Microelectrode Ion Flux Estimation (MIFE) technique in microbiology

A cutting edge approach to study bacterial stress responses

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Introduction

The idea of measuring net ion fluxes at the proximity of a living organism using non-invasive microelectrodes was first proposed by Prof B Lucas at the NATO Advanced Studies Institute in Italy in 1984[1]. This led to creation of the National Vibrating Probe Facility at Woods Hole, USA, which is widely used for electrophysiological studies in medical and animal physiology research[2].

An alternative approach was developed by Dr Ian Newman (School of Mathematics and Physics, University of Tasmania) and resulted in the construction of the MIFE^R system, which is now commercially available. Since the late 1990s, MIFE has been successfully applied to the study of various aspects of membrane-transport processes in plants and protoplasts derived from the plant tissues.

However, neither the Woods Hole 'vibrating probe' facility, nor the MIFE technique were applied to smaller organisms like bacteria until 2001, when studies on membrane transport processes in food-related microorganisms exposed to a variety of stresses were pioneered by Dr L Shabala. Currently, it is possible to use the MIFE to measure net fluxes of H^+, Ca^{2+}, K^+, Na^+, Cl^-, Mg^{2+}, NH^{4+}, NO^{3-}, Cd^{2+}, Zn^{2+}, Cu^{2+}, O_2, and redox potential in real time from higher plants, protoplasts derived from plant tissue, muscle tissues, fungi, yeast, and bacteria.

Cell membranes are traditionally considered to act as a semi-permeable barrier, allowing the preferential uptake of some nutrients, and preventing or restricting accumulation of undesirable chemicals[3]. When membrane integrity is lost, the cells are freely permeable to all solutes, finally leading to their death[4]. Membrane transporters are involved in response to any environmental or endogenous stimuli. Transport of ions is crucial for maintaining optimal osmotic potential and pH_i homeostasis of an organism. Transport of nutrients into and waste out of the cell is a necessary part of metabolism. Therefore, a better understanding of mechanisms of adaptation of microorganisms may offer insights into methods of controlling their growth.

Principle of MIFE

The MIFE system uses a stepper motor-driven micromanipulator to move 4 ion selective electrodes close to tissue surface and back (Figure 1). The principle of ion flux calculations is based on measurements of electrochemical gradients between two different points (close to tissue and at some distance)[5, 6]. Recorded at two positions, voltage characteristics are transformed into concentration parameters using calibration curves and software is used for calculation of the net ion flux that is proportional to concentration gradient between these two positions. The latter provides the tabulated results of measurements as net ion fluxes (in nmol $m^{-2}s^{-1}$) for import into a spreadsheet.

Together with high spatial (a few microns) and temporal (5s) resolution, MIFE has become a unique tool for kinetic studies of transport processes. Therefore, MIFE provides quantitative estimates of the rate of the measured process. An advantage is that, being non-invasive, the microelectrodes do not interfere with cell integrity. Furthermore, the MIFE technique allows measurements of several ions simultaneously and essentially from the same site which enables stoichiometry between ions to be established (Figure 2).

MIFE applications

Researchers at AFSC use MIFE to study responses of food-related micro-organisms to stresses commonly applied in food preservation. Different treatments are employed to ensure food safety but using traditional

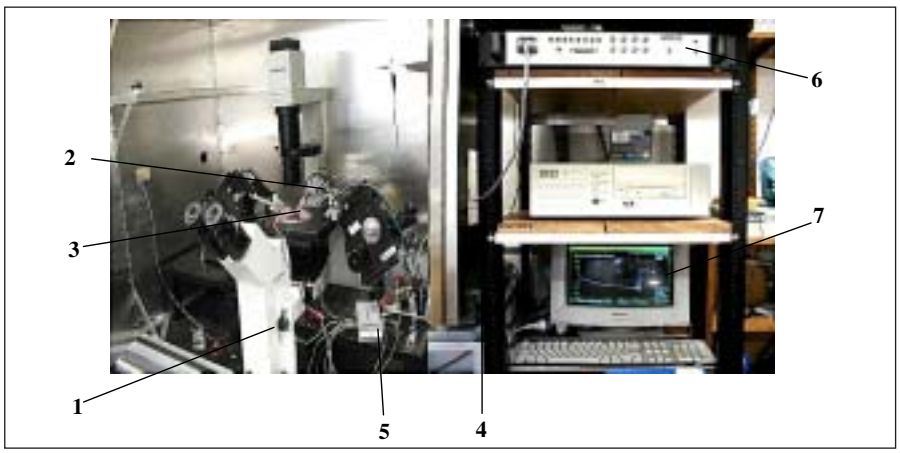

Figure 1. The MIFE setup: microscope (1); an electrode holder (2); a measuring chamber (3); a stepper motor and controller (4); a pre-amplifier (5); a main amplifier and controller (6) connected to a personal computer (7).

microbiological techniques to screen for optimal combinations of treatments is a lengthy, empirical process. MIFE applications include rapid screening of stress responses (low and high temperature, salt and osmotic stresses, pH stress, antimicrobial agents, effects of antibiotics and probiotics, oxidative stress), a rapid viability assay, assessment of starter culture vitality, studies on biofilms, mechanisms of adaptation and resistance, and functional genomics of microorganisms.

Some examples of MIFE application are given in our publications[7-11]. MIFE studies show that energy availability is critical for *L. monocytogenes* subjected to acid stress. H^+ ions enter the bacterial cell in the absence of glucose, but addition of glucose enables expulsion of H^+ from the cell and effectively maintains a constant pH_i crucial for pH homeostasis (Figure 3). The strong relationship between temperature and the activity of the ion transport systems measured by MIFE (Figure 4) may indicate its potential to rapidly screen bacterial growth limits.

The practical outcome of MIFE studies will be a novel, rapid technology to screen combinations of food preservatives, increasing Australia's capacity for innovation in food preservation technology and reducing economic losses to Australia associated with food spoilage and food-borne bacterial disease. MIFE studies will advance knowledge of bacterial adaptive responses to food processing treatments.

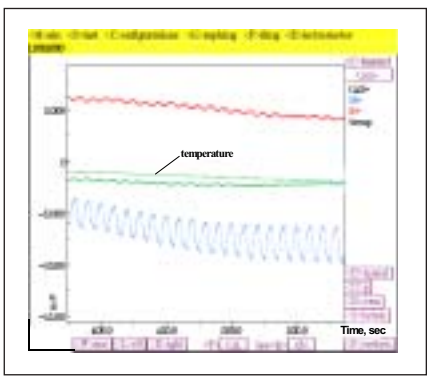

Figure 2. Computer screen display of the MIFE recordings showing four concurrent voltage records for Ca^{2+}, H^+, K^+, and temperature. Fluxes of ions were calculated from the voltage using recorded concentration values. H^+ and K^+ concentrations are decreasing slowly. Net K^+ and H^+ fluxes are steady, while Ca^{2+} flux decreases to zero. Temperature (reversed scale) increases slowly.

Once the adaptive mechanisms are understood, it will be easier to exploit fully the potential of existing preservation methods in the food industry as well as to identify possible new antimicrobial technologies, e.g. design of new preservatives specifically targeted to identified bacterial physiological function.

While the MIFE technique by itself is a powerful tool, capable of providing valuable information about the 'blueprints' of cell adaptive responses to the environment, its power can be increased many-fold higher when combined with other electrophysiological or molecular techniques.

References

1. Lucas WJ & Kochian LV. Ion transport processes in corn roots: an approach utilizing microelectrode techniques. In: Gensler WG (Ed). *Advanced Agricultural Instrumentation: Design and Use.* Dordrecht: Matinus Nijhoff, 1986, p.402-425.
2. Smith PJS. Non-invasive ion probes – tools for measuring transmembrane ion flux. *Nature* 1995; 378:645-646.

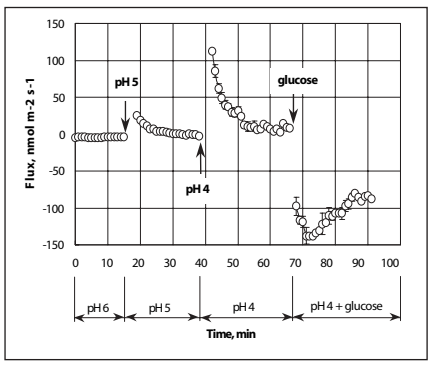

Figure 3. Effect of glucose availability on kinetics of *L. monocytogenes* Scott A after acid treatment. Cells were adapted at pH 6.0 in the absence of glucose for 1h. Acid treatment was applied by a change of the medium to one with desired pH. Glucose was added as indicated in amounts to achieve 10mM in the experimental solution. Error bars are SEM (n=4).

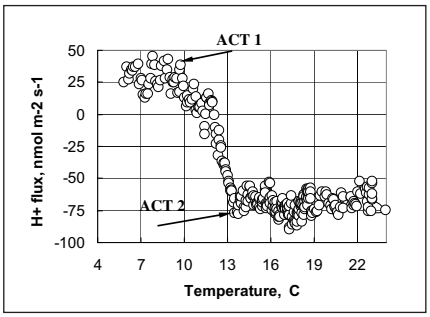

Figure 4. Kinetics of net H^+ flux recovery from 4°C to 23°C for a thraustochytrid culture. Cells were adapted at 4°C for 2h. Two apparent critical temperatures were identified (ACT1 and ACT2) at 9°C and 14°C which have been attributed to the re-activation of plasma membrane transporters for H^+ at ACT1 and complete recovery at ACT2, respectively.

3. Maloney PC & Wilson TH. Ion-coupled transport and transporters. In: Neidhardt P (Ed). Escherichia coli *and* Salmonella. *Cellular and Molecular Biology.* ASM Press, Washington, DC, 1996, p.1130-1148.
4. Yeagle PL. Lipid regulation of cell membrane structure and function. *FASEB J* 1989; 3:1833-42.
5. Shabala SN, Newman IA & Morris J. Oscillations in H^+ and Ca^{2+} ion fluxes around the elongation region of corn roots and effects of external pH. *Plant Physiol* 1997; 113:111-118.
6. Newman IA. Ion transport in roots: measurement of fluxes using ion-selective microelectrodes to characterize transporter function. *Plant Cell Enviro* 2001; 24:1-14.
7. Shabala SN & Shabala LI. Kinetics of net H^+, Ca^{2+}, K^+, Na^+, NH^{4+}, and Cl^- fluxes associated with post-chilling recovery of plasma membrane transporters in *Zea mays* L. leaf and root tissues. *Physiologia Plantarum* 2001; 114:47-56.
8. Shabala L, Ross T, Newman I, McMeekin TA & Shabala S. Measurements of net fluxes and extracellular changes of H^+, Ca^{2+}, K^+ and NH^{4+} in *Escherichia coli* using ion-selective micro-electrodes. *J Micro Methods* 2001a; 46:119-129.
9. Shabala L, Shabala S, Ross T & McMeekin T.A. Membrane transport activity and ultradian ion flux oscillations associated with cell cycle of *Thraustochytrium* sp. *Aus J Plant Physio* 2001b; 28:87-99.
10. Shabala L, Budde B, Ross T, Siegumfeldt H & McMeekin TA. Responses of *Listeria monocytogenes* to acid stress and glucose availability monitored by measurements of intracellular pH and viable counts. *Int J Food Micro* 2002a ; 75:89-97.
11. Shabala L, Budde B, Ross T, Siegumfeldt H, Jakobsen M & McMeekin T.A. Responses of *Listeria monocytogenes* to acid stress and glucose availability revealed by a novel combination of fluorescence microscopy and microelectrode ion-selective techniques. *App Enviro Micro* (2002b); 68:1794-1802.