



Cloning and expression of hyaluronan synthase (*hasA*) in recombinant *Escherichia coli* BL21 and its hyaluronic acid production in shake flask culture

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ABSTRACT

Aims: Hyaluronic acid (HA) is a high molecular weight polymer and a major component of mucoid capsule in bacteria and extracellular matrix (ECM) of vertebrate tissue. Due to its unique characteristics, HA is used extensively in medical and cosmetic field. However, because of the exotoxins production from animal tissues extraction and *Streptococcus zooepidemicus*, HA production by recombinant microorganisms has gained interest. The present study was aimed at cloning of *hasA* gene in *Escherichia coli* and optimization of the medium components for HA production.

Methodology and results: A fragment of an approximate size of 1.5 kb that encodes the hyaluronan synthase (*hasA*) gene from *S. zooepidemicus* ATCC 39920 was amplified by PCR using *hasA*-specific primers. The *hasA* gene was ligated into the bacterial expression vector pLbADH and transformed into the expression host, *Escherichia coli* BL21 strain. Then, genetically engineered *E. coli* strain BL21 was used for the production of HA by fermentation using different glucose concentration (10-50 g/L) and different IPTG concentration (0.1, 0.5 and 1.0 mM) in shake flask culture. Amongst varying glucose concentrations, results showed that 50 g/L glucose with nutrient rich media containing nitrogen source was able to produce the highest HA concentration (0.115 ± 0.002 g/L). With addition of 1.0 mM IPTG, HA production reached a peak 0.532 ± 0.026 g/L which is around fivefold higher compared to without IPTG.

Conclusion, significance and impact of study: The *hasA* gene was cloned from *S. zooepidemicus* and successfully expressed in recombinant *E. coli* BL21 cells. This low molecular weight HA is gaining more importance in medical and cosmetic application due to possess pronounced free radical scavenging and antioxidant activities.

Keywords: Recombinant *E. coli* BL21, hyaluronic acid, hyaluronan synthase, IPTG

INTRODUCTION

Hyaluronic acid (or hyaluronan or HA) is a polysaccharide that belongs to glycosaminoglycan family and consists of D-glucuronic acid (GlcUA) and N-acetylglucosamine (GlcNAc), linked alternately by β-1,3 and β-1,4 glycosidic bond. HA is a high molecular weight linear polysaccharide with the molecular weight ranging from 10⁴ to 10⁷ Da (Liu *et al.*, 2011). HA is abundantly present in the umbilical cord, synovial fluid and vitreous humor (eye). Due to its biocompatibility, viscoelasticity and hygroscopicity, HA is extensively used for the treatment of ulcer and burn, in ophthalmology, rheumatology and dermatology (Chong *et al.*, 2005). In conjunction with the properties of HA, it is also widely used in cosmetic products, medicine and specialty foods. According to Widner *et al.* (2005), the market value of HA was estimated to be over a billion dollars. The global hyaluronic acid market size is

expected to reach USD 15.25 billion by year 2026 according to a new report by Grand View Research, Inc (Grand View Research, 2019).

Previously, HA was extracted from rooster comb. According to a study conducted by Rosa *et al.* (2012), they concluded that rooster comb was composed of about 90% HA, making it suitable for extraction. However, use of animal-derived biochemical can be risky due to zoonosis and unpremeditated infection transfer. Hence, currently, HA production from microbial fermentation is the commonly used method to meet the demands of the market. Since the 1980s, HA was produced by fermentation of group C Streptococci (Yamada and Kawasaki, 2005). However, these microorganisms are potential human pathogens, thus making HA purification costly. Genetic engineering of microorganisms for HA synthesis has allowed HA production from non-pathogenic microorganisms. Once the genes that encode

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for HA biosynthesis pathway were determined, a number of bacteria (*Bacillus*, *Agrobacterium*, *E. coli* and *Lactococcus*) were genetically modified to express the genes and produce HA. Most of the recombinant hosts used for HA production lacks the hyaluronan synthase (*hasA*) gene. For bacteria to synthesise HA capsule they must contain the *hasA* gene. A study conducted by Kumari and Weigel (1997) suggested that incorporation of *hasA* gene is sufficient for HA biosynthesis when the bacteria is already producing GlcUA and GlcNAc. HA production is commonly observed in Gram-positive bacteria, but by genetic engineering of Gram negative bacteria such as *E. coli*, synthesis of HA has been possible and they can be used as potential host candidate for industrial HA production (Yamada and Kawasaki, 2005). Numerous findings have done using glucose as a sole carbon source for the production of HA with concentrations such as 10 g/L of glucose producing 1.8 g/L of HA by *S. zooepidemicus* ATCC 35246 (Chong *et al.*, 2005), 40 g/L of glucose, producing 0.3-0.4 g/L of HA by recombinant *Corynebacterium glutamicum* ATCC 13032 pJH174.1 (Hmar *et al.*, 2014) and 3.8 g/L of HA is produced by recombinant *E. coli* JM109 using 45 g/L of glucose (Mao *et al.*, 2009). However, there is less study showed the molecular weight of HA using recombinant strains.

Although *E. coli* is a Gram-negative bacterium with the cell wall structure composed of lipopolysaccharide, peptidoglycan and lipoprotein, its metabolic pathway for HA synthesis is very similar to that of *Streptococcus* spp. Since the biosynthesis pathway of HA is partially similar to cell wall biosynthesis (Figure 1), the heterologous expression of *hasA* gene alone is sufficient to induce production of hyaluronic acid in these organisms. The effort of using recombinant systems to produce HA started when the *hasA* gene encoding for the enzyme that polymerizes UDP-GlcNAc and UDP-GlcUA into hyaluronan was isolated from *Streptococcus pyogenes* (DeAngelis, 1999). The carbon source essentially provides the necessary energy for both cell growth and exopolysaccharides production (Lai *et al.*, 2011). In addition to that, there is stiff competition for metabolites between HA synthesis and cellular growth due to glucose-6-phosphate and fructose-6-phosphate being consumed in the pentose phosphate pathway and glycolysis respectively (Mao *et al.*, 2009). Glucose is the most common carbon source for the production of HA since it allows for high productivities and production. There is limited study on the biosynthetic capabilities of recombinant *E. coli* BL21 for the production of HA. Hence, the aim of this research study was to clone and express *hasA* gene into *E. coli* BL21 and use this recombinant *E. coli* strain BL21 as host in order to explore the biosynthetic capabilities for HA production (concentration and molecular weight) in batch fermentation using shake flask culture.

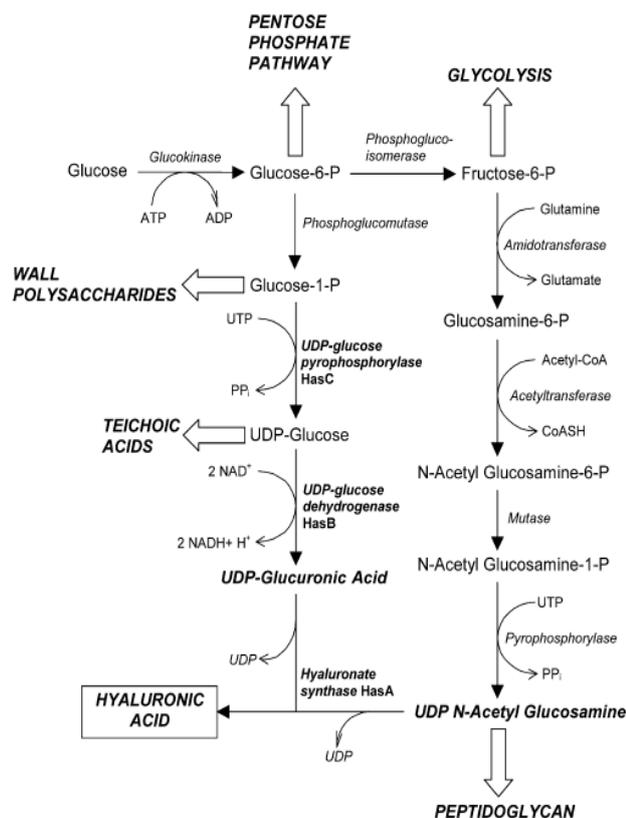


Figure 1: Biosynthetic pathway for the production of HA in *Streptococci* (Chong *et al.* 2005).

MATERIALS AND METHODS

Bacteria strains and plasmid

Streptococcus equi sub sp. *zooepidemicus* (American Type Culture Collection® 39920™) was used and stored at -80 °C in 50% (v/v) glycerol stock. *E. coli* BL21 were used as the recombinant host cell and plasmid pLbADH was used as bacterial expression vector.

Isolation of *hasA* gene using PCR amplification from *S. zooepidemicus*

The *hasA* gene was isolated from *S. zooepidemicus* and amplified by PCR using the designed forward (5' GGG GAA TTC ATG AGA ACA TTA AAA AAC CTC 3') and reverse (5'GCC AAG CTT ATT ATA ATA ATT TTT TAC GTG TTC CCC 3') primers, which named HasA_01F and HasA_01R, respectively. With the bacteria *S. zooepidemicus* used as the template, polymerase chain reaction (PCR) was performed with KOD Hot Start DNA polymerase in a thermal cycler using the following program: 1 cycle at 95 °C for 3 min, 30 cycles of 95 °C for 15 sec, 60 °C for 30 sec, 72 °C for 90 sec and 1 cycle of 72 °C for 5 min. PCR product was analyzed on 1% (w/v) agarose gel and viewed using gel documentation system.

Cloning and transformation of *hasA* gene

The PCR product and the plasmid pLbADH were digested separately with FastDigest enzyme EcoRI and HindIII (New England Biolabs, United States) in an incubator at 37 °C for 1 h. The enzymes were then heat inactivated at 80 °C. Both digestion products were ligated together using T4 DNA ligase enzyme (Fermentas, United States) at 22 °C for overnight. The ligation product was then used for transformation. The ligation product was transformed into *E. coli* BL21 competent cells using heat shock method. The transformed *E. coli* BL21 culture was spread onto a Luria-Bertani (LB) agar plate containing 100 mg/ml ampicillin. All the colonies grown on the LB agar plates were collected and screened with PCR using the following program: 1 cycle at 95 °C for 3 min, 30 cycles of 95 °C for 15 sec, 60 °C for 30 sec, 72 °C for 90 sec and 1 cycle of 72 °C for 5 min. HasA_01F and HasA_01R were used as primer in selection of positive transformant. PCR products were analysed with 1% agarose gel electrophoresis.

Medium and fermentation

Recombinant *E. coli* was grown at 37 °C with agitation for 5 h in the LB broth (as an inoculum) that supplemented with 100 mg/mL of ampicillin. The fermentation medium contained two different types of components; one is LB and glucose (10-50 g/L) only; one is nutrient rich medium which consisted of (in g/L) glucose 50, tryptone 15, yeast extract 5, KH₂PO₄ 2, K₂HPO₄ 2, MgSO₄·7H₂O 0.5. Fermentations were carried out in triplicates and the mean value of each experiment was obtained. To investigate the effect of glucose concentrations (10–50 g/L) for HA biosynthesis by the recombinant *E. coli* strain, experiments were carried out using a 500 mL erlenmeyer flask. The flask cultures were incubated in rotary shaker at 200 rev/min with different glucose concentrations. To initiate the fermentation, 1 loop of colony culture was transferred to 50 mL of LB inoculum medium for 5 h at pH 7 and 10% (v/v) of inoculum was inoculated into the 500 mL shake flask containing 180 mL of the production medium and run for another 7 hours at initial pH 7, 37 °C. Four different isopropyl β-D-1-thiogalactopyranoside (IPTG) concentrations (0, 0.1, 0.5 and 1.0 mM) were investigated in the optimized medium and incubated at 37 °C, 200 rev/min and with initial pH 7. IPTG was added to induce the expression of *hasA* genes when OD600 reached about 0.6.

Analytical methods

All samples were withdrawn at regular time intervals for analysis of cell, HA and glucose concentration. Cell growth was observed by measuring the optical density of the culture broth at 600 nm using a spectrophotometer. Correlation between the dry cell weight (DCW) and OD was estimated from several batch experiments using the equation: DCW (g/L) = 2.2511 × OD.

The supernatants were used for HA and reducing sugar determination. After the removal of the cell pellet for cell

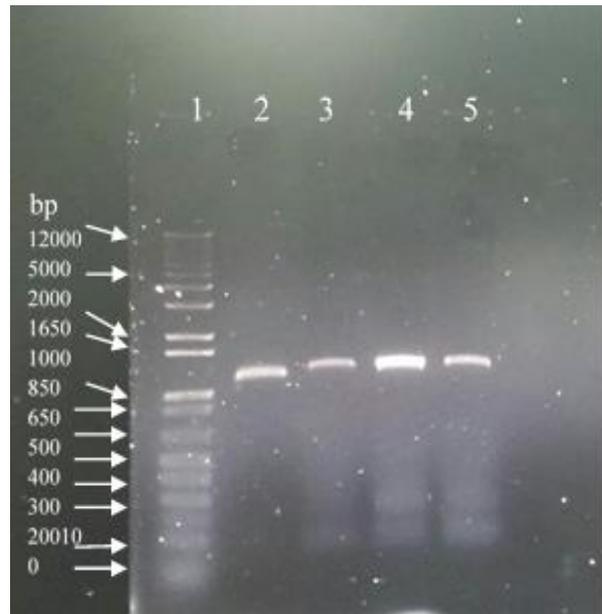


Figure 2A: Screening for positive transformant. Lane 1: 1kb plus DNA ladder; Lane 2: positive control of *hasA* gene; lane 3-5: Samples 1-3.

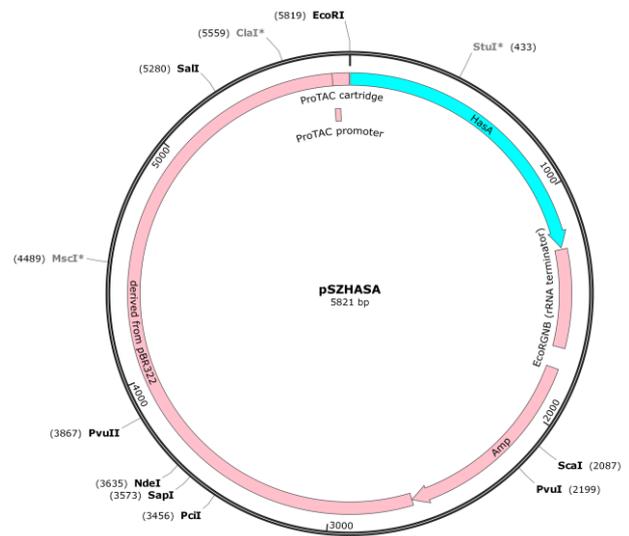


Figure 2B: Vector map of pSZHASA.

growth determination, two volumes of ethanol were added to one volume of the supernatant in a 15 mL centrifuge tube and the solution was refrigerated at 4 °C for 1 h to precipitate HA. The precipitate was re-dissolved in 1 mL of 0.15 mol/L of NaCl solution and centrifuged at 3,000 × g for 20 min; then was re-dissolved with a 2 to 3-fold volume of distilled water. The HA concentration was determined using the carbazole method (Bitter and Muir, 1962) and the optical density was measured at 530 nm. The HA concentration was calculated using a standard

curve prepared at different concentrations of HA standards (Sigma-Aldrich, Malaysia). For reducing sugar concentration, 3,5-dinitrosalicylic acid (DNS) method was used (Miller, 1959). The HA molecular weight was analyzed by following Lai *et al.* (2012) method.

RESULTS AND DISCUSSION

Cloning of *hasA* gene from *S. zooepidemicus*

The *hasA* gene from *S. zooepidemicus* was successfully amplified by PCR and cloned into the plasmid pLbADH as shown in Figure 2A. In this study, the original *hasA* gene from *S. zooepidemicus* was used to produce HA in recombinant *E. coli* with no change in Codon Adaptation index (CAI). The expression of *hasA* gene is driven by the TAC promoter with the vector of pSzHasA as shown in Figure 2B. There was several plasmid constructed containing different promoters to transformed *hasA* gene into other recombinant hosts. Jeong *et al.* (2014) utilized two types of plasmid, pAO815 and pGAPZB which have an inducible promoter (AOX) and a constitutive promoter (GAP), respectively. They used the vector with constitutive promoters to accumulate both sugar precursors of HA in the cell, then activate the inducible promoter preceding the *hasA* gene, leading to an increased production and higher MW of HA. In this study, a strong *E. coli* TAC promoter was used to provide strong expression of *hasA* gene in *E. coli* BL21 host.

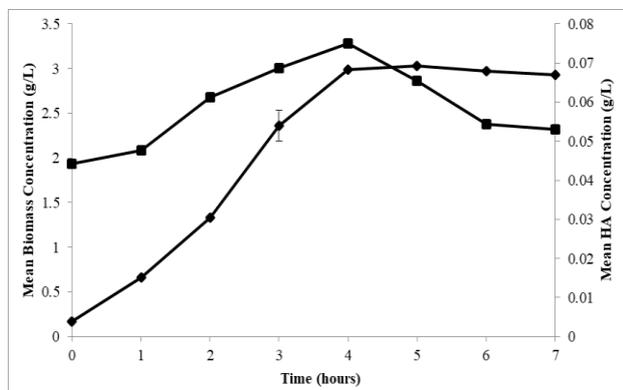


Figure 3: Graph of mean biomass and HA concentration (g/L) by recombinant *E. coli* BL21 in shake flask culture using 50 g/L glucose. All concentrations were represented as the mean value of triplicated results from independent fermentation. ■) HA (♦) Biomass

Effect of different glucose concentrations on HA production by recombinant *E. coli* BL21

Based on the kinetic parameter values (Table 1), highest biomass concentration (X_m) and highest HA concentration (P_m) was 3.031 ± 0.004 g/L and 0.074 ± 0.001 g/L, respectively, both achieved at 50 g/L glucose Concentration. However, lowest X_m and P_m was 2.161 ± 0.002 g/L and 0.018 ± 0.013 g/L respectively, both

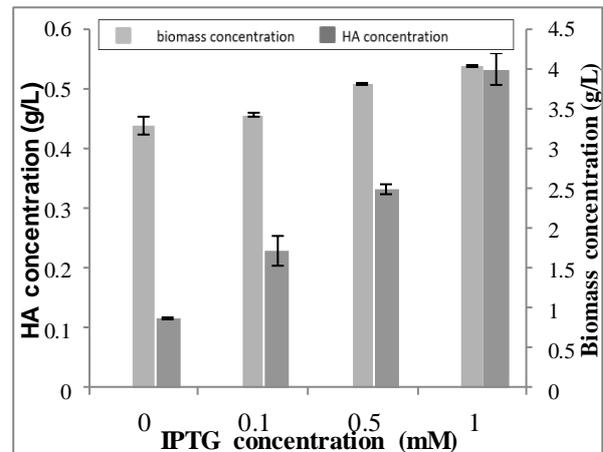


Figure 4: Comparison of HA concentration (g/L) using different IPTG concentration in 50 g/L of glucose concentration with nutrient rich media. All HA concentrations were represented as the mean value of triplicated results from independent fermentation.

achieved in control condition. This is in accordance to our expected results, as the bacteria requires carbon source for cell growth and HA production. In control, there was no glucose present, so there was insufficient precursor for HA production. However, as glucose concentration increased, the precursors, GlcUA and GlcNAc were made and HA concentration increased. According to a study conducted by Mashitah and Noor (2010), highest HA concentration of 0.589 g/L was achieved at 50 g/L glucose concentration. However, in their experiment, when glucose concentration was increased to 60 g/L, HA concentration decreased.

Highest cell yield coefficient ($Y_{X/S}$) was achieved at 20 g/L glucose concentration. This result suggests that highest $Y_{X/S}$ does not mean highest productivity. At 50 g/L, the $Y_{X/S}$ value was the least, 0.313 g cell/ g substrate, suggesting that less cells were produced per gram of glucose. It could be concluded that glucose concentration above 50 g/L causes reduction in water activity which may lead to growth inhibition. Glucose is a monosaccharide and hence its' osmotic pressure will be higher, so microorganisms will detect the changes in osmotic pressure and alter their metabolic pathway. Ding and Tan (2006) also stated that high initial glucose concentration in batch fermentation method can cause low cell mass due to substrate inhibition.

At 50 g/L, product yield per substrate produced ($Y_{P/S}$) and maximum productivity (P_r) had maximum values of 0.004 g HA/g cell and 0.018 g HA/L/h, respectively. At 40 g/L, $Y_{P/S}$ was 0.003 g HA/ g cell and at 50 g/L, $Y_{P/S}$ was 0.004 g HA/g cell suggesting that glucose was more efficiently consumed at 50 g/L. Values for product yield per biomass produced ($Y_{P/X}$) increased with increase in glucose concentration, reaching a maximum value of 0.010 g HA/g cell at 50 g/L glucose concentration. This suggested that as the number of cells increased, the

Table 1: Kinetic parameter values of HA production by recombinant *E. coli* BL21 using different glucose concentrations.

Kinetic parameters	Glucose concentration (g/L)				
	10	20	30	40	50
X_m (g cell /L)	2.501 ± 0.010	2.602 ± 0.011	2.656 ± 0.002	2.879 ± 0.004	3.031 ± 0.004
P_m (g HA/L)	0.026 ^e ± 0.001	0.029 ^d ± 0.0001	0.037 ^c ± 0.0001	0.057 ^b ± 0.003	0.074 ^a ± 0.001
μ_m (1/h)	0.500	0.520	0.531	0.575	0.606
$Y_{X/S}$ (g cell/g substrate)	0.669	0.682	0.415	0.446	0.313
$Y_{P/S}$ (g HA/g substrate)	0.001	0.001	0.001	0.003	0.004
$Y_{P/X}$ (g HA/g cell)	0.002	0.002	0.004	0.005	0.010
P_r (g HA/L/h)	0.006	0.007	0.009	0.014	0.018

All results are expressed as means ± standard deviation of means; Values are means of triplicates from three separate runs (n = 3). p-value for average HA concentration in accordance to glucose concentration was obtained using two way ANOVA statistical analysis ($\alpha=0.05$). a, b, c, d and e: Values with different lowercase superscript are significantly different.

Table 2: Kinetic parameter values of HA production by recombinant *E. coli* BL21 using different media.

Kinetic parameters	Glucose concentration (g/L)	
	50 without nutrient rich components	50 & nutrient rich media
X_m (g cell/L)	3.031 ± 0.004	3.280 ± 0.108
P_m (g HA/L)	0.074 ^b ± 0.001	0.115 ^a ± 0.002
μ_m (1/h)	0.606	0.546
$Y_{X/S}$ (g cell/g substrate)	0.313	0.259
$Y_{P/S}$ (g HA/g substrate)	0.004	0.006
$Y_{P/X}$ (g HA/g cell)	0.010	0.0195
P_r (g HA/L/h)	0.018	0.0287

All results are expressed as means ± standard deviation of means; Values are means of triplicates from three separate runs (n = 3). p-value for average HA concentration in accordance to different media was obtained using two way ANOVA statistical analysis ($\alpha=0.05$). a and b: values with different lowercase superscript are significantly different.

Table 3: HA concentration and molecular weight in different IPTG concentrations

IPTG concentration (mM)	HA concentration (g/L)	HA molecular weight (kDa)
0	0.115 ^d	8.7 ^d
0.1	0.229 ^c	12.5 ^c
0.5	0.331 ^b	17.3 ^b
1.0	0.532 ^a	34.6 ^a

All results are expressed as means ± standard deviation of means; Values are means of triplicates from three separate runs (n = 3). p-value for average HA concentration and molecular weight in accordance to IPTG concentration was obtained using one way ANOVA statistical analysis ($\alpha=0.05$). a, b, c and d: values with different lowercase superscript in same column are significantly different.

amount of HA production also increased. Hence, the results showed that HA fermentation by recombinant *E. coli* BL21 is highly growth associated as shown in Figure 3. However, studies conducted by Huang *et al.* (2007) suggested that HA production was delayed-growth

associated which was in accordance to several other reports.

At 50 g/L, $Y_{X/S}$ was the least and $Y_{P/S}$ was the highest suggesting glucose was mainly used for HA production. Precursors such as glucose-1-phosphate, UDP-glucose and UDP-N-acetylglucosamine are shared in HA

synthesis and cell growth. Hence, competition between these two pathways exists to consume the same precursors. In addition, competition for carbon flux exists between HA synthesis and glycolysis. Metabolites such as glucose-6-phosphate and fructose-6-phosphate are consumed by hexomonophosphate shunt and glycolysis, respectively. Hence, there is strong competition for metabolites between HA production and cell growth. HA production is an energy intensive process and total of 4 mol ATP is required for production of 1 mol of repeating unit of HA disaccharide. Therefore, in order to balance between biomass and HA production, maintenance of metabolic balance is important as well (Izawa *et al.*, 2011).

Effect of nutrient rich media on HA fermentation by recombinant *E. coli* BL21

The use of nutrient rich media for HA production yielded higher biomass concentration and HA concentration when compared to use of glucose alone. As shown in Table 2, maximum biomass concentration (X_m) was 3.280 ± 0.108 g/L when 50 g/L glucose along with nutrient rich media was used. However, when only 50 g/L glucose was used, the biomass concentration was 3.031 ± 0.004 g/L. Studies by other researchers were conducted with constant glucose concentration and varying yeast extract concentration (as nitrogen source). The results supported our findings, suggesting that presence of organic nitrogen source improves the microorganism growth, with maximum cell growth when C: N ratio is 2:1 (Chen *et al.*, 2009). The increase in biomass concentration is because yeast extract is a good substrate for many microorganisms as it contains important components such as amino acid, peptide, water-soluble vitamins and carbon source which are required for growth and metabolism. With nutrient rich media, maximum HA concentration (P_m) was 0.115 ± 0.002 g HA/L, whereas with 50 g/L glucose concentration it was only 0.074 ± 0.001 g HA/L. This might have been due to availability of amino acid and B-vitamins provided by organic nitrogen source, yeast extract and tryptone. Additionally, presence of these organic nitrogen sources provides large portion of carbon for the growth and production of cells. The specific growth rate (μ_m) was slightly higher at 50 g/L glucose concentration. This can be explained by the cell yield coefficient ($Y_{X/S}$) which is also higher for 50 g/L glucose concentration, which suggests that more cells are produced per substrate consumed at 50 g/L. In nutrient rich media, more of the metabolised carbon source might have been used for HA production, as a result slightly lower specific growth rate was achieved. The product yield coefficient ($Y_{P/S}$) and product yield per biomass coefficient ($Y_{P/S}$) was higher with nutrient rich media containing 50 g/L glucose. The productivity is also higher in nutrient rich media, with a value of 0.028 g HA/L/h which is in accordance to results obtained by other researchers which suggests that presence of nitrogen source increases the production of HA (Pan *et al.*, 2014).

There is limited information about the consequence of medium carbon-to-nitrogen (C/N) ratios which will cause an effect on the metabolic flux. Studies showed that presence of potassium dihydrogen phosphate (KH_2PO_4) and magnesium sulphate heptahydrate ($MgSO_4 \cdot 7H_2O$) also contribute to increase in biomass and HA productivity (Aroskar *et al.*, 2012). Hence, the presence of these substances in nutrient rich media may have also contributed to higher biomass and HA concentration.

Effects of different IPTG concentrations on HA fermentation

Different IPTG concentrations are used to induce the expression of *hasA* in recombinant *E. coli* in nutrient rich media. As shown in Figure 4, 1.0 mM of IPTG was able to achieved the highest biomass concentration (X_m) (4.03 ± 0.020 g/L), the highest HA concentration (P_m) (0.532 ± 0.026 g/L), and the highest productivity (P_r) (0.076 g/L/h). This is expected as IPTG acts as an inducer to induce the higher expression of HAS genes in recombinant *E. coli*, thus producing more HA. The result obtained was significantly higher when compared to Yu and Stephanopoulos (2008), as they were able to produce 95.9 mg/L of HA. In contrast, the control experiment (without the addition of IPTG) showed a lower biomass concentration of 3.28 ± 0.024 g/L with HA concentration (P_m) of 0.115 ± 0.002 g/L, and HA productivity (P_r) of 0.028 g/L/h. A possible explanation is that recombinant *E. coli* BL21 is able to express the *has* genes without any help from IPTG inducer.

In nutrient rich medium with 50 g/L of glucose, the medium with added 1.0 mM of IPTG produced the highest among the rest. It is an advantage as higher biomass allows IPTG to rapidly enter into the cell (Fernández-Castané *et al.*, 2012). Moreover, it revealed that the lac promoter was fully induced by 1.0 mM of IPTG and there was a competition for metabolites between biomass growth and HA production. According to Madurawe *et al.* (2000) the concentrations of IPTG (between 0.1-2.0 mM) vary differently with clones in order for a complete induction.

Comparing the control experiment with 0.1 mM of IPTG, the production of biomass and HA is almost similar. This could be due to the fact that HA expression is not fully induced by IPTG as the intracellular inducer does not bind tightly to the receptor to release all the repressor molecules from various operator sites. Another possibility is that when using low concentrations of IPTG, it is not enough to induce all the cells, thus leaving some of the cells un-induced (Fernández-Castané *et al.*, 2012). On the other hand, reduction of the biomass concentration will occur if there is an excess of IPTG present in the medium. A study carried out by Yee and Blanch (1993) supports that statement where they used IPTG concentration more than 1.0 mM, resulting in reduced growth rate in *E. coli*. Therefore, production of HA can be regulated from low to highly induced expression level by altering the IPTG concentration. Molecular weight is an important parameter for microbial HA synthesis because it

determines the quality of HA. Low molecular weight HA is widely used in cosmetics as moisturizers and possesses pronounced free radical scavenging and antioxidant activities. In this study, the HA molecular weight 34.6 kDa obtained at 50 g/L glucose with nutrient rich media plus 1.0 mM IPTG (Table 3). This result is comparable to Lai *et al.* (2016) who were able to produce 40.9 kDa HA using combination of *hasA* and *hasE* genes in recombinant *E. coli*.

CONCLUSION

The *hasA* gene was cloned from *S. zooepidemicus* and successfully expressed in recombinant *E. coli* BL21 cells. 50 g/L glucose concentration along with nitrogen source such as yeast and tryptone is the preferred combination to produce the highest concentration of HA through fermentation by recombinant *E. coli* BL21 using shake flask culture. The results obtained from this study showed that high HA production (0.115 ± 0.002 g/L) was achieved in nutrient rich media with 50 g/L glucose concentration. With addition of 1.0 mM IPTG, the highest HA production (0.532 ± 0.026 g/L) and molecular weight at 34.6 kDa was achieved which is around fivefold higher compared to without IPTG. To further improve the productivity of HA in recombinant *E. coli*, gene integrative system will be used for *hasA* gene and *hasABC* gene cassette in future.

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