Construction and expression of indonesian hepatitis B core antigen (HBcAg) in *Lactococcus lactis* as potential therapeutic vaccine

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Abstract

The hepatitis B core antigen protein (HBcAg) may induce proliferation of B, T, and cytotoxic T cells; induce immune responses; and provide T-cell resources for antibody responses after vaccination (Freivalds et al., 2011). Thus, HBcAg may be used as a therapeutic vaccine in patients with chronic hepatitis B virus (HBV) infection. This article describes a study on the construction and expression of HBcAg in *Lactococcus lactis* by the nisin-controlled expression (NICE) system. An HBcAg gene, the HBV subgenotype B3 that is dominant in Indonesia, was successfully cloned into a pNZ8148 vector and resulted in the formation of the transformant *L. lactis* NZ3900 pNZ818-HBcAg. The HBcAg protein measured 21 kDa. Induction with 10 ng/ml nisin significantly increased the concentration of the expressed protein. Western blotting and dot blot hybridization analysis indicated that the HBcAg proteins confirmed the expression of an antibody, with potential to become an HBV therapeutic vaccine.

Key words: hepatitis B virus, HBcAg gene, *Lactococcus lactis*, NICE system

Introduction

The HBV belongs to the Hepadnaviridae family; it is a small, enveloped DNA virus that replicates via reverse transcription (Azizi et al., 2000). Nine HBV genotypes have been identified and classified as A, B, C, D, E, F, G, H, and J; the existence of I remains controversial (Utsumi et al., 2009). The HBV sequence is characterized by 8% and 4–8% differences in nucleotide (nt) sequences among the genotypes and the subgenotypes, respectively (Lusida et al., 2008). Globally, HBV infections constitute a major health problem, and has two stages: acute and chronic. A World Health Organization (WHO) report indicates over 257 million people have chronic HBV infection (WHO, 2015). Although high virus levels have been reported after treatment with antiviral drugs in chronic HBV infection (Bertoletti and Ferrari, 2012; Buchmann et al., 2013), they continue to be used. Despite their limited long-term therapeutic efficacy and adverse effects, antiviral drugs are incapable of eradicating the covalently closed circular DNA (cccDNA) of HBV and cannot restore immunity. Consequently, HBV replication and hepatic damage resume when antiviral pharmacotherapy ceases (Buhmann et al., 2013). There is a need for therapeutic vaccines to induce strong immunity against HBV infections, particularly in chronic infections. Maini et al. (2000) found that patients with chronic HBV infection with controllable HBV replication levels have noticeably higher levels of the hepatitis B core antigen protein (HBcAg) compared to patients with HBV replication levels that are refractory to treatment. HBcAg is encoded in the HBV genome (3.5 kb, 22 kDa) together with a viral nucleocapsid protein, has an icosahedral conformation, and forms two spherical shells that form small and large capsids with 180 and 240 copies of core protein, respectively (Wingfield et al., 1995). Chisari and Ferrari (1995) reported that HBcAg can induce the proliferation of B, T, and cytotoxic T cells in patients with HBV infection. Moreover, HBcAg can induce immune

responses and T-cell proliferation after vaccination (Frei-valds et al., 2011).

Given the importance of HBcAg as a therapeutic vaccine, HBcAg production has been investigated in several expression systems. The expression of recombinant HBcAg in *Escherichia coli* has been induced through a pFP15 vector under a tryptophan promoter, followed by purification with 30% ammonium sulfate and a Q-Sepharose Fast Flow Column (Azizi et al., 2000). However, further analyses revealed the presence of inclusion bodies formed by the long peptides of 26 amino acids present at the N-terminus region of HBcAg. Rolland et al. (2011) reported the expression and purification of recombinant HBcAg in *Pichia pastoris* expressed under an alcohol oxidase gene promoter. The yield of the expressed protein was high, but the process required many days of yeast culture growth under methanol induction.

We report a novel method for the construction and expression of HBcAg *Lactococcus lactis* NZ3900 by the NICE system. As *L. lactis* is known to be non-hazardous, it is considered safe for use in the biopharmaceutical development and mucosal administration of *L. lactis* live recombinant therapeutic vaccines (Bermudez et al., 2011). *Lactococcus lactis* does not contain endotoxins in the membrane; therefore, it is a safe agent to secrete recombinant proteins into growth medium (Jorgensen et al., 2014). Several advantages support the use of *L. lactis* as protein expression host strain – for instance, the efficacy of transformation, the existence of a strong promoter, the small size of its genome, rapid bacterial growth, and, importantly, the fact that the secreted proteins do not form inclusion bodies. We used the HBV subgenotype B3 of the HBcAg gene that is dominant in Indonesia. This HBV subgenotype B3 is commonest, followed by HBV/C1, in Indonesia; however, diverse novel genotypes have been isolated (Heriyanto et al., 2012). We aimed to develop an expression system for a therapeutic HBV vaccine with the HBcAg gene constructed in a pNZ8148 expression vector and transformed into NZ3900 *L. lactis*.

Materials and methods

Bacterial strains, plasmids, and culture conditions

The bacterial strains and vectors used in this study are listed in Table 1. The *E. coli* strains were cultivated in Luria–Bertani (LB) medium with aeration at 37°C,

whereas the *L. lactis* NZ3900 strains were cultivated at 30°C, without shaking, in M17 media (Himedia) with 0.5% glucose.

Vector constructions

The HBcAg gene used in this research was a 549 bp-long synthetic gene of the 1839 Java isolate of HBV (GenBank EF473972.1). An 81 bp-long signal peptide (SP_{usp45}; GenBank M60178.1) was added to the gene-coding sequence and inserted into the pMAT vector. The recombinant pMAT-HBcAg vector was digested with restriction endonucleases *Nco*1 and *Xba*1 (BioLabs Inc™). The excised fragment was purified using a DNA Purification Kit (Qiagen) and ligated into the pNZ8148 vector; then, the recombinant vector (pNZ8148-HBcAg) was inserted into the *E. coli* MC1061 strain. Transformants were selected on agar-containing chloramphenicol (Sigma™), and the length of the insert was verified using PnisA_F (5′–TTC CCT CGA GGG ATC TAG TCT TAT AAC–3′) and TpNZ8148_R (5′–GCT AAA ACG TCT CAG AAA CG–3′) primers. PCR amplification was done as follows: pre-denaturation at 94°C for 3 min, 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, elongation at 72°C for 30 s, followed by a final elongation at 72°C for 6 min.

Transformation of L. lactis

The recombinant pNZ8148-HBcAg vector was inserted into *L. lactis* NZ3900 by using the electroporation method. The *L. lactis* culture was grown overnight in M17 media containing 0.5% (wt/vol) glucose, and then was transferred into M17 media containing 0.5% (wt/v) glucose, 0.5 M sucrose, and 2.5% (wt/v) glycine. Culture growth was continued until the optical density of the medium at 600 nm (OD_{600}) reached ~0.3. Subsequently, cells were collected by centrifugation at 6,000 × *g* and 4°C for 10 min, before resuspension in a cold buffer composed of 0.5 M sucrose and 10% (v/v) glycerol. This procedure was repeated with the same buffer, but with the addition of 0.05 M EDTA. Thereafter, the recombinant pNZ8148-HBcAg vector was added to the cell suspension and the mixture was electroporated at 2 000 V, 25 μF, and 260 Ω. After electroporation, the cells were transferred directly into recovery medium (M17 containing 0.5% (wt/v) glucose) and incubated at 30°C for 3 h, without shaking. The cells were then transferred onto M17 agar to which 10 μg/ml chloramphenicol was

Table 1. Bacterial strains and vectors used in the study

Strains and vector	Characteristics	Source
Bacterial strains		
Escherichia coli Top 10	cloning host strain	Novagen
Escherichia coli MC 1061	cloning host strain	NICE
Lactococcus lactis NZ3900	expression host strain HBcAg	NICE
Vectors		
pMAT	Amp^r, cloning vector	Invitrogen
pNZ8148	Cmp^r nisin-inducible promoter	NICE

Amp^r – ampicillin resistance; Cmp^r – chloramphenicol resistance

added (Holo and Nes, 1995). The transformants were screened for the presence of an insert using PCR (PnisA_F and TpNZ8148_R primers) and *Nco*1 and *Xba*1 restriction enzymes (BioLabs Inc™). The sequence of the construct was verified through sequencing using the Sanger method.

Expression of HBcAg gene in *L. lactis* NZ3900 on transcription level

Total RNA isolation was undertaken using the TRIzol method (Rio et al., 2010). First, 10 ml of the culture with *L. lactis* carrying the pNZ8148-HBcAg vector was added to M17 medium supplemented with 0.5% glucose and 10 μg/ml chloramphenicol. Next, the culture was incubated at 30°C until the OD_{600} of the medium reached 0.4–0.5; at this point, protein production was induced with nisin (5, 10, and 50 ng/ml). Uninduced bacteria were used as a control; the *L. lactis* NZ3900 culture was additionally used as another negative control. The bacterial culture was transferred to a 10 ml tube and centrifuged at 13 000 rpm for 10 min at 4°C; 1 ml TRIzol was added to the cell pellet, which was then resuspended before the addition of 200 μl cold chloroform. Lysis was induced by vortexing for 1 min, followed by incubation on ice for 15 min. The mixture was transferred to a new tube and centrifuged at 12 000 rpm for 15 min at 4°C. The top layer was then transferred into a fresh tube, 500 μl cold isopropanol was added, the mixture was thoroughly vortexed for 30 sec, and incubated for 1 h at –20°C. After incubation, the mixture were centrifuged at 12 000 rpm for 10 min at 4°C to sediment the RNA. The RNA was washed by adding 70% ethanol and centrifuging the pellet again at 9 000 rpm for 10 min at 4°C.

The pellet was dried overnight on a laboratory bench before being dissolved in Diethylpyrocarbonate (DEPC)-treated ddH$_2$O and incubated at 37°C for 30 min. Subsequently, RNA was quantified by using a spectrometer at 260 nm.

A one-step RT-PCR kit (Bioline) was used to reverse transcribe total RNA to cDNA. We used 0.1 μg total RNA; the reverse transcription mix contained 5 μl MyTaq One-Step Mix, 0.1 μl reverse transcriptase, 0.2 μl RiboSafe RNase Inhibitor, and 3.3 μl DEPC. To amplify HBcAg cDNA, 400 nM each of the primers HBcAgBamHI-HindIII-F (5′–ATTTGGATCCATGGACATTGACCCGT AT–3′) and HBcAgBamHI-HindIII-R (5′–ACGCAAGCT TCTAACAATGAGAATCCCG–3′) were used. Reverse transcription was undertaken at 45°C for 30 min. Thereafter, cDNA amplification was conducted as follows: 5 min of predenaturation at 94°C, denaturation at 94°C for 1 min, annealing for 1 min at 50°C and elongation for 3 min at 72°C for 35 cycles, followed by a final elongation for 3 min at 7°C. Agarose electrophoresis was carried out to visualize PCR products using 0.5 μg/ml ethidium bromide.

Quantitative PCR (qPCR) analysis was conducted according to the protocol by Schmittgen dan Livak (2008). The first step of qPCR was a standard curve formulation using for 16S rRNA. cDNA were diluted to final concentrations of 0.2, 0.02, and 0.002 ng/μl; then, the remaining qPCR reagents were added: 5 μl SYBR qPCR mixture, 300 nM 16S rRNA F primer, 300 nM 16S rRNA R primer, 0.2 μl 50× ROX Refer, and ddH$_2$O added to a final volume of 50 μl. The PCR was set as follows: predenaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 1 min, and annealing at 50°C for 1 min.

Next, levels of HBcAg mRNA were measured in samples induced with 0, 5, 10, and 50 ng/ml nisin using the qPCR method.

Expression of HBcAg gene in L. lactis NZ3900 and partial purification with size-exclusion chromatography (SEC)

Protein expression was undertaken according to modified Le Loir and Rajalingam methods (Le Loir et al., 1998; Rajalingam et al., 2009). Recombinant *L. lactis* NZ3900 pNZ8148-HBcAg were cultivated in M17 media to which 0.5% glucose and 10 μg/ml chloramphenicol were added. Protein expression in recombinants was induced with either 5, 10, or 50 ng/ml nisin (Sigma-Aldrich). The supernatant was harvested by 30-min centrifugation at 10 000 rpm (4 °C), and precipitated overnight at 4 °C using 45% (wt/v) ammonium persulfate. The protein pellet was harvested through centrifugation and resuspended in 50 mM Tris HCl. Purification with SEC was undertaken as described by Hong et al. (2012). Protein samples were loaded onto a pre-equilibrated Sephadex G-50 pre-packed column (GE Healthcare™), and elution was carried out using 50 mM Tris HCl (pH 7.8) with a 0.1 ml/min flow rate. Sample fractions were collected every 5 min and analyzed using a spectrophotometer at 280 nm. Additionally, protein fractions were analyzed on 12% SDS-PAGE and stained by a silver staining kit (Thermo Scientific). The concentration of total proteins was determined using the BCA Protein Assay Kit (Thermo Scientific, USA) according to manufacturer instructions.

Western blotting and dot blot hybridization assays

Western blotting and dot blot hybridization assays were conducted in accordance with protocols by Sambrook and Russell (Sambrook and Russell, 2001). In Western blotting, protein fractions were electrophoresed on 12% SDS-PAGE, followed by the transfer of proteins onto a nitrocellulose membrane. The membrane was blocked with 10% skimmed milk in 1× Tris Borate EDTA (TBE) for 1 h, rinsed with 1× Tris Borate EDTA (TBE) – 0.1% Tween, incubated with the HBcAg Primary Monoclonal Antibody (dilution 1 : 1 000; cat. no. MA1–7608, ThermoFisher Scientific–) and then with secondary anti-Ig G Mouse Alkaline Phosphatase Conjugates antibody (cat. no. WP20006, ThermoFisher Scientific), before it was stained with 5-bromo-4-chloro-3-indolylphosphate/ nitro blue tetrazolium (BICP/NBT; SigmaFast™). In the
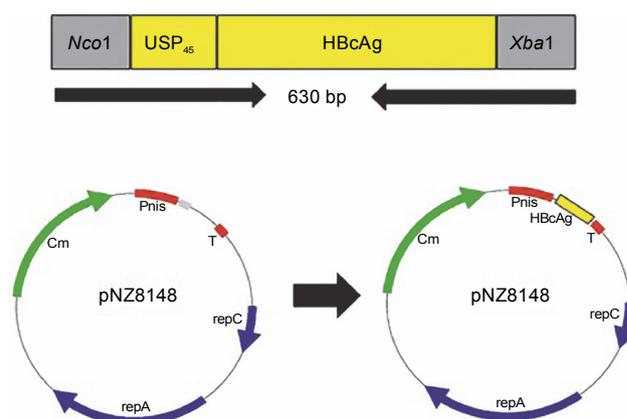
Fig. 1. The construction of the pNZ8148-HBcAg expression vector. The fusion of the SP$_{usp45}$ HBcAg gene was ligated into the expression vector pNZ8148
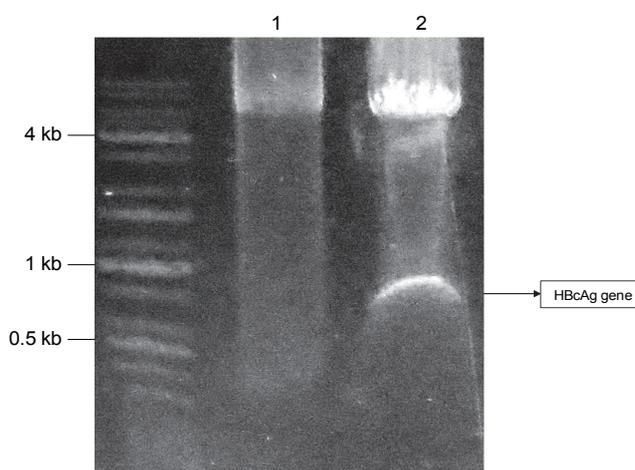
Fig. 2. Analysis of a DNA construct pNZ8148-HBcAg using *Nco*1 and *Xba*1 restriction enzymes: visualization of a DNA vector and target HBcAg gene using ethidium bromide staining of agarose gel; lane 1 – undigested sample; lane 2 – digested sample

dot blot hybridization assay, proteins were transferred onto a nitrocellulose membrane and then detected using Western blotting analysis as previously described.

Results

Construction of recombinant vector

The full-length HBcAg gene encoding the HBcAg protein that we used in this study belonged to the HBV B3 subgenotype – an Indonesian 1839 Java isolate of HBV. Figure 1 presents a construction map of the expression vector harboring the HBcAg gene. The HBcAg gene was inserted into the multicloning site of the pNZ8148 expression vector to generate a recombinant pNZ8148-

Fig. 3. Sequence analysis of the final HBcAg construct. RBS is the ribosome-binding site; * stop codon; marked in blue: +1; transcription start position, in yellow; SP$_{usp45}$, in green; HBcAg gene-coding sequence

Table 2. Concentration of cDNA from *L. lactis* pNZ8148-HBcAg

Sample	cDNA concentration [ng/μl]
HBcAg uninduced	442
HBcAg induced 5 ng/ml of nisin	527
HBcAg induced 10 ng/ml of nisin	544
HBcAg induced 50 ng/ml of nisin	578

Table 3. ΔC_T of uninduced and induced of *L. lactis* pNZ8148-HBcAg

Sample	C_T of HBcAg gene	C_T of 16S rRNA gene	ΔC_T
HBcAg uninduced	6.73	3.89	2.85
HBcAg induced 5 ng/ml of nisin	7.69	3.47	4.23
HBcAg induced 10 ng/ml of nisin	8.37	3.32	5.05
HBcAg induced 50 ng/ml of nisin	7.35	3.85	3.50

HBcAg cassette and was subsequently transformed to *E. coli* MC1061 for use as a cloning host strain. The selection of transformants was undertaken on the basis of chloramphenicol resistance, PCR amplification (PNisA_F and TpNZ8148_R), and restriction enzyme analysis using *Nco*1 and *Xba*1. Recombinant pNZ8148-HBcAg from positive transformants was purified and successfully transformed to *L. lactis* NZ3900, which was used as the expression host strain. The vector transformants were screened by PCR amplification (using PNisA_F and TpNZ8148_R primers) and digestion by *Nco*1 and *Xba*1 restriction enzymes. The pNZ8148-HBcAg recombinant

vector was 3815 bp-long with a 630 bp-long HBcAg gene (Fig. 2). DNA sequence analysis of recombinant pNZ8148-HBcAg was conducted to determine the sequence of the inserted HBcAg gene (Fig. 3). DNA sequencing revealed that the recombinant cassette had no mutations. Moreover, a translation analysis showed there was no amino acid substitution in the protein sequence, and the recombinant vector carried the desired gene. HBcAg had an open reading frame of 549 bp that encoded 183 amino acid residues with a predicted molecular weight of 21 kDa. The restriction analysis (Fig. 2) and DNA sequencing (Fig. 3) confirmed that the HBcAg gene had been successfully inserted into the pNZ8148 expression vector and transformed into *L. lactis* NZ3900.

Verification of expression of HBcAg mRNA level

The first step of the analysis was RNA isolation from the *L. lactis* culture that carried the recombinant pNZ8148-HBcAg plasmid induced with nisin. The highest RNA concentration was obtained when expression was induced with 50 ng/ml nisin (172. ng/µl), whereas the lowest was observed in uninduced samples (89.2 ng/µl). The cDNA was synthesized from total RNA using reverse transcriptase. The highest HBcAg cDNA concentration was obtained upon the induction of *L. lactis* pNZ8148-HBcAg with 50 ng/ml nisin. Subsequently, cDNA was used for qPCR, performed using a SYBR green kit and a pair of HBcAg BamHI-HindIII primers. The results are presented in Table 3, and indicate successful expression of the HBcAg gene.

Analysis of HBcAg protein expression

The recombinant HBcAg protein was expressed in *L. lactis* NZ3900. The 21-kDa HBcAg protein was present in isolates from samples of recombinant *L. lactis* NZ3900 pNZ8148-HBcAg (Fig. 5). The protein was analyzed by Western blotting and dot blot hybridization using a purified monoclonal HBcAg antibody. The results indicate that recombinant pNZ8148-HBcAg protein (21 kDa) was recognized and bound by the HBcAg antibody. However, in a sample derived from bacteria containing an empty vector, there was no antibody signal on the nitrocellulose membrane. Concentrations of total protein samples from different groups are shown in Table 5. The total protein level was found to be highest and significantly increased in samples grown with
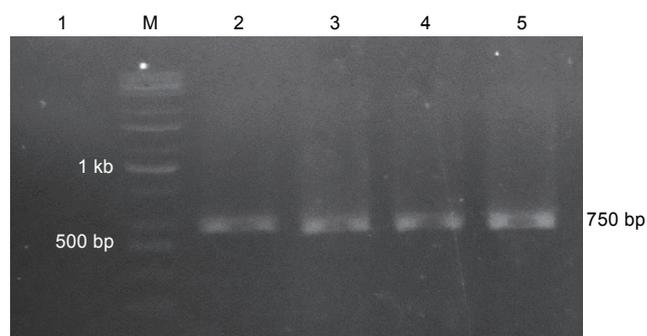
Fig. 4. The result of HBcAg cDNA amplification from total cDNA 1) *L. lactis* NZ3900 as negative control 2) *L. lactis* pNZ8148-HBcAg uninduced 3) induced by 5 ng/ml nisin 4) induced by 10 ng/ml nisin 5) induced by 50 ng/ml nisin
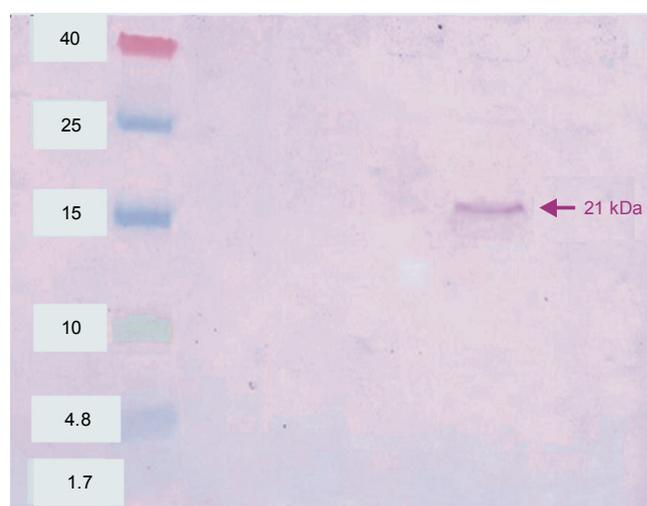
Fig. 5. Western blotting analysis of a fraction sample after purification with size-exclusion chromatography; the band of HBcAg protein was identified at the 21 kDa position

10 ng/ml nisin (11.852 ± 0.182 mg/ml), when compared with uninduced samples, or those with 5 or 50 ng/ml nisin. The level of protein expression was markedly increased by the addition of nisin to the growth media when compared with uninduced samples; however, the highest induction was observed for samples to which 10 mg/ml nisin was added. Bacteria containing an empty vector were used to measure the level of total protein expression; therefore, it was used for the quantification of basal protein in the recombinant sample. Estimation of HBcAg protein concentration in the recombinant sample was carried out by subtracting the total protein content of recombinant samples from the total protein content produced by cells containing an empty vector.

Table 4. $\Delta\Delta C_T$ of induced of *L. lactis* pNZ8148-HBcAg

Sample	$\Delta\Delta C_T$	Enhancement of protein expression levels between induced and uninduced samples
HBcAg induced 5 ng/ml of nisin	1.38	2.60 ×
HBcAg induced 10 ng/ml of nisin	2.20	4.59 ×
HBcAg induced 50 ng/ml of nisin	0.65	1.57 ×

Table 5. Concentration of protein expression of total fractions in recombinant cells containing the HBcAg-expressing vector

Samples	Total protein [mg/ml]
Empty vector (*L. lactis* pNZ8148)	5.316 ± 0.249
HBcAg uninduced	6.760 ± 0.902
HBcAg induced 5 ng/ml of nisin	7.109 ± 0.162
HBcAg induced 10 ng/ml of nisin	11.852 ± 0.182
HBcAg induced 50 ng/ml of nisin	8.950 ± 0.490

Discussion

The HBcAg protein has been used as a therapeutic vaccine because it can induce strong B-, T-, and cytotoxic T-cell proliferation (Chisari and Ferrari, 1995). In addition, the HBcAg protein can induce an immune response and provide immunogenicity against HBV (Freivalds et al., 2011). Here, we report the construction and expression of HBcAg in *L. lactis*. This study is the first research on the expression of HBcAg proteins using *L. lactis* as the host. The HBcAg gene was designed according to the HBcAg nucleotide sequence of the dominant HBV subgenotype in Indonesia. Sugauchi et al. (2008) reported that the HBV B2 substrain (HBV/B2) was discovered mostly in Indonesians of Chinese ethnic origin, and HBV/B3 was dominant among the Javanese, who constitute the largest ethnic group. Thus, the protein so obtained can be used to produce an HBV vaccine that has high compatibility and, therefore, the potential ability to prevent HBV infection in Indonesia. The HBcAg protein is a dimer and forms Cys61–Cys61 disulfide bonds connecting the two monomers (Zlotnick et al., 2012). In addition to its capability of being generated in recombinant *E. coli*, the HBcAg protein can be assembled into core virus particles within prokaryotic and eukaryotic hosts (Schodel et al., 1992). In this work, we produced for the first time an HBcAg protein in recombinant *L. lactis* NZ3900 by utilizing the inducible

NICE system promotor. *Lactococcus lactis* NZ3900 is a strain derived from *L. lactis* NZ9000 that is commonly used in food-grade products and biopharmaceutical industries. From a cluster of nisin genes, the *nisK* and *nisR* signals transduction genes were isolated and inserted into *L. lactis* subsp cremoris MG1363 to produce *L. lactis* NZ9000 (Mierau et al., 2005). These signal transduction genes are used to induce protein expression in the NICE system because it comprises the *nis* operon (*Pnis*A as a nisin-inducible promotor) and *nis*RK (sensor of regulatory function). The addition of nisin into a culture medium is detected by *Nis*K, which induces its autophosphorylation. Furthermore, a phosphate group is transferred to *Nis*R which then activates transcription (Platteeuw et al., 1996). The addition of a signal peptide (SP$_{usp45}$) to the coding sequence causes the HBcAg protein to be secreted extracellularly. This signal peptide is generally utilized for heterologous protein production in *L. lactis* and is commonly fused with the inducible NICE system (Le Loir et al., 1998; Mierau et al., 2005; Novotny et al., 2005). The *usp45* gene encodes a major extracellular protein in *L. lactis* (Dieye et al. 2001).

This system has been used to produce high levels of recombinant Plantaricin W (Pln W) protein in *L. lactis*, and maximum expression of recombinant PlnW was obtained by induction with 50 ng/ml nisin (Lages et al., 2015). In the present research, induction with 10 ng/ml nisin ge-

nerated the highest HBcAg protein expression, which was further confirmed by immunoblotting and concentration of the total protein fraction (Table 5 and Fig. 5).

This work revealed that recombinant *L. lactis* NZ3900 pNZ8148-HBcAg can produce HBcAg. The qPCR data analysis was conducted to confirm the production of HBcAg mRNA. The qPCR uses the C_T comparison method ($\Delta\Delta C_T$ method) to calculate the difference between the C_T values (ΔC_T) of a desired gene (from each induction treatment) and the housekeeping gene (16s rRNA) to find the expression level of the HBcAg gene. The ΔC_T of *L. lactis* pNZ8148-HBcAg induced by 10 ng/ml nisin generated the highest output when compared to that of induction with 0. 5 and 50 ng/ml nisin. Comparison of the fold-change in the expression level of the induced and uninduced samples revealed that induction with 10 ng/ml nisin had the highest fold-change (4.59×).

Protein identification was confirmed by Western blotting, as it was recognized by the monoclonal HBcAg antibody. A DNA sequencing analysis showed there was no mutation in the coding sequence. A translational analysis showed that the recombinant protein has no amino acid substitution. Purification of the HBcAg protein with size-exclusion successfully produced a band of protein in the 21-kDa position on Western blotting analysis.

Conclusions

The recombinant *L. lactis* pNZ8148-HBcAg genic sequence was successfully constructed and expressed in *L. lactis* NZ3900 by using the NICE system. The highest HBcAg expression level was achieved by the addition of 10 ng/mL nisin to the culture medium. Proteins were detected using a specific HBcAg antibody in Western blotting and dot blot hybridization assays. The results of the study could facilitate HBcAg protein production in *L. lactis* and has the potential to form the basis for an HBV therapeutic vaccine.

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