LIPOPOLYSACCHARIDE INDUCES THE PRODUCTION OF DIAGNOSTIC MONOCLONAL ANTIBODY BY HYBRIDOMA CELLS AGAINST CONGENITAL ADRENAL HYPERPLASIA

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Abstract
The purpose of this research is to screen and identify the potential inducers in maximizing the production of monoclonal antibody by hybridoma 192 cell line for Congenital Adrenal Hyperplasia diagnostic. There are nine inducers used in this research, namely lysozyme, aldolase, sodium butyrate, sodium phosphate, potassium phosphate, dimethyl sulfoxide, lipopolysaccharide, essential amino acids, and nonessential amino acids. Hybridoma 192 cell was cultured in 5% CO2 incubator at 37°C and >80% humidity in the medium with different concentrations of inducer agents. The inducers were added at the beginning of the culture and the samples were taken after 72 h of culture. The performance of these inducer agents was assessed based on the maximum monoclonal antibody titer achieved using Enzyme-linked Immunosorbent Assay. Lipopolysaccharide was found to increase the maximum monoclonal antibody titer when supplemented at 8 to 12 µg/mL. After optimization using one-factor central composite design at this range, the optimum point was determined to be 8 µg/mL. Verification experiments shows that lipopolysaccharide enhanced the average specific monoclonal antibody production rate by 56% relative to control. In conclusion, lipopolysaccharide at 8 µg/mL is able to increase the monoclonal antibody specific production of hybridoma 192 cell line.

Keywords: Lipopolysaccharide, Inducer agent, Monoclonal antibody, Hybridoma, Congenital adrenal hyperplasia.

1. Introduction
Adrenal glands of people with Congenital Adrenal Hyperplasia (CAH) are unable to produce enough amounts of cortisol and aldosterone hormones due to defective genes that regulate the production of enzymes which involve in adrenal steroidogenesis [1]. Thus, it impairs the synthesis of cortisol and aldosterone. The most common type of CAH is due to the deficiency of 21-hydroxylase, which
most common type of CAH is due to the deficiency of 21-hydroxylase, which accounts for 95% of the cases. This type of CAH can be detected by the presence of high levels of 17-hydroxyprogesterone (17-OHP) in the serum [2,3]. 21-Hydroxylase deficiencies can be divided into classic and nonclassic CAH. Classic CAH is usually found in the stage of infancy and early childhood. It can be considered as the most severe type of CAH. Early screening is a must because classic CAH is life threatening. Nonclassic CAH, on the other hand, manifests during or after puberty. Though it is not life threatening, nonclassic CAH can cause accelerated bone maturation and results in a reduced final height [4]. As the prevalence of classic CAH is very high at about 1:15,000 [5], a quick and reliable screening method for newborns is necessary.

Current newborns screening methods for CAH include radioimmunoassay, time-resolved fluorescence spectroscopy, tandem mass spectrometry, DNA analysis, gas chromatography tandem mass spectrometry, high-performance liquid chromatography tandem mass spectrometry, and enzyme-linked immunosorbent assay (ELISA) [4,6-8]. In hospitals, the most suitable assay to be used is ELISA method. It is sensitive, specific, fast, accurate, and cost effective. However, most of the current ELISA methods are based on the polyclonal antibody for diagnosis of CAH. Polyclonal antibody is not as specific as the monoclonal antibody (MAb), thus may give a higher frequency of false positive results. In view of this, Chong et al. [3] has developed an in-house mouse-mouse hybridoma (hybridoma 192), which produces the monoclonal antibody that recognizes 17-OHP with high specificity and sensitivity [3,9]. Even though there is one commercial monoclonal antibody recognizing 17-OHP available in the market, the cost per test kit is rather high. Thus, Chua [9] has attempted to optimize the medium used to culture hybridoma 192 and successfully reduced the medium cost up to 65%.

In order to produce antibodies specific for human antigens, the cell from mouse or rat is always the production source [10]. Similarly, hybridoma 192 cell line that produces MAb recognizing 17-OHP is also originated from a mouse. However, mouse-mouse hybridomas usually produce a low level of MAbs [11]. This means that, to produce sufficient amount of MAb for clinical trials, or commercial use, it will require the handling of multiple and/or huge volume cultures, which is not feasible in terms of time and cost. Hence, a technique to improve the production shall be sought. Enhancing the immunoglobulin or MAb production can be done in
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many ways, for example, by modification of media supplements in the culture media [12], temperature control [13], or a combination of several factors including cell density and the duration of the culture [14]. Among these three ways of enhancing MAb productivity, modification of media supplements is the most suitable method for lab scale production. Temperature control and cell density control, on the other hand, are suitable for the production in a reactor. One type of culture media modifications is by adding inducer into the cell culture either at the beginning or at any appropriate time during the culture. A few researchers have shown that inducers such as amino acids [15,16], sodium phosphate [11], sodium butyrate [17], potassium phosphate [11], dimethyl sulfoxide (DMSO) [18-19], lipopolysaccharide (LPS) [20,21], aldolase [22], and lysozyme [12,23] were effective in inducing the production of antibodies. Therefore, the purpose of this research is to identify the most effective inducer that potentially maximizes the production of monoclonal antibody by hybridoma 192 cell line.

2. Materials and Methods

2.1. Materials and cell line

Mouse hybridoma 192 cell line was a gift from Universiti Malaya. All chemicals were obtained from Sigma-Aldrich, USA, except fetal bovine serum that was bought from Gibco, Life Technologies, USA. ABTS [2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)] buffer was purchased from Roche Diagnostics, Switzerland.

2.2. Screening of potential inducer agent

The nine selected components [essential amino acids (EAA), non-essential amino acids (NEAA), sodium butyrate, sodium phosphate, potassium phosphate, aldolase, lysozyme, DMSO, and LPS] were screened for their effectiveness in inducing the production of MAb. The concentration range studied is depicted in Table 1. In this study, cells inoculum with a viability of greater than 90% was centrifuged and inoculated at the cell density of 2 × 10^5 cells/mL into the test medium. The test medium was consisted of high glucose Dulbecco’s Modified Eagle’s Medium (DMEM), fortified with 0.4% fetal bovine serum, 4 mM of L-glutamine, 312 µM of ferric citrate, 4.5 µM of zinc sulfate, and 17 nM of sodium selenite [9] and added with the specified amount of inducers tested. The experiments were run in the 6-well plate (TPP, Switzerland) in triplicate. A control experiment (medium without inducer) was run together for each inducer. All cultures were kept in a humidified CO₂ incubator (Shel Lab, Cornelius, USA) with 5% of CO₂ at 37°C and greater than 80% humidity. Samples were harvested at 72 h, spun (500g, 10 min; Eppendorf Centrifuge 5415 R, Germany) and the supernatant was kept at -20°C until further analysis. The MAb titer attained at the time of harvest was monitored as the only response.

2.3. Optimization and validation of effective inducer

Based on the screening experiments with nine inducers (see Section 3.1), only LPS was selected for the further detailed study. The optimum concentration of LPS was determined using the response surface methodology approach. One factor central composite experimental design was chosen and the experiment was designed using Design Expert version 7.0. The LPS concentration range chosen for optimization was based on the results of screening (8-12 µg/mL). There was a
total of seven runs. The MAb titer attained at 72 h and the average specific MAb production rate were the responses monitored throughout the experiment. Data obtained from duplicate independent runs were used for the analysis (average of six measurements). Similar to the screening process, $2 \times 10^5$ cells/mL were spun and resuspended in the test medium with different concentrations of LPS. T-25 flasks were used with a total volume of 10 mL. All cultures were kept in humidified CO$_2$ incubator with 5% of CO$_2$ at 37°C. The sampling was done at 24 h intervals. Cell count was done immediately after the sampling and the supernatant was kept at −20°C until further analysis. The verification run was analogous to the optimization process experiments. LPS concentration used was according to the optimum point suggested by the software. The experiment was run in duplicate and the sampling was done at 24 h intervals for both cell density and MAb titer analysis. Data reported were the average of six measurements.

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration range studied</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Butyrate</td>
<td>0-5 mM</td>
</tr>
<tr>
<td>Sodium Phosphate</td>
<td>0-25 mM</td>
</tr>
<tr>
<td>Potassium Phosphate</td>
<td>0-25 mM</td>
</tr>
<tr>
<td>Aldolase</td>
<td>300-550 µg/ml</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>0-500 µg/ml</td>
</tr>
<tr>
<td>LPS</td>
<td>0-25 µg/ml</td>
</tr>
<tr>
<td>Essential Amino Acids</td>
<td>0-5% (v/v)</td>
</tr>
<tr>
<td>Non Essential Amino Acids</td>
<td>0-5% (v/v)</td>
</tr>
<tr>
<td>DMSO</td>
<td>0-5% (v/v)</td>
</tr>
</tbody>
</table>

2.4. Cell density determination

Cell count was done by using hemocytometer. Trypan blue dye was used to differentiate between dead cells and living cells. Percentage viability was determined from the ratio of living cells to total cells calculated.

2.5. MAb titer determination

The concentration of total MAb was determined using the standard sandwich enzyme linked immunosorbent assay (ELISA) as described by Chong et al. [3]. All analyses were run in triplicate. Briefly, 1 µg/mL of anti-mouse IgG from goat in 0.1 M of coating buffer at pH 9.6 was coated on all wells of a 96-well plate (maxisorp; Nunc A/S, Roskilde, Denmark) and incubated for 2 h at 37°C. The plate was then manually washed for several times with the washing buffer and tapped to dry. A blocking solution containing 1% w/v of bovine serum albumin and 0.1% v/v of Tween 20 was used to treat the plate before it was incubated overnight at 4°C. The plate was then washed with the washing buffer as explained above. Serially diluted mouse IgG was used as the standard. The standard and the diluted samples were added to separate wells and incubated at 37°C for 1.5 h. The washing process was then repeated. Next, diluted anti-mouse IgG peroxidase from goat was added to each well and incubated for 1.5 h at 32°C. The washing process was repeated. ABTS [2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)] buffer prepared in accordance with the instructions accompanying the product was added to the wells. The plate was left to stand for 25 min at 35°C for optimum color development. The plate was then read at 405 nm using a microplate reader (Columbus, Tecan, Switzerland).
3. Results and Discussion

3.1. Screening of inducing agent

In this experiment, nine inducing agents were screened to determine their effect on MAb production by mouse hybridoma 192 cell lines. They were selected based on the positive inducing effects reported in the literature. A different range was selected for each inducer based on the reported optimum concentration in the previous studies. In order to determine the effect of each inducer in this study, the other factors such as temperature, pH, or cell density were fixed. Hybridoma 192 was cultured in the complete DMEM as described in the methodology section without any addition of inducer for each run as a control. The MAb titer reported was the average of triplicate runs, and the results plotted were the average value of six measurements.

Figures 1(a) to (c) show that the presence of EAA, NEAA, and sodium butyrate in the medium did not produce any significant inducing effect on MAb production. In addition, the addition of aldolase, potassium phosphate, and lysozyme reduced the MAb production, as shown in Figs. 1(d) to (f). At the time of harvesting for the medium which contained lysozyme, the MAb titer was about 60% of that in the control [Fig. 1(f)]. On the other hand, the addition of aldolase or potassium phosphate into the medium reduced the MAb production to only about 30% of their respective control. The results obtained in this study are in contrast to the earlier findings in relation to lysozyme [12, 23], amino acids [15, 16], sodium butyrate [17], aldolase [22], and potassium phosphate [11].

By feeding amino acids solution, Gong et al. [16] were able to increase the MAb production of JJ-1 cells from 116 mg/L to 707 mg/L. A similar observation was reported by Ducommun et al. [15], where balanced feeding of amino acids and vitamins increased the specific IgA production of Zac 3 cells from 0.60 pg/cell.h to 1.10 pg/cell.h. However, the addition of both essential and nonessential amino acids in this study did not enhance the MAb production. A possible reason is that the other researchers specially formulated the amino acids solution and fed according to the consumption requirements of the cells, but in this study, the general essential or nonessential amino acids solution (obtained from Gibco) was added at the beginning of the culture without considering the consumption by the cells. Franĕk et al. [24] reported that the addition of free amino acids [glycine (Gly)/lysine (Lys) mixture] did not enhance MAb production, but the addition of Gly-Lys-Gly tripeptide improved the production. Thus, the enhancing effect of amino acids is specific to the cells and highly dependent on the type of amino acids being fed and the time of feeding. Amino acids consumption profiles were not scrutinized in this study, hence, specially formulated amino acids feeding were not tested. Moreover, the requirement of specially formulated amino acids feeding has violated the intention of maximizing yield with minimal cost and time.

Kantardjieff et al. [17] treated the Chinese hamster ovary (CHO) cells with 2 mM of sodium butyrate at 37°C and then changed the culture temperature to 33°C during log phase. The antibody secreted has raised up to 520 µg/mL after 170 hours of fermentation. Sodium butyrate is able to induce antibody production because of its ability to modulate the transcription of gene by inhibiting histone deacetylase and thus induces histone hyperacetylation [25]. However, antibody production for this experiment did not increase with the addition of sodium
butyrate. This may be because of the culture time that was too short and the temperature was higher at 37°C. Hence, the production of MAb was low as the condition was not ideal for triggering the MAb production by the cell. Another possible reason might be due to different cell line that contributes to the dissimilar responses to sodium butyrate addition.

Fig. 1. MAb titer at 72 h in media supplemented with various concentrations of (a) essential amino acids, (b) non-essential amino acids, (c) sodium butyrate, (d) potassium phosphate, (e) aldolase, and (f) lysozyme.

Aldolase was found to give significant effect toward HB4C5 cell and helped to enhance IgM four times better than IgG [22]. Suguhara et al. [22] proved that at a concentration of more than 1000 µg/mL, aldolase enhanced the concentration of IgM. This was in contrary with the results obtained in this study. Figure 1(e) shows that the production of MAb was the highest in the control culture. Hence, aldolase did not enhance the MAb production in this study. This may be due to different in cell line used in this study as compared to Suguhara and co-workers.
On top of this, aldolase might only enhance the production of IgM but not too significant for inducing the IgG production, the monoclonal antibody type produced in our study.

The optimum concentration of potassium phosphate studied by Sato et al. [11] was 15 mM in the culture of hybridoma HB4C5. Sato and co-workers [11] used a cell density of $5 \times 10^5$ cells/mL, and the production of MAb was stimulated more than 2-fold. Potassium phosphate was more effective in enhancing the production when seeded with higher cell density. The results obtained in this study was not in line with the findings of Sato et al. [11] in which the cell density used was $2.0 \times 10^5$ cells/mL and the result shows that at 15 mM concentration, the MAb production was less than 50% of the production of MAb by control cells. The plausible explanation is that the addition of potassium phosphate has disturbed the Na$^+/K^+$ balance which may have some complex effects on cellular metabolism [26]. This, in turn, affects the antibody production as cellular energy and nutrients have been concentrated to maintain the normal function of the cells. The effect is especially obvious at low cell density. Another possibility is the instability of MAb in the presence of potassium phosphate, which changes the conformational structure and makes it inactive [27]. Murakami et al. [23] found that 380 µg/mL of lysozyme stimulated the production of IgM up to 13-fold by HB4C5 hybridoma cells. The finding in this research, however, was that lysozyme at the range of 0-500 µg/mL had no stimulatory effect on MAb production by hybridoma 192 cells. Therefore, the effect of lysozyme might be cell specific.

Ling et al. [19] reported that the productivity of their hybridoma culture increased 2-fold following the addition of 0.2% (v/v) of DMSO at the time of the maximal viable cell densities. A 3-fold increase in the productivity of hepatitis B surface antigen by CHO cells, when supplemented with 1.5% (v/v) of DMSO at 96 h of culture, has been reported [18]. The present work studied the stimulating effect of DMSO in the concentration range of 0-5% (v/v). Two independent sets of experiments were run. The first duplicate set of experiments was run without spinning the cells prior to resuspending into the fresh test medium that contained 0-2.5% (v/v) of DMSO. The results are depicted in Fig. 2(a). Due to the spent medium carry over in the inoculum, the real concentration of DMSO in the study was less than the values shown in Fig. 2(a). When the error was realized, the experiment was repeated with a broader concentration range of DMSO and the cells were spun prior to resuspending. The results of the repeated experiment were shown in Fig. 2(b). Figures 2(a) and (b) display a totally different behavior. There is a significant increase in MAb titer at the time of harvesting (i.e., 72 h) for the experiment with a low concentration of DMSO and spent medium carry over [Fig. 2(a)]. In addition, MAb productivity was also found to increase in this experiment. Cells tend to lyse and growth is suppressed when DMSO is added to the culture as previously reported by others [18-19]. Lysed cells release the antibody that may not have fully formed into the culture [28]. However, in Fig. 2(b) where the cells were spun and the concentration range of DMSO was broader, the MAb titer declined with increased concentration of DMSO. In fact, the MAb titer at the time of harvesting was very much lower in DMSO-containing media as compared to the control. Thus, the response of cells towards DMSO was inconsistent. This was in agreement with the findings of Allen et al. [29], who found that the effect of DMSO on the cells could only be seen at the concentration of above 1% (v/v), but this effect was not consistent.
Fig. 2. MAb titer at 72 h in media supplemented with various concentrations of DMSO (%v/v); (a) no spinning and (b) with spinning.

Figure 3(a) shows that the production of IgG is the highest when the cell was treated with 25 mM of sodium phosphate. Compared to MAb produced in control media, the production increased about 2-fold. Sato et al. [11] identified 15 mM as the optimum concentration for MAb production by human-human hybridoma HB4C5. However, for hybridoma 192 cells, the production of MAb fluctuated when the concentration of sodium phosphate was increased as depicted in Fig. 3(a). Thus, the results were considered not valid.

Fig. 3. MAb titer at 72 h in media supplemented with various concentrations of (a) sodium phosphate and (b) LPS.

As the stimulatory effect of DMSO and sodium phosphate could not be assured, while amino acids and sodium butyrate produced no benefit along with lysozyme, and aldolase and potassium phosphate had a negative effect, all these inducers were excluded from further study.

Figure 3(b) shows the MAb titer attained at 72 h with the various concentrations of LPS in the medium. LPS concentration of 5-15 µg/mL shows a significant increase in MAb titer as compared to the control (without LPS). The stimulation effect of LPS seemed to be quite promising because the preliminary study using LPS at a similar concentration range also enhanced the MAb titer as compared to control. As a result, LPS was further optimized within the concentration range of 5-15 µg/mL.
3.2. Determination of optimum LPS concentration

Table 2 illustrates the results of analysis of variance (ANOVA) for the optimization using central composite design. It reveals that the increasing LPS concentration will have a negative effect on the MAb titer and specific MAb production rate. In other words, the increase in LPS concentration would decrease the MAb titer attained and reduce the specific MAb production rate. The significant term in both models is the quadratic term of LPS concentration ($A^2$). LPS concentration (A) is also significant in the average specific MAb production rate model.

Table 2. Analysis of variance table for maximum MAb titer and average specific MAb production rate.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Maximum MAb titer</th>
<th>Average specific MAb production rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Effect</td>
<td>F-ratio</td>
</tr>
<tr>
<td>A - LPS concentration</td>
<td>-0.38</td>
<td>2.32</td>
</tr>
<tr>
<td>$A^2$</td>
<td>1.69</td>
<td>12.36</td>
</tr>
<tr>
<td>Lack of Fit</td>
<td>-</td>
<td>0.49</td>
</tr>
</tbody>
</table>

* Significant at p < 0.05

The experimental results obtained were fitted to the quadratic model equations in coded form as follows:

Maximum MAb titer $= 6.25 - 0.38A + 1.69A^2$ (1)

Average Specific MAb production rate $= 0.11 - 0.008A + 0.048A^2$ (2)

The goodness of fit ($R^2$) and the goodness of prediction ($Q^2$) are two parameters used to evaluate the models. $R^2$ for average specific MAb production rate in this study is 0.9367, which is greater than 0.75, revealing that the model fits well. However, the model for the maximum MAb titer could only be considered as a fair model because the $R^2$ is 0.6788, which is less than 0.75. As for the prediction power, the $Q^2$ value for maximum MAb titer is 0.3215, while the $Q^2$ value for average specific MAb production rate is 0.8791. The value well above 0.75 is considered as having a good prediction power, while it is considered fair if the $Q^2$ value is between 0.25 and 0.75. Therefore, the models are expected to be able to predict the optimum values reasonably well. Based on the models, the optimum point is predicted to be at an LPS concentration of 8 µg/mL. At this optimum point, the maximum MAb titer and the specific MAb production rate are predicted to be 8.3 µg/mL and 0.16 pg/cell.h, respectively. This optimum point was verified by running a duplicate experiment at the predicted optimal LPS concentration of 8 µg/mL. A control was also run in duplicate for comparison.

3.3. Verification of the optimum point

Table 3 compares the actual experimental values of the maximum MAb titer and the average specific MAb production rate with the predicted values from the model and the actual values of control run. It can be seen that the actual maximum MAb titer obtained is lower than the predicted and control values. This is obviously in contrast to the results obtained during screening and optimization processes. The reason
behind this is not clear and needs further investigation. Yet, Table 3 also reveals that the actual average specific MAb production rate obtained is very much higher than the predicted and control values. The increment of productivity is up to 56% (or 1.6-fold) as compared to the control. The addition of LPS into the culture medium has a suppression effect on growth resembling that of DMSO [18-20]. However, LPS did not lyse the cells. Through microscopy observation during cell counting, LPS only suppressed the growth of cells for the first 24 to 48 hours (Fig. 4). Cell grew as usual afterward. This suppression effect reduced the cell number at the time of harvesting. The average specific MAb production rate was calculated based on the maximum MAb titer achieved using the viable cell density attained at the instance of maximum MAb titer, which led to the increase in the value of the average specific MAb production rate.

**Table 3. Comparison of the predicted, actual, and control values for maximum MAb titer and average specific MAb production rate in the verification experiments.**

<table>
<thead>
<tr>
<th>Type of response</th>
<th>Maximum MAb titer (µg/mL)</th>
<th>Average specific MAb production rate (pg/cell.h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predicted</td>
<td>8.3</td>
<td>0.16</td>
</tr>
<tr>
<td>Actual*</td>
<td>6.2 ± 0.3</td>
<td>0.25 ± 0.02</td>
</tr>
<tr>
<td>Control*</td>
<td>7.3 ± 0.8</td>
<td>0.16 ± 0.01</td>
</tr>
</tbody>
</table>

* Value at t = 72 hr

![Fig. 4. Growth profiles and MAb titer during verification runs. (Solid line = growth profile, dash line = MAb titer; ● = optimum point, ■ = control).](image)

Even though LPS would suppress growth, its influence was inconsistent. The suppression effect was not observed in the screening experiment, but was obviously seen in the optimization experiment and also in the verification run. This may be the reason that maximum MAb titers obtained were higher in the screening experiment, but not in the optimization experiment or the verification run. At the time of harvesting (i.e., 72 h), cells supplemented with LPS had just returned to their normal growth rate and the cell number was lower than the control culture.
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As a result, the MAb titer produced was also lower than the control culture that contained a higher cell number. If the culture was harvested at a later time, the maximum MAb titer achieved might have improved. LPS is believed to be able to stimulate the immune response of mammalian cells but suppresses growth [20,21]. Thus, this could be a plausible explanation for the enhancement of MAb production rate in this case.

Martín-López et al. [20] found that the addition of 5 µg/mL of LPS to the culture of 55-6 murine B cell hybridoma increased the MAb production rate by 2.5-fold as compared to the control. In this study, the LPS concentration used was 8 µg/mL, which was 1.5-fold greater than that used by Martín-López et al. [20]. Nevertheless, the enhancement of MAb production was only 1.6-fold. Different cells may differ in their responses to the LPS at different concentrations [21]. For example, Oliver et al. [21] observed that the ability of marginal zone B cells and follicular B cells to proliferate and differentiate into plasma cells depended on the LPS concentration. Furthermore, the difference in response to the LPS may also due to the medium used. Martín-López et al. [20] and Oliver et al. [21] used RPMI 1640 supplemented with 10% serum, while DMEM supplemented with a minimal percentage of serum (0.4%) was used in this study. It is known that the undefined components in serum which include growth factor and antioxidant have the function of protecting the cells and promoting growth and production. Thus, this may be the reason for higher survival and production rates of the previous studies even though they were using lower cell density as compared to this study.

4. Conclusions

LPS at a concentration of 8 µg/mL was found to be suitable for stimulating the MAb production of hybridoma 192. The verification process confirmed that LPS enhanced the average specific MAb production rate by 1.6-fold relative to control, but not the maximum MAb titer. Even though an effective inducer was identified, but the level of enhancement of the average specific MAb production rate could not compensate for the high cost of the inducer. Thus, it is recommended to study a few other factors such as the effect of temperature or the time of addition in order to maximize the MAb production and counterbalance the high cost of inducer.

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