Cell growth in monodisperse water-in-oil emulsion droplets using microfluidics

Oprea A.M., Hirata K., Washio N., Ono T*.
Okayama Univ., Okayama, Japan (aoprea@icmpp.ro)

INTRODUCTION

Directed evolution is a powerful methodology to create new biocatalysts. For this purpose, engineered genes "genotype" and the expressed proteins "phenotype" should be accommodated in the same segregated spaces to select the best gene. We describe the compartmentalization of *E. coli* in microdroplets and *in situ* protein expression. Cell growth in water-in-oil emulsion droplets has been achieved by monitoring the fluorescence of expressed proteins which facilitates the selection of active *E. coli* in a suitable medium.

MATERIALS AND METHODS

Preparation of E. coli cell suspensions
The transformation of BL21 (DE3) cells was performed with PTO-10 (EGFP), pNW-1 (DsRed-monomer) and the pNW-2 (EBFP) plasmids. After 12 hours incubation in LB-kanamycin liquid medium at 37 ^{0}C and 250 rpm the suspensions were centrifuged and the cells pellet were stored at -80 ^{0}C. In order to prepare monodisperse gel particles the *E. coli* pellets were resuspended and diluted with LB medium to predetermined concentrations of: 0.20, 0.10, 0.05 and 0.01 cells/particle.

E. coli encapsulation in agarose gel particles
Aqueous phase : The aqueous solution was prepared by ultrapure water addition to LB-agarose-L medium and autoclave 20 minutes at 121 ^{0}C. The solution was stirred and mixed with kanamycin (50 µg/ml), IPTG (80 µM) and 1/100 *E. coli* suspension at 37 ^{0}C.

Oil phase : Span 80 (4.5 v/v %) and Tween 80 (0.5 v/v %) were dissolved in decane the obtained solution being used as oil phase. The water-in-oil (w/o) emulsions were maintained at 37 ^{0}C for avoiding gelation. The flow rate of aqueous phase was 5 µm/min and for oil phase the flow rates were 20, 50, 100 µm/min. The w/o emulsion preparation method was followed by a reduction of temperature using an ice bath to allow gelation of the agarose droplets. The *E. coli*-encapsulating agarose gel particles were incubated in decane at 20, 25, 30 and 37 ^{0}C, the proteins expression being monitored by fluorescence microscopy.

RESULTS AND DISCUSSION

Influence of medium concentration on EGFP expression process in monodisperse droplets and agarose gel particles
The EGFP expression was correlated with the medium concentration and incubation time for both monodisperse droplets and agarose gel particles (figures 1 and 2). Because the volume of each drop is restricted, EGFP amount secreted by *E. coli* cells can rapidly attain detectable concentrations.

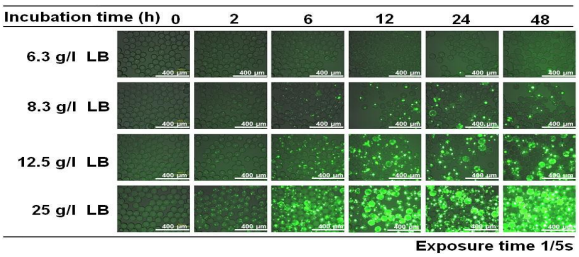

Figure 1 : Fluorescence micrographs obtained for EGFP expression in agarose gel particles by variation of medium concentration within 48 hours

The fluorescence was visible at 25 g/l LB medium concentration from the first hour after sample preparation, the EGFP synthesis being faster and more intense between 6-48 hours incubation in case of agarose gel particles (figure 1).

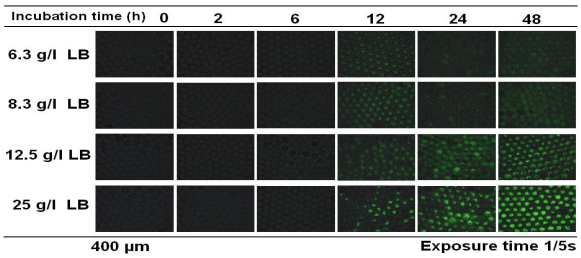

Figure 2 : Fluorescence micrographs obtained for EGFP expression in monodiperse droplets by variation of medium concentration within 48 hours

For monodisperse droplets (figure 2) an increased fluorescence was noticed between 12-48 hours of incubation at 12.5 g/l LB and 25 g/l LB medium concentrations.

Influence of incubation time and concentration of E. coli suspensions on DsRed-monomer, EGFP and EBFP expression process in monodisperse agarose gel particles
The 0-6 hours droplets incubation interval showed a very weak or absence of protein fluorescence. Between 12-48 hours the fluorescent proteins

expression is becoming stronger, the synthesized amount increasing with increasing of *E. coli* suspension concentrations (figure 3).

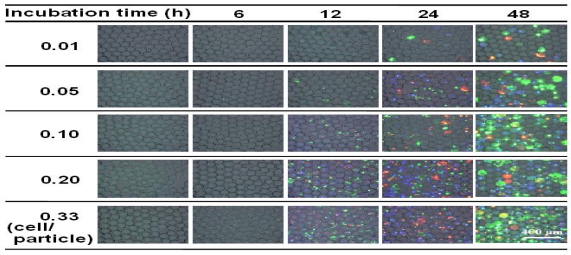

Figure 3 : Fluorescence micrographs of monodisperse agarose gel particles at different incubation time and concentrations of *E. coli* suspensions (exposure time: 1/5s)

Influence of temperature on EGFP expression in agarose gel particles and monodisperse droplets

The micrographs showed similar protein expression behaviour for both agarose gel particles (figure 4) and monodisperse droplets (figure 5) within 0 and 6 hours for all temperatures, the fluorescence intensity being weak or even absent. By increasing the *E. coli* incubation temperature at 25 ^{0}C and 30 ^{0}C the fluorescence intensity increased within 12-48 hours. At 37 ^{0}C, the appropriate *E. coli* proliferation temperature, the fluorescence is almost absent (figure 4 and 5).

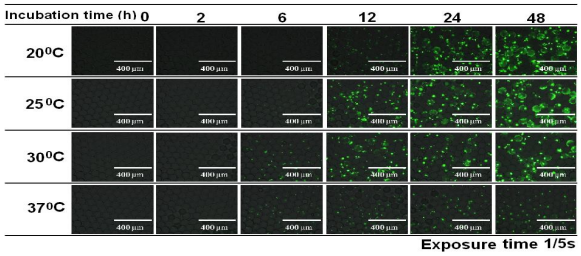

Figure 4 : Fluorescence micrographs of EGFP expression in agarose gel particles at different incubation temperatures within 48 hours

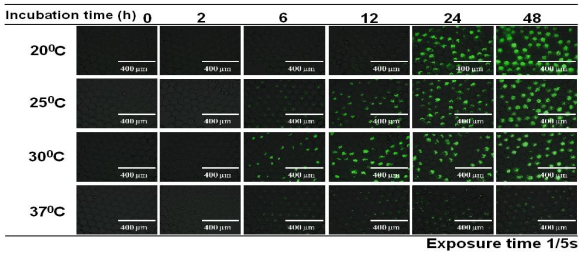

Figure 5 : Fluorescence micrographs of EGFP expression in monodisperse droplets at different incubation temperatures within 48 hours

From the fluorescent intensity of *E. coli*-encapsulated droplets or gel particles, we have estimated the growth curve of *E. coli* in these compartmentalized media (figure 6).

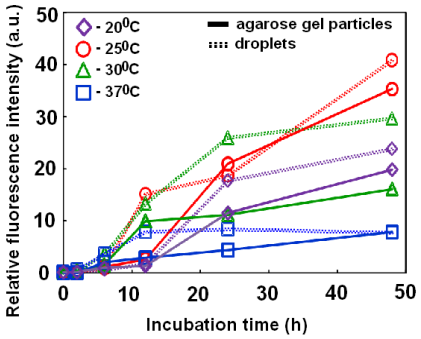

Figure 6 : The relative fluorescent intensity of the *E. coli*-encapsulated monodisperse droplets and agarose gel particles at different incubation time

Figure 7 presents the influence of IPTG factor on *E. coli* growth behaviour and EGFP and DsRed-monomer expression in batch culture.

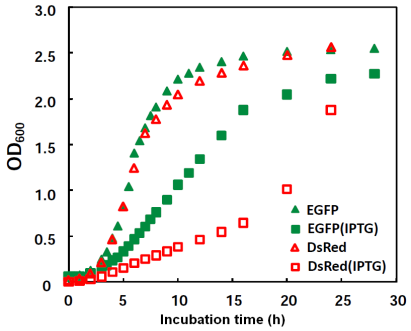

Figure 7 : The influence of IPTG factor on *E. coli* growth behaviour and EGFP and DsRed-monomer expression at 25 ^{0}C (in batch)

The IPTG-free *E. coli* growth curves showed similar aspect, the expression of DsRed-monomer and EGFP did not affect the cell proliferation. In the presence of IPTG the *E. coli* proliferation is slower in the first 5 hours, a logarithmic growth being noticed between 15 and 30 hours.

CONCLUSIONS

We have demonstrated the individual cell growth in water-in-oil emulsion droplets and the monitoring *in situ* protein expression in the compartmentalization system.