academicJournals

Vol. 12(31), pp. 4874-4880, 31 July, 2013
DOI: 10.5897/AJB12.1360
ISSN 1684-5315 ©2013 Academic Journals
http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Production optimisation of a DNA vaccine candidate against leishmaniasis in flask culture

Myriam Sanchez-Casco[1], Eric Dumonteil[2] and Jaime Ortega-Lopez[1]*

[1]Departamento de Biotecnología y Bioingeniería, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (Cinvestav), Av. Instituto Politécnico Nacional # 2508, Col. San Pedro Zacatenco, CP 07360, México D. F., México.
[2]Laboratorio de Parasitología, Centro de Investigaciones Regionales "Dr. Hideyo Noguchi", Universidad Autónoma de Yucatán, Av. Itzáes # 490, C.P. 97000, Mérida, Yucatán, México.

Accepted 25 July, 2013

Plasmid DNA (pDNA) vaccines are promising means to prevent and treat infectious diseases, such as leishmaniasis, but immunisation protocols require large amounts of supercoiled plasmid DNA (sc-pDNA). Although pDNA can be produced at a reasonable cost in bioreactors; this scale of production may not be the best method at the initial step of a vaccine development when many antigens need to be tested. Then, with the goal of improving the production of VR1012-NH36 and pVAX-NH36 pDNA vaccines against leishmaniasis, the effect of the culture medium and temperature on the pDNA yield was studied in flask cultures. The results indicate that the plasmid volumetric yield increased up to 65 mg/l in flask cultures by using a semi-defined medium, and shifting the culture temperature from 37 to 42°C at the late exponential growth phase. This pDNA production, with at least 80% of sc-pDNA at a laboratory scale seems sufficient to evaluate this and other pDNA vaccine candidates in the initial steps of vaccine development.

Key words: Plasmid DNA vaccine, plasmid DNA production, growth medium, flask culture, trace metal optimisation, leishmaniasis.

INTRODUCTION

Leishmaniasis is a neglected tropical disease affecting about 12 million poor people in developing countries (Hotez, 2008). Research devoted to finding alternative methods of preventing and treating this infection has shown that plasmid DNA (pDNA) vaccines containing a DNA fragment encoding a 36 kDa nucleoside hydrolase from *Leishmania donovani* (VR1012-NH36) induced protective or therapeutic immune responses in a murine model (Aguilar-Be et al., 2005; Gamboa-Leon et al., 2006). New antigen candidates were identified recently by mining the *Leishmania major* genome (Herrera-Najera et al., 2009). To assess their potential as pDNA vaccines, pure supercoiled pDNA (sc-pDNA) at mg scale must be obtained for each antigen.

The production of pDNA in *Escherichia coli* depends on many factors, including interactions between the host, plasmid vector and growth conditions, such as media components and the fermentation strategy (O'Kennedy et al., 2000, 2003), as well as downstream processing (Ferreira et al., 2000). The production of pDNA has been a very active study field in the last decade, many attempts to improve the yield and quality of pDNA by engineering *E. coli* (Borja et al., 2012; Phue et al., 2008), optimising media (Duttweiler and Gross, 1998;

*Corresponding author. E-mail: jortega@cinvestav.mx. Tel: 52-55-5747-3800 ext. 4381. Fax: 52- (55) 5747 3315.

O'Kennedy et al., 2000; Wang et al., 2001; Danquah and Forde, 2007; Zheng et al., 2007) and fermentation conditions have been studied reaching volumetric yields of up to 2 to 2.6 g/l in bioreactors (Phue et al., 2008; Carnes et al., 2011). Moreover, several patents have been issued in large scale upstream processing of plasmid DNA (Tejeda-Mansir and Montesinos, 2008). Nevertheless, this large-scale production of pDNA may not be reasonable at the initial step of plasmid DNA vaccine development when many antigens should be tested. Furthermore, many laboratories do not have access to bioreactor facilities or lack the resources to pay for custom production of pure sc-pDNA from commercial providers. Hence, improving laboratory protocol in flask cultures to increase sc-pDNA yields could help those laboratories involved in the initial step of plasmid DNA vaccine development. The pDNA yields can be improved by modifying the culture medium to increase the cell density or find culture conditions to increase the pDNA copy number. Duttweiler and Gross (1998) reported pDNA yields from 12.6 to 48 mg/l in *E. coli* strain DH5α by using a strongly buffered medium containing RNA and RNase A, glucose, yeast extract, and casamino acids. O'Kennedy et al. (2000) using a semi-defined medium (SDCAS), also with casamino acids, reported an increase in biomass from 0.13 to 2.5 g/l and up to a 50% increase in pDNA specific yield with respect to Luria-Bertani (LB); medium commonly used in laboratory protocols for the production and purification of pDNA. Moreover, Wang et al. (2001) based on a stoichiometric model, formulated the defined medium M1 using amino acids and nucleosides. They reported pDNA yields of 60 mg/l with M1 and 30 mg/l without nucleosides (M2), as compared with 11 mg/l obtained with LB. The economic impact analysis of M1 medium lead to the replacement of pure amino acids and nucleosides with yeast extract and tryptone. This medium (PDM) was optimised for pUC19 production, and a yield up to 23.8 mg/l of plasmid was obtained (Danquah and Forde, 2007). In addition to optimise the culture medium, the pDNA yields in bioreactors have greatly improved by shifting the culture temperature to increase the pDNA specific yield (copy number) (Carnes et al., 2011; Ongkudon et al., 2011; Phue et al., 2008).

Recently, PDM medium was also optimised for pcDNA3F production and an oscillatory temperature shift strategy from 35 to 45°C for 5 h was tested in 500 ml flask culture previously to studies at bioreactor scale, and pDNA yields increased up to 20 mg/l, as compared with 13.65 mg/l in flask cultures at 37°C (Ongkudon et al., 2011). Moreover, Studier (2005) specifically formulated a semi-defined medium for the production of pDNA (ZMY505), with casamino acids, yeast extract and glycerol, reaching a high cell densities (OD_{600} = 10) in a flask culture. Here, we report that trace metals optimisation of the ZMY505 medium, combined with a shift in culture temperature from 37 to 42°C in the late exponential growth phase, increased production of VR1012-NH36 and pVAX-NH36, pDNA vaccines candidates against leishmaniasis in amount and quality suitable for the initial test on experimental animals.

MATERIALS AND METHODS

Yeast extract was purchased from Difco (NJ, USA), NZ Casamino acids, kanamycin, and ethidium bromide were from Sigma-Aldrich (MO, USA), and PicoGreen dsDNA quantitative reagent from Molecular Probes Inc. (USA). All other reagents and chemicals used in this study were of analytical grade (Sigma-Aldrich or J.T. Baker, USA).

Plasmids and bacterial strain

Plasmids VR1012-NH36 (5850 bp) from previous work (Aguilar-Be et al., 2005) and a new construct pVAX-NH36 (3936 bp) containing a 936-bp DNA fragment coding the NH36 antigen of *L. donovani* were used in this study. The pVAX-NH36 was obtained by cloning the *nh36* DNA fragment in a pVAX1 vector (Invitrogen) as described elsewhere (Sambrook et al., 1989). Briefly, *nh36* was amplified by PCR using VR1012-NH36 as a template DNA, and oligonucleotides 5'-TGGCGCGGATCCGCCACCATGGCGCG CAAGATTATTCTC-3' and 5'-GGATGCTCTAGATTATTGAGGATCGCCGATGC-3' containing *BamH*I and *Xba*I restriction enzyme sites, respectively. After digestion of the amplicon with *BamH*I and *Xba*I restriction enzymes, the *nh36* DNA fragment was ligated into the pVAX1 plasmid. Once the *nh36* sequence was corroborated, *E. coli* DH5α cells were transformed with pVAX-NH36 or VR1012-NH36 and used for plasmid production.

Culture media

First, three different media were tested for the production of pVAX-NH36 in flasks: LB, which is commonly used in pDNA standard purification laboratory protocols (Sambrook et al., 1989), SDCAS (O'Kennedy et al., 2000) and ZMY505 (Studier, 2005); two semi-defined media previously reported for pDNA production (Table 1). Then, the production of VR1012-NH36 plasmid was tested using the best of the three media (ZMY505). To improve the pDNA yields, the trace metals of ZMY505 were optimised by using a Plackett-Burman design (Table 2).

Cell propagation and monitoring

Single colonies of *E. coli* DH5α harbouring pVAX-NH36 or VR1012-NH36 were selected from the LB-agar kanamycin plates and subcultured overnight in 5 ml of LB medium containing kanamycin (50 µg/ml) at 100 rpm and 37°C. Subsequently, 30 ml medium supplemented with kanamycin (50 µg/ml) was inoculated with 5% v/v (OD_{600} = 0.6) of the inoculum. Flasks cultures were incubated at 37 °C and 200 rpm for 18 h in a temperature controlled orbital shaker incubator (MRC, USA). One milliliter (1 ml) of culture was taken every 2 h to determine the dry cell weight, specific and volumetric pDNA yields, as well as sc-pDNA, and final pH. Cell growth was determined by measuring the OD_{600} of every sample, and the dry cell weight was estimated using a standard curve of OD_{600} versus dry cell weight previously obtained (data not shown). The maximum specific growth rate was determined by the plot

Table 1. Composition of LB, SDCAS, ZMY505, and ZMY505m media.

Nutrient	LB (g/l)	SDCAS (g/l)	ZMY505 (g/l)	ZMY505m (g/l)
Yeast Extract	5	-	5	5
Casamino acids*	10	10	10	10
Glucose	-	25	0.5	0.5
Glycerol	-	-	5	5
$MgSO_4 \cdot 7H_2O$	-	1.2	0.49	0.49
NaCl	10	-	-	-
$(NH_4)_2SO_4$	-	4.0	-	-
NH_4Cl	-	-	2.67	2.67
KH_2PO_4	-	13.3	3.04	3.04
Na_2HPO_4	-	-	3.55	3.55
Na_2SO_4	-	-	0.71	0.71
Citric acid	-	1.7	-	-
Thiamine HCl	-	0.0045	-	-

*or Peptone. Trace element concentration: SDCAS: 22.6 µM $Na_2EDTA \cdot 2H_2O$, 10.5 µM $CoCl_2 \cdot 6H2O$, 67.3 µM $MnSO_4 \cdot 4H_2O$, 7.7 µM $CuSO_4 \cdot 2H_2O$, 48.4 µM H_3BO_3, 10.3 µM $Na_2MoO_4 \cdot 2H_2O$, 95.9 µM $ZnCl_2$, 408 µM Fe(III) citrate; ZMY505: 0.4 µM $CoCl_2 \cdot 6H_2O$, 0.4 µM H_3BO_3, 0.4 µM $Na_2MoO_4 \cdot 2H_2O$, 10 µM $FeCl_3$, 4 µM $CaCl_2$, 2 µM $MnCl_2 \cdot H_2O$, 2 µM $ZnSO_4$, 0.4µM $CuCl_2 \cdot H_2O$, 0.4µM $NiCl_2$, 0.4 µM $NaSeO_3$; ZMY505m: 10 µM $CoCl_2 \cdot 6H_2O$, 0.4 µM H_3BO_3, 10 µM $Na_2MoO_4 \cdot 2H_2O$, 10 µM $FeCl_3$, 7 µM $CaCl_2$, 4 µM $MnCl_2$ H_2O, 4 µM $ZnSO_4$, 10 µM $CuCl_2$ H_2O, 1 µM $NiCl_2$, 0.4 µM $NaSeO_3$.

Table 2. Plackett-Burman experimental design for the evaluation of trace metals on pDNA production[a].

Trial	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11
1	+	+	-	+	-	+	-	-	-	+	+
2	-	+	+	-	+	-	+	-	-	-	+
3	+	-	+	+	-	+	-	+	-	-	-
4	+	+	-	+	+	-	+	-	+	-	-
5	-	+	+	-	+	+	-	+	-	+	-
6	-	-	+	+	-	+	+	-	+	-	+
7	-	-	-	+	+	-	+	+	-	+	-
8	+	-	-	-	+	+	-	+	+	-	+
9	-	+	-	-	-	+	+	-	+	+	-
10	+	-	+	-	-	-	+	+	-	+	+
11	-	+	-	+	-	-	-	+	+	-	+
12	+	-	+	-	+	-	-	-	+	+	-

[a] *E. coli* DH5α harbouring the pVAX-NH36 were grown in 250 ml flasks containing 30 ml of different media (12 trials with base medium like ZMY505 as the control medium), incubated at 37 °C and 200 rpm. Specific and volumetric pDNA yields were determined. Concentrations in the parenthesis indicate the low (-) and high (+) levels of trace metals used. Trace metals: X1 = $FeCl_3$ (10 - 100 µM); X2 = $MnCl_2 \cdot H_2O$ (1 - 4 µM); X3 = $CoCl_2 \cdot 6H_2O$ (1 - 10 µM); X4 = $ZnSO_4$ (4 - 40 µM); X5 = dummy variable; X6 = $CaCl_2$ (1 - 7 µM); X7 = $NiCl_2 \cdot$(1 - 10 µM); X8 = $CuCl_2 \cdot H_2O$ (1 - 10 µM); X9 = $NaSeO_3 \cdot$(1 - 10 µM); X10 = $Na_2MoO_4 \cdot 2H_2O$ (1 - 10 µM); X11 = dummy variable.

slope of the logarithm of dry cellular weight versus culture time.

Trace metals optimisation

The trace metals of the ZMY505 medium were optimised by using a Plackett–Burman design (Ahuja et al., 2004; Plackett and Burman, 1946) (Table 2). Nine trace elements and two dummies variables, to estimate the error, were analysed in twelve experiments done at least by triplicate. Specific and volumetric pDNA yields were used as a response parameter, and the F-test was performed to test the significance of the results. All experiments were done with *E. coli*

Table 3. Production of pDNA vaccine candidates against Leishmaniasis in flask cultures[a].

Plasmid	Media	Specific growth rate (h^{-1})	Dry cellular weight (g/l)	Specific pDNA yield (mg/g)	Volumetric pDNA yield (mg/l)	sc-pDNA (%)	pH[b]
pVAX-NH36	LB	0.71	1.19 ±0.01	8.93±0.8	10.62 ±0.7	72	8.4
	SDCAS	0.64	2.40 ±0.01	6.15 ±0.5	14.76 ±1.2	83	5.2
	ZMY505	0.73	2.72 ±0.07	9.15 ±0.7	24.88 ±1.1	80	5.4
VR1012-NH36	ZMY505	0.73	2.56±0.02	8.81±1.0	22.55 ±1.3	83	5.9

[a]*E. coli* DH5α harboring pVAX-NH36 were grown in 250 ml flasks containing 30 ml of LB, SDCAS, or ZMY505 media, VR1012-NH36 was produced only in ZMY505, at 37 °C and 200 rpm for 18 h. Dry cellular weight, specific growth rate and specific and volumetric pDNA yields were determined every 2 h as indicated in the Materials and Methods section. Data represent the mean ± SD of three replicates of the highest production of pDNA (14 h) observed. [b]pH at the end of the culture (18 h).

DH5α harbouring pVAX-NH36 in 250 ml flasks containing 30 ml of designated and control media (Table 2). The flasks were incubated at 37°C and 200 rpm for 18 h, and 1 ml samples were taken every 2h to determine the dry cell weight, specific and volumetric pDNA yields. The best ZMY505 modified medium (ZMY505m) was selected to perform next experiments.

Induction of plasmids with a shift in the culture temperature

Flasks with 30 ml of medium ZMY505m containing kanamycin (50 µg/ml) were inoculated with *E. coli* DH5α harbouring pVAX-NH36 and incubated at 37°C and 200 rpm. Then, the culture temperature was shifted from 37 to 42°C when the OD_{600} = 7 (late exponential growth phase) was maintained for 5 h. Dry cell weight, specific and volumetric pDNA yields, and sc-pDNA percentage were determined every hour.

Specific and volumetric pDNA yields

To determine specific and volumetric pDNA yields, clarified cell lysate containing plasmid DNA was prepared according to a protocol previously described (Sambrook et al., 1989). Cells from 1 ml samples, previously normalised by dilution to an OD_{600} = 0.6 – 0.8, were resuspended, alkaline lysed, neutralised and clarified by centrifugation.

The pDNA samples were resolved by 0.8% agarose gel electrophoresis using TBE buffer (45 mM Tris HCl, 45 mM H_3BO_3, 1 mM EDTA pH 8.0) at 70 V for 120 min and stained with ethidium bromide (5 µg/ml). Specific total pDNA and sc-pDNA yields were estimated by the densitometric analysis of pDNA bands by using the Quantity One Software version 4.6.5 (Bio-Rad, USA) and a known amount of a calibrated 1 kb DNA Ladder (New England Biolabs, USA) as a standard. Alternatively, the total and sc-pDNA were estimated by a fluorescence method reported by Levy et al. (2000); pDNA samples were incubated for 5 min at 25 or 95°C, then for 5 min at 4°C before adding PicoGreen dsDNA fluorophore (Invitrogen, USA). The fluorescence emission intensity of each sample was measured at 520 nm with excitation at 480 nm in a spectrofluorometer FluoroMax 3 (Jobin Yvon Horiba, USA). Volumetric pDNA yield (mg/l) was estimated with specific pDNA yield (mg/g) and dry cell concentration (g/l). The total pDNA and sc-pDNA percentage were estimated by the difference in fluorescence intensity between pDNA incubated at 25 and 95°C (Levy et al., 2000). No significant differences were observed in sc-pDNA yield determined with the two techniques.

RESULTS AND DISCUSSION

Cell growth and plasmid production in different media

To improve the sc-pDNA production, *E. coli* DH5α harbouring pVAX-NH36 was grown in LB, SDCAS, or ZMY505; two semi-defined media previously reported as specific for the production of pDNA. Specific growth rates (µ), dry cellular weight at the maximum specific pDNA, and volumetric yields for pVAX-NH36 in the three media and for VR1012-NH36 in the ZMY505 are shown in Table 3. Specific growth rates (0.64 to 0.73 h^{-1}) were similar in all media. The cellular growth (1.9 g/l) and pDNA yields obtained with LB (7.3 to 10.62 mg/l) and SDCAS (14.76 mg/l) were similar to values previously reported with the same medium (O'Kennedy et al., 2000), while the maximum volumetric yields obtained with ZMY505 (22.5 to 24.8 mg/l), with a dry cellular weight similar to those reported by Studier (2005), were indeed greater than with LB or SDCAS medium. In addition to the improvement in the volumetric and specific pDNA yields in SDCAS and ZMY505 as compared with LB medium, the quality of pDNA increased from 72 to 80-83% sc-pDNA for both plasmids. The final pH for SDCAS (5.2) and ZMY505 (5.4-5.9) indicates an accumulation of acetate at the end of the culture. These pH values are similar to those reported by Zheng et al. (2007) after 24 h incubation in semi-defined medium. Then, ZMY505 was chosen to optimise the trace metals by using a Plackett-Burman design (Table 2); results are shown in Figure 1. The best pDNA yield was obtained in trial 5 (37 mg/l); about 50% greater than the control medium (24.7 mg/l). Moreover, a plasmid quality higher than 80% sc-pDNA was obtained with trials 5 and 8. However, specific and volumetric yields of trial 8 were clearly lower. Then, the following experiments were done using the ZMY505 modified medium (ZMY505m) as formulated in trial 5 (Tables 1 and 2).

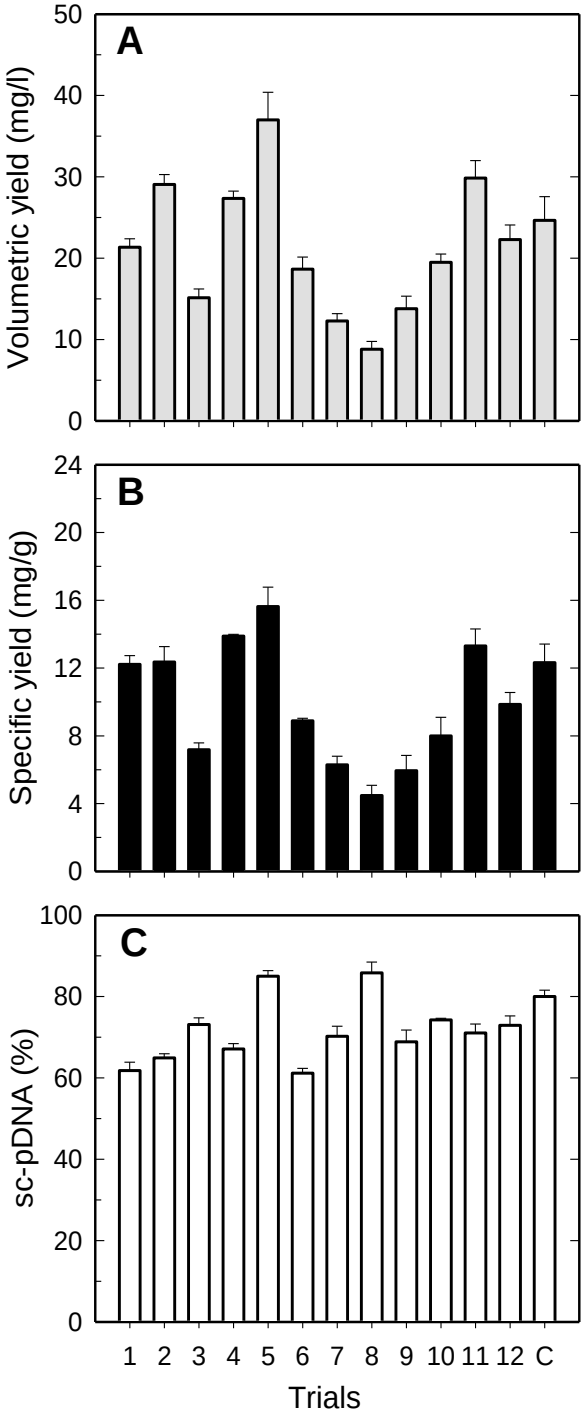

Figure 1. Optimisation of trace metals of the medium for pDNA vaccine production. *E. coli* DH5α harbouring pVAX-NH36 was grown in 250 ml flasks containing 30 ml of different media at 37°C and 200 rpm. **A**) Volumetric (gray bars). **B**) Specific pDNA yields (black bars). **C**) sc-pDNA percentage (white bars) were estimated by densitometry analysis of ethidium bromide stained 1% agarose gel and by fluorescence. Numbers represent twelve trials of Plackett-Burman design and **C** represents ZMY505 control. Each point represents the mean ± SD (error bars) of five replicates.

Cell growth and plasmid production in the ZMY505m medium

Figure 2 shows the dry cell weight (A), specific sc-pDNA (B), volumetric sc-pDNA yields (C), and percentage of sc-pDNA (D) profiles as a function of incubation time of *E. coli* DH5*α* harbouring pVAX-NH36 (empty symbols) or VR1012-NH36 (filled symbols) grown at 200 rpm and 37°C in ZMY505m. The dry cellular weight of *E. coli* DH5α harbouring pVAX-NH36 was slightly higher than VR1012-NH36 during the 18 h incubation, with values similar to those reported by Studier (2005), and consistent with the volumetric yields during the exponential phase. At the beginning of the stationary phase, (12 - 14 h) the pDNA yields reached the maximum with similar values for both constructs. After 14 h of culture, the specific and volumetric pDNA yield decreased; nevertheless the percentage of sc-pDNA was maintained about 80% for both constructs. Although, the volumetric yields obtained in this study were lower, the quality of pDNA is similar to previous reports using *E. coli* DH5α (Duttweiler and Gross, 1998; Wang et al., 2001; Zheng et al., 2007). Then, to induce an increase in the pDNA specific yield (plasmid copy number) a shift in the culture temperature from 37 to 42°C was tested.

Shift in the culture temperature

E. coli DH5*α* harbouring pVAX-NH36 (empty symbols) or VR1012-NH36 (filled symbols) were grown at 200 rpm and 37°C in ZMY505m, and after 12 h incubation (OD_{600} = 7), the culture temperature was shifted to 42°C (arrow) for 5 h. Figure 3 shows the dry cellular weight (A), specific pDNA (B), volumetric pDNA yields (C), and percentage of sc-pDNA (D) profiles as a function of the incubation time. As previously observed (Figure 2), the bacteria transformed with the smallest size construct (pVAX-NH36), reached a higher cell density, however, when the temperature was shifted (12 h, OD_{600} = 7), both cultures had a similar cell density and both reached a stationary phase at 16 h (Figure 3A).

Clearly, both specific (Figure 3B) and volumetric (Figure 3C) pDNA yields increased for both plasmids after shifting the culture temperature (arrow). The volumetric pDNA yields of 57 to 65 mg/l were two-fold higher than those obtained by maintaining the culture at 37°C (33 mg/l). Additionally, the specific and volumetric yields steadily increased during the 5 h incubation after shifting the culture temperature to 42°C, indicating that this temperature induced the plasmids production, even with a clear reduction in growth rate (Figure 3A). Interestingly, the maximum specific pDNA yield obtained at 37°C (10.67 to 13.67 mg/g) (Figure 2B) increased to 21 mg/g for pVAX-NH36 and 18 mg/g for VR1012-NH36 with a sc-pDNA profile higher than 80% for both plasmids. This volumetric pDNA yield was similar to that obtained with a defined medium (M1) formulated with

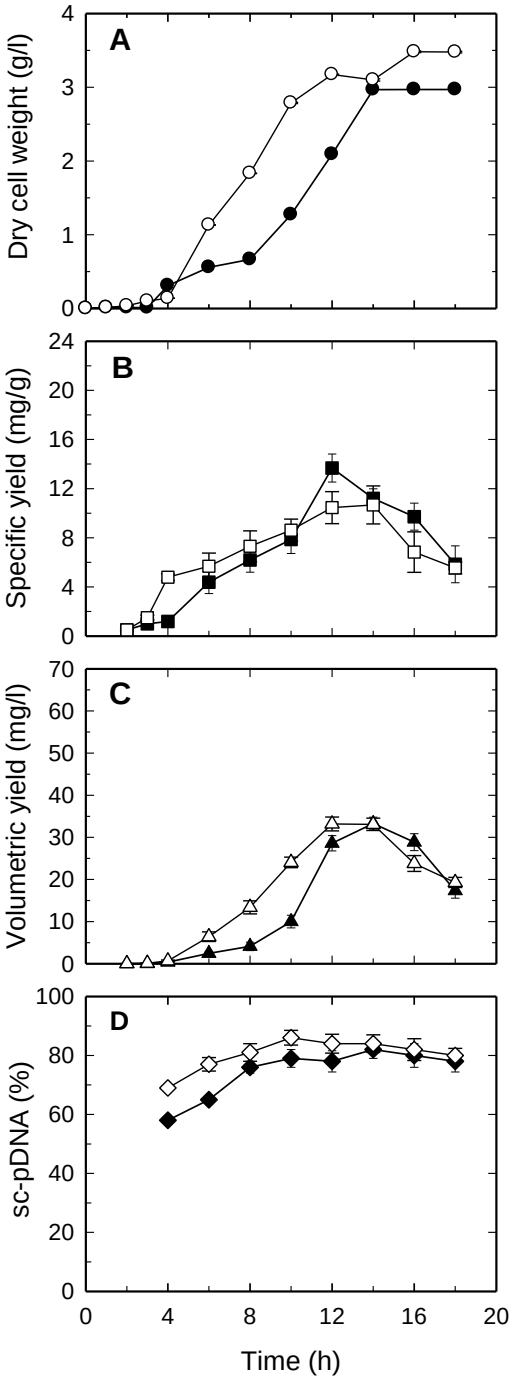

Figure 2. Production of pDNA vaccine candidates against Leishmaniasis in a semi-defined medium. *E. coli* DH5α harbouring VR1012-NH36 (filled symbols) or pVAX-NH36 (empty symbols) were grown in 250 ml flasks containing 30 ml of ZMY505m medium at 37 °C and 200 rpm. **A**) Dry cell weight (g/l). **B**) specific (mg/g). **C)** volumetric yield profiles (mg/l). D) sc-pDNA percentage were determined as a function of the incubation time. Dry cell weight (DCW) was estimated by OD_{600} using a standard curve of OD_{600} vs DCW previously obtained. The specific, volumetric pDNA yields, and sc-pDNA percentage were estimated by the densitometric analysis and fluorescence. Each point represents the mean ± SD (error bars) of five replicates.

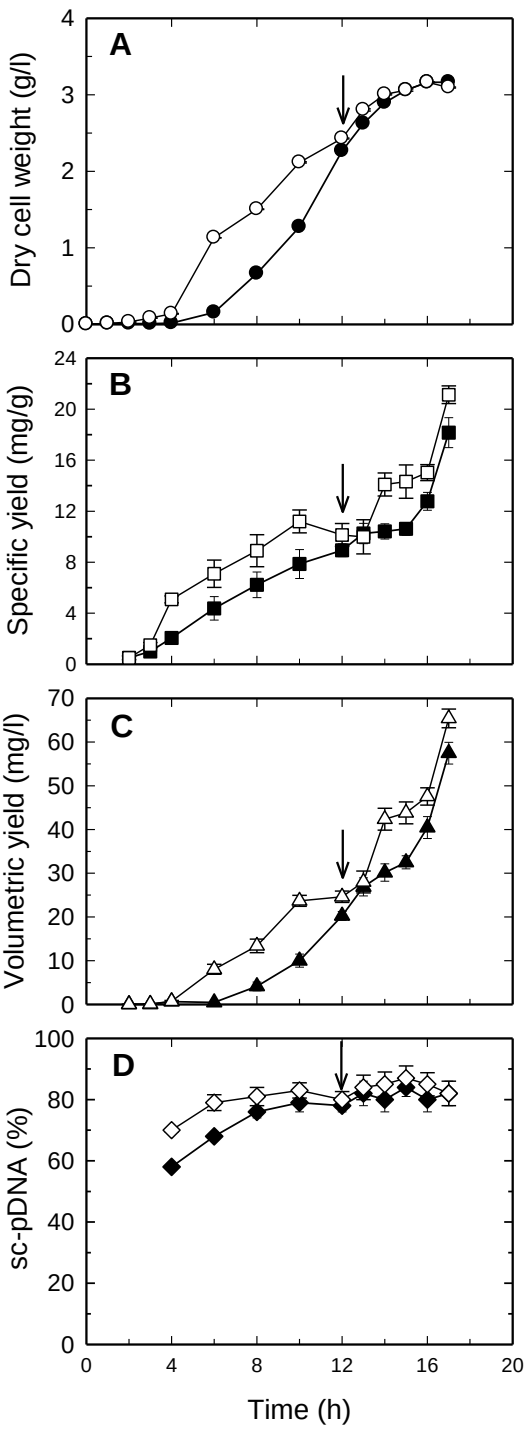

Figure 3. Temperature-induced amplification of plasmid DNA vaccine candidates against Leishmaniasis in a semi-defined medium. *E. coli* DH5α harbouring VR1012-NH36 (filled symbols) or pVAX-NH36 (empty symbols) were grown in 250 ml flasks containing 30 ml of ZMY505m medium at 37 °C and 200 rpm. When the OD_{600} = 7 (late exponential phase), the incubation temperature was shifted from 37 to 42 °C (arrow). **A**) Dry cell weight (g/l). **B**) specific (mg/g). **C**) volumetric (mg/l) pDNA yield profiles. **D**) sc-pDNA percentage were determined as a function of the incubation time. Each point represents the mean ± SD (error bars) of five replicates. See materials and methods for details.

amino acids and nucleosides, but was two-fold higher than pDNA yield obtained with (M2) medium without nucleosides (Wang et al., 2001), and about three-fold higher than pDNA yield reported with PDMR medium (Danquah and Forde, 2007) without the culture temperature shifting. Zheng et al. (2007), also without the temperature shifting, reported a pDNA yield of 51.80 mg/l but with incubation time of 24 h instead of 16 h in this work. Moreover, Duttweiler and Gross (1998) reported a production up to 109 to 87 mg/l of pDNA. Nevertheless, these yields were obtained for only two plasmids and by using a different *E. coli* strain. However, with *E. coli* DH5α, the other six plasmids using a strong buffered medium, dubbed “H15”, and incubation time of 24-30 h or even up to 48 h, obtained 12.6-48 mg/l pDNA yields that are lower than those reported in this work.

Conclusions

In summary, we have shown that the production of sc-pDNA in flask cultures by standard laboratory protocols can be improved up to six-fold by using a semi-defined medium and shifting the culture temperature from 37 to 42°C in the late exponential phase. The plasmid volumetric (65 - 57 mg/l) and specific yields (21 - 18 mg/g), as well as the quality of pDNA (> 80% sc-pDNA) obtained in flask cultures, seem sufficient for the purification of these pDNA vaccine candidates against leishmaniasis at a laboratory scale. This strategy could be a cost-effective alternative to obtain sc-pDNA at the initial steps of pDNA vaccine development.

ACKNOWLEDGMENTS

The authors acknowledge the support of Cinvestav, UADY, Instituto de Ciencia y Tecnología del Distrito Federal (ICyTDF) grant PIFUTP08-108 (to J.O.L.) and Consejo Nacional de Ciencia y Tecnología (CONACyT) for a scholarship 167077 for M.S.C. We thank Claudia Ivonne Flores-Pucheta and Maria Eugenia Zuñiga Trejo for their technical assistance.

REFERENCES

Aguilar-Be I, Silva-Zardo R, et al. (2005). Cross-protective efficacy of a prophylactic Leishmania donovani DNA vaccine against visceral and cutaneous murine leishmaniasis. Infect. Immun. 73 (2): 812 – 819.

Ahuja SK, Ferreira GM, et al. (2004). Application of Plackett-Burman design and response surface methodology to achieve exponential growth for aggregated shipworm bacterium. Biotechnol Bioeng 85(6): 666-675

Borja GM, Meza Mora E, Barron B, Gosset G, Ramirez OT, Lara AR (2012). Engineering Escherichia coli to increase plasmid DNA production in high cell-density cultivations in batch mode. Microb. Cell. Fact. 11: 132

Carnes A, Luke J, et al. (2011). Plasmid DNA fermentation strain and process-specific effects on vector yield, quality and transgene expression. Biotechnol. Bioeng. 108 (2): 354 – 363

Danquah M and Forde G (2007). Growth medium selection and its economic impact on plasmid DNA production. J. Biosci. Bioeng. 104 (6): 490 – 497

Ferreira GN, Monteiro GA, et al. (2000). Downstream processing of plasmid DNA for gene therapy and DNA vaccine applications. Trends Biotechnol. 18 (9): 380 – 388.

Duttweiler HM, Gross DS (1998). Bacterial growth medium that significantly increases the yield of recombinant plasmid. BioTechniques 24: 438 - 444

Gamboa-Leon R, Paraguai de Souza E, et al. (2006). Immunotherapy against visceral leishmaniasis with the nucleoside hydrolase-DNA vaccine of Leishmania donovani. Vaccine 24(22): 4863-4873.

Herrera-Najera C, Pina-Aguilar R, et al. (2009). Mining the Leishmania genome for novel antigens and vaccine candidates. Proteomics 9(5): 1293-1301.

Hotez P (2008). Forgotten People, Forgotten Diseases: The Neglected Tropical Diseases and Their Impact on Global Health and Development, ASM Press. Washington D.C., USA. 95-102.

Levy M S, Lotfian P, et al. (2000). Quantitation of supercoiled circular content in plasmid DNA solutions using a fluorescence-based method. Nucleic Acids Res. 28(12): E57.

O'Kennedy R D, Baldwin C, et al. (2000). Effects of growth medium selection on plasmid DNA production and initial processing steps. J Biotechnol. 76(2-3): 175-183.

O'Kennedy R D, Ward JM, et al. (2003). Effects of fermentation strategy on the characteristics of plasmid DNA production. Biotechnol. Appl. Biochem. 37(Pt 1): 83-90.

Ongkudon CM, Pickering R, et al. (2011). Cultivation of *E. coli* carrying a plasmid-based Measles vaccine construct (4.2 kbp pcDNA3F) employing medium optimisation and pH-temperature induction techniques. Microb. Cell. Fact. 10: 16.

Phue JN, Lee SJ, et al. (2008). Modified *Escherichia coli* B (BL21), a superior producer of plasmid DNA compared with *Escherichia coli* K (DH5α). Biotechnol. Bioeng. 101(4): 831-836.

Plackett RL, Burman J P (1946). The design of optimum multifaltorial experiments. Biometrika. 33(4): 305-325.

Sambrook J, Fritsch EF, et al. (1989). Molecular Cloning: A Laboratory Manual. New York, USA, Cold Spring Harbour Laboratory Press. 1.21-1.31, 5.4 - 5.12, 8.18 - 8.20.

Studier FW (2005). Protein production by auto-induction in high density shaking cultures. Protein Expr. Purif. 41(1): 207-234.

Tejeda-Mansir A, Montesinos RM (2008). Upstream processing of plasmid DNA for vaccine and gene therapy applications. Recent Pat. Biotechnol. 2 (3): 156-172.

Wang Z, Shi Y, Wegrzyn G (2001). Medium design for plasmid DNA production based on stoichiometric model. Process Biochem. 36: 1085 - 1093.

Zheng S, Friehs K, He N, et al. (2007). Optimization of Medium Components for plasmid production by Recombinant E. Coli DH5α pUK21CMVβ1.2. Biotechnol. Bioprocess Eng. 12: 213 – 221