gel were mixed with 1 mL of the yeast cell YPD solution/culture when the temperature of agarose gel was cooled to 40°C. The mixture was transferred into the well and covered with a microscope cover slide (Esco 24×40 No.1 thickness, ERIE SCIENTIFIC COMPANY, Portsmouth, NH, USA). After it

Corresponding Author: Hui-Hui Liu, MD PhD, Department of Gastroenterology, The Third Hospital of Wuhan, Wuhan, Hubei, People's Republic of China and Genomic Medicine Institute, Cleveland Clinic Foundation, Cleveland, OH, USA

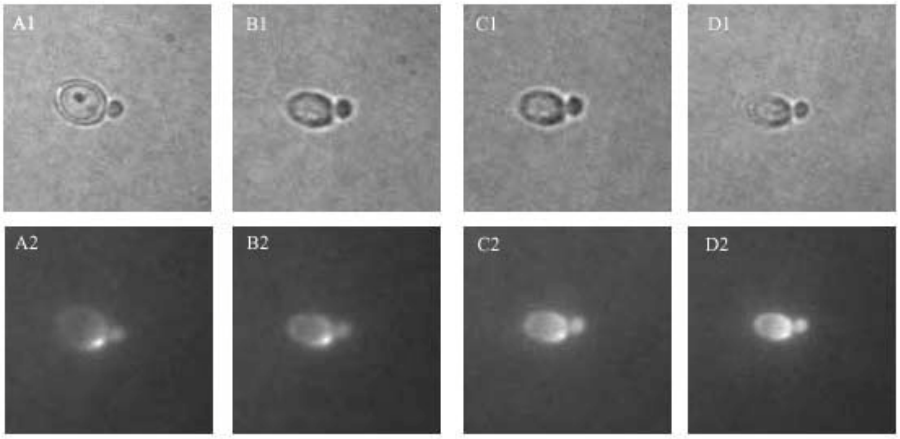

Fig. 2: Dynamic investigation on one yeast cell treated by H_2O_2. Yeast cell stained with DAPI and viewed by inverted Olympus microscope continuously. (A1-2) yeast cell immobilized in agarose gel in the first hour of investigation. (B1-2) After 1 h, the yeast cell was treated with H_2O_2 for about 70 min (C1-2) The same cell treated with H_2O_2 for about 100 min. (D1-2) The cell treated with H_2O_2 for a total of about 140 min. The images show the nuclear of the cell was desegregating and the body of the cell was shrinking when treated with H_2O_2

had been coagulated for 20 min, the cover glass was removed and 1% DAPI (4,6-diaminidino-2-phenylindole, Sigma) was dropped on the agarose gel. Then the slide was placed in a dark box for 30 min. The gel was covered again before view.

To monitor process of apoptosis, yeast cells were embedded in 0.5% agarose gel and plated on the slide we designed. One percent H_2O_2 was dropped on the gel to induce apoptosis (Fig. 2a). Images were collected every 1 h for a total of 4 h. The images show clearly that the nuclear of the cell was disaggregating and the cell body was shrinking after H_2O_2 was applied (Fig. 2b-d). In this case, it is feasible to technician to change the culture environment of the observed cell repeatedly by dropping media or any solution on the gel. The media or the solution can also be removed by being adsorbed by filter paper. It is noteworthy that the humidity and the concentration of the gel should be carefully kept during experiment.

To perform the real-time observation on movement of subcellular structures, a similar method was employed. In detail, yeast cells were immobilized on the slide with 1% agarose after cultured. Movement of subcellular structures' were monitored and recorded with the inverted microscope. As a result, an object was discovered in the about 10% of yeast cells and its movements were recorded in a real-time manner. It is clear that the size of the object was bigger than mitochondria's in photos (Fig. 3a) and it can be stained by DAPI (Fig. 3b). In addition, the object can move inside the vacuole as well as outside cells. Unlike the techniques such as Pendent drop and the microchannel (Xu *et al*., 2003; Lim *et al*., 2010; Luo *et al*., 2008). Our method was successfully used to record the dynamic process of subcellular structures. We believe that this technique would also make it easy to monitor the macromolecular movement within living cells. Moreover, life cycle of microbial cell won't be disturbed by the procedures of our method. *P. aeruginosa* PAO1 cells were cultured in LB-broth medium containing 1% tryptone, 0.5% yeast extract and 1% NaCl with shaking at 150 rpm and 28°C. The cells were then inoculated and cultured for additional 1 h. The cell solution was immobilized with 0.3% agarose gel and observed at 28°C with inverted Olympus microscope for 40 min. Four of the images were selected to show the progress of proliferation of a single *P. aeruginosa* PAO 1 cell (Fig. 4). Different from other methods (Korobkova *et al*., 2004; Pogliano *et al*., 2001), this method has little effect on physiological behaviors of living cell, such as division, sporulation and proliferation. The entire cell cycle can thus be monitored and recorded.

Agar has long been used as a common material to immobilize the microbial cells (Perrot *et al*., 1998; Khattar *et al*., 1999).

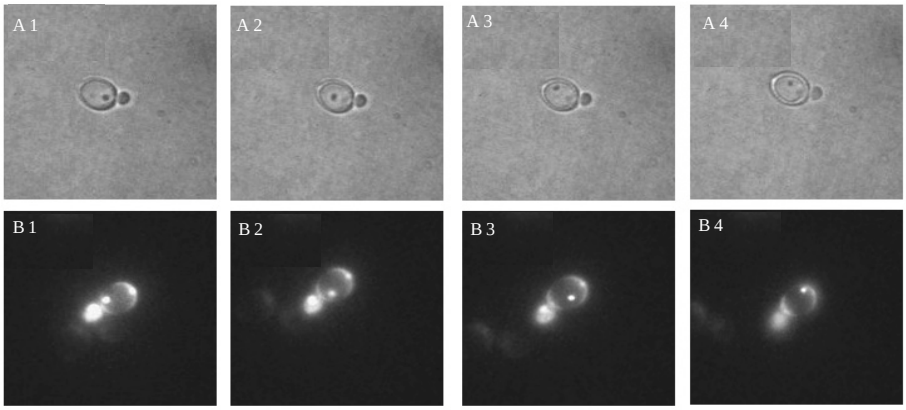

Fig. 3: The movement of an object in a yeast cell. A yeast cell was stained with DAPI after immobilized with 1% agarose gel. Images were collected every 50 msec for a total of 5 sec. And 4 out of 100 photos were selected. (A) A cell was observed under bright field (B) Another cell was viewed with fluorescence. The results suggest, there was a moving object in the cell vacuole since the body of the cell was fixed

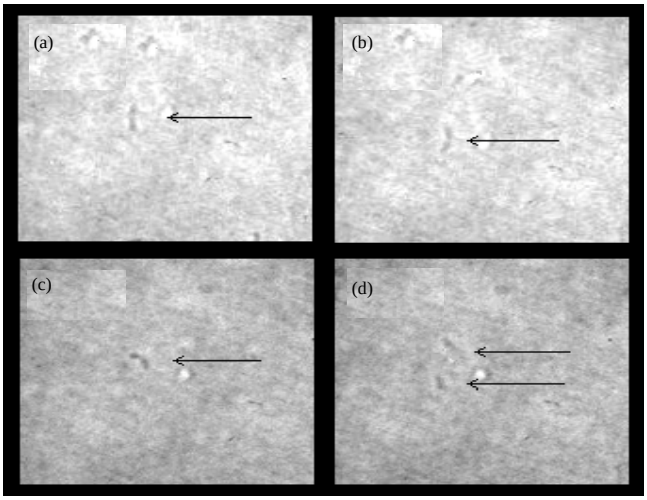

Fig. 4(a-d): Dynamic investigation on the proliferation of *P. aeruginosa* PAO 1. The cells of *P. aeruginosa* PAO 1 were immobilized with 0.3% agarose gel. The photos were taken every 1 min for 40 min. (a) The photos of 30, (b) 31, (c) 32 and (d) 33 min were shown which recorded the dynamic process of the proliferation of the cell of *P. aeruginosa* PAO 1. The result suggests the bacterial cell can move freely in the low concentration of agarose

CONCLUSIONS

In these experiments, higher concentration of agar restricted movements of microbial cells. Most of studies focus on population of bacteria or eukaryotic cells. Our method can be used not only to monitor real-time responses of a microbial cell toward change of environment, but also to offer a quick and simple way for investigating movement of the substance/structure within a living cell. It can also be applied to record cellular phenotypes during cell cycle detailedly.

LIMITATIONS AND RECOMMENDATIONS

The technique we mentioned here has some limitations. For example, the device that we used to

monitor cell behavior or particle movement around cell is mechanically simple. It has not been combined with any robot or automatic. Hence, we have to re-localize cell of interest after we change the culture environment of the cell. But the simple device can be produced, we believe, in most of labs over the world. The technique is also feasible to almost all biological labs. Additionally, in our experiments, the performance of single cell was not consistent all along, which might be due to difference between individual cells. So repeat of experiment was strongly suggested to obtain reliable result. Although analyses and operations on single cell remain challenging, we believe experiments based on this technique can provide useful information about single-cell microbiology or medical science.

REFERENCES

Cluzel, P., M. Surette and S. Leibler, 2000. An ultrasensitive bacterial motor revealed by monitoring signaling proteins in single cells. Science, 287: 1652-1654.

Deich, J., E.M. Judd, H.H. McAdams and WE. Moerner, 2004. Visualization of the movement of single histidine kinase molecules in live *Caulobacter* cells. Proc. Nat. Acad. Sci., 101: 15921-15926.

El-Ahmady, N., A. El-Naggar, M.S. El-Hersh, H.A. El-Fadaly and W.I.A. Saber, 2011. Bioconversion of some agro-industrial by-products into single cell oil using *Candida albicans* NRRL Y-12983 and *Lipomyces starkeyi* NRRL Y-11557. Res. J. Microbiol., 6: 784-795.

Gato, W.E. and J.C. Means, 2011. Single cell gel electrophoretic analysis of 2-aminoanthracene exposed F-344 rats. Am. J. Biochem. Mol. Biol., 1: 275-283.

Huang, L.R., 2006. Growth and cell cycle regulation by silibinin in chang liver cells. J. Pharmacol. Toxicol., 1: 527-536.

Kehr, J., 2003. Single cell technique. Curr. Opinion Plant Biol., 6: 617-621.

Khattar, J.I.S., T.A. Sarma and D.P. Singh, 1999. Removal of chromium ions by agar immobilized cells of the cyanobacterium *Anacystis nidulans* in a continuous flow bioreactor. Enzyme Microbial. Technol., 25: 564-568.

Khorshid, F.A., S.A. Rahimaldeen and J.S. Al-Amri, 2011. Apoptosis study on the effect of PMF on different cancer cells. Int. J. Biol. Chem., 5: 150-155.

Korobkova, E., T. Emonet, J.M.G. Vilar, T.S. Shimizu and P. Cluzel, 2004. From molecular noise to behavioural variability in a single bacterium. Nature, 428: 574-578.

Lim, F.Y., S. Abdullah and I. Ahmad, 2010. Numerical study of fluid flow and heat transfer in microchannel heat sinks using anisotropic porous media approximation. J. Applied Sci., 10: 2047-2057.

Luo, W.J., K.C. Chang and J.S. Wang, 2008. Proportional mixing of microfluidic flows utilizing DC and AC electric fields in a T-type microchannel. J. Applied Sci., 8: 1632-1641.

Nasseri, A.T., S. Rasoul-Amini, M.H. Morowvat and Y. Ghasemi, 2011. Single cell protein: Production and process. Am. J. Food Technol., 6: 103-116.

Perrot, F., T. Jouenne, M. Feuilloley, H. Vaudry and G.A. Junter, 1998. Gel immobilization improves survival of *Escherichia coli* under temperature stress in nutrient-poor natural water. Water Res., 32: 3521-3526.

Pogliano, J., N. Osborne, M.D. Sharp, A.A. de Mello, A. Perez, Y.L. Sun and K. Pogliano, 1999. A vital stain for studying membrane dynamics in bacteria: A novel echanism controlling septation during *Bacillus subtilis* sporulation. Mol. Microbiol., 31: 1149-1159.

Pogliano, J., T.Q. Ho, Z. Zhong and D.R. Helinski, 2001. Multicopy plasmids are clustered and localized in *Escherichia coli*. Proc. Natl. Acad Sci. USA., 98: 4486-4491.

Pogliano, K., E. Harry and R. Losick, 1995. Visualization of the subcellular location of sporulation proteins in *Bacillus subtilis* using immunofluorescence microscopy. Mol. Microbiol., 18: 459-470.

Rahmat, A.., E. Susi, I. Patimah, Y.Y.H. Taufiq and A.B.M. Fadzelly, 2006. Chemical constituents, antioxidant activity and cytotoxic effects of essential oil from *Strobilanthes crispus* and *Lawsonia inermis*. J. Biol. Sci., 6: 1005-1010.

Rajendran, P., R. Venugopal, G. Ekambaram, A. Aadithya and D. Sakthisekaran, 2008. Rehabilating activity of mangiferin in benzo(a) pyrene induced lung carcinogenesis. Asian J. Biochem., 36: 118-125.

Xu, X.H.N., W. Brownlow, S. Huang and J. Chen, 2003. Single-molecule detection of efflux pump machinery in *Pseudomonas aeruginosa*. Biochem. Biophys. Res. Commun., 305: 79-86.