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Viability of the recombinant hepatitis B surface antigen expressed -*Hansenula polymorpha* yeast cells in Master Cell Bank

Win Aung,Jae Seung Kim ,**Nam Joong Le,*
***Yeon Hee Kim &* Khin Pyone Kyi*

*Hepatitis B Vaccine Plant
Department of Medical Research (LM)
**Research & Development Centre
Institute of Sciences and Technology
CJ Corporation, Republic of Korea

For production of the recombinant hepatitis B (HB) vaccine, freeze-dried form of Master Cell Bank (MCB) containing the HBsAg expressed-*Hansenula polymorpha* yeast cells , stored at 4ºC has been used as a starting material, followed by seed cultivation, fermentation and purification processes. In this study, freeze-dried form of MCB was reconstituted and viability of the HBsAg expressed-*Hansenula polymorpha* yeast cells was determined by observing cell morphology, growth pattern and presence of contamination during cultivation. It was found that there were actively growing viable yeast cells at different stages of cell divisions with specific characteristics. These cells also showed an increase in growth during cultivation with normal pattern of growth curve and were free from any contamination. Therefore, the recombinant hepatitis B surface antigen expressed -*Hansenula polymorpha* yeast cells in long-term storage at 4ºC in MCB were found to be viable, stable and safe satisfactorily thus being suitable for further processing recombinant HB vaccine production.

INTRODUCTION

For production of vaccine using recombinant DNA technology, a gene that codes for a specific product can be isolated and propagated by insertion into a suitable vector with the aid of highly specific restriction endonuclease and ligase enzymes. The vector can then be introduced into host organisms, and individual clones that carry the desired genes can be selected and propagated in mass culture to obtain gene expression under controlled conditions with efficient synthesis of the encoded product [1]. At the C J Pharmaceutical Corporation, Republic of Korea, the hepatitis B surface antigen (HBsAg) expressed-*Hansenula polymorpha* yeast cell was successfully developed using recombinant DNA technology in year 2000 for production of recombinant HB vaccine [2]. After cultivation of these cells with selective media, followed by mixing with skim milk solution, 0.5 ml of sterile cell media was aliquoted into individual containers. Then, lyophilization and sealing were done under sterile conditions. They were stored at 4ºC and considered as Master Cell Bank (MCB). In this study, the viability of MCB which was stored at 4ºC for about 4 years was determined with the aim to confirm the morphological stability and sterility of transformed yeast cells required for further production processes.

MATERIALS AND METHODS

The lyophilized form of MCB in a sterile glass ampoule, stored at 4ºC for about

4 years, a product of CJ Corporation, Republic of Korea was reconstituted with 1 ml of autoclaved distilled water. The suspension was further used for the following tests.

Growth pattern

One-hundred microliter of MCB suspension was inoculated into 50 ml of 0.7% Yeast Nitrogen Base (YNB) with 2% glucose media broth in 250 ml conical culture flask, followed by incubation at 37ºC in a shaking incubator (200 rpm) for 24 hours. The samples were taken from the above culture media every 4 hours for 24 hours and the optical density (i.e. cell growth) of each sample was measured in a spectrophotometer at 600 nm. The cell growth curve was determined by plotting the different OD against different times.

Morphological stability

The morphology and activity of the yeast cells in the culture samples were observed using an optical microscope at 1000 times magnification.

Contamination test

The presence of other microorganisms (bacteria or fungi) except the HBsAg-expressed yeast strain was checked in MCB strain as follows:

Culture plates for growth of different micro-organisms such as Brain Heart Infusion agar, Lactose agar, Nutrient agar, Sabouraud Dextrose agar and Tryptic Soy agar were prepared. Then, 100 µl of MCB suspension in different dilutions i.e. 10^{-2}, 10^{-4}, 10^{-6}, and 10^{-8} were inoculated to the above mentioned culture plates. These plates were kept at respective temperatures in an incubator for 72 hours and were checked every day for colony formation. After 3 days, the number, size and shape of colonies were observed. Among them, 10 colonies from each plate were randomly picked up and checked under an optical microscope for confirmation of shape of cells and colonies, and microbial species.

RESULTS

The growth pattern of MCB in culture broth is illustrated in Fig. 1. The yeast cells started to grow at 8 hours and growth rapidly increased at 16 hours and reached the peak level at 24 hours of cultivation.

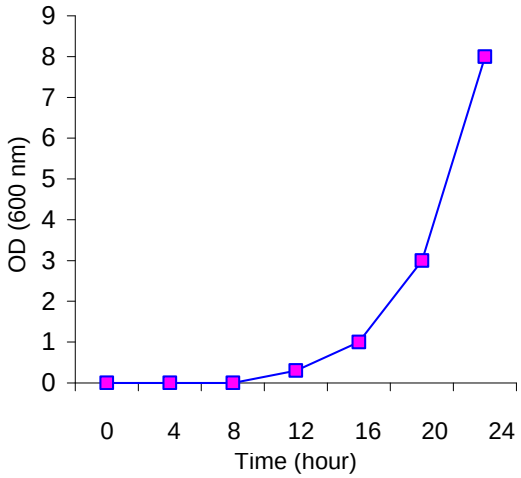

Fig.1. Growth pattern of the HBsAg expressed (MCB) during cultivation

The morphology and activity of these cells under the microscope are shown in Fig. 2. The specific appearance of *H. polymorpha* yeast cells was confirmed and actively growing cells with different stages of cell division were observed.

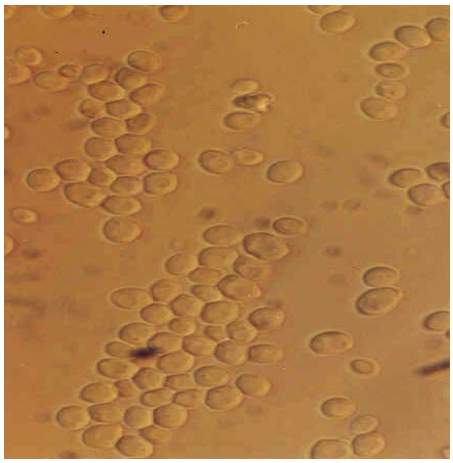

Fig. 2. Morphological observation of MCB by optical microscope

On daily checking of culture plates, there was no isolation of other microorganisms except *Hansenula polymorpha* cells which

showed their specific size and shape. In morphological observation under microscope, they showed specific appearance of *Hansenula polymorpha* cells only. It confirmed the identification of viable *Hansenula polymorpha* and free of contamination of other organisms of MCB strain during the growth period of 3 days culture (Table 1).

Table.1. Contamination test for microorganisms in different cultures

Types of culture media	Condition (Temp)	Microorganisms allowed	Microorganisms detected	
			H. polymorpha	Other microbes
Brain Heart Infusion agar	30 ºC	fastidious microorganisms fungi, yeasts	+	–
Lactose agar	37 ºC	coliform bacteria	+	–
Nutrient agar	37ºC	majority of the less fastidious microorganisms	+	–
Sabouraud Dextrose agar	30ºC	Yeasts, molds, acidic micro-organisms	+	–
Tryptic Soy agar	37ºC	Fastidious & nonfastidious microorganisms	+	–

DISCUSSION

Hepatitis B viral infection is a major health problem worldwide and caused by the HB virus .There is no cure for chronic HB infection, that is why prevention is so important. The HB vaccine is the best protection against HB infection [3]. In Myanmar, since the beginning of year 2004, the Hepatitis B Vaccine Plant, Department of Medical Research (LM), Ministry of Health has been producing recombinant HB vaccine and plasma derived HB vaccine as test run under the supervision of CJ / Samsung Corporation through the EDCF loan agreement from the government of the Republic of Korea. According to the contract, the plant will have a capacity of producing 5-million and 2-million pediatric doses of recombinant and plasma derived HB vaccines respectively after complete transfer of technology. For production of recombinant HB vaccine, the CJ corporation has provided a starting material i.e MCB containing the HBsAg expressed-*Hansenula polymorpha* yeast cells which have to further undergo fermentation and purification processes to produce the desired HBsAg protein for production of recombinant HB vaccine.

In this study, freeze-dried form of MCB stored at 4ºC for about 4 years was found to be viable and stable with normal growth pattern on cultivation. Specific morphological appearance and identification of yeast cells were also confirmed. Moreover, no growth of other microorganisms and absence of contamination were confirmed. Therefore, stored MCB containing the HBsAg expressed-*Hansenula polymorpha* cells was still viable with no notable difference from that in the pre-lyophilization stage. It could be concluded that both lyophilization procedure and long term storage of freeze-dried form of MCB at 4ºC do not affect the viability and sterility of the HBsAg expressed-*Hansenula polymorpha* cells. Therefore, the lyophilized form of MCB strain can be used effectively and safely in further processes of recombinant HB vaccine production in the HB Vaccine Plant.

REFERENCES

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