JOURNAL OF PURE AND APPLIED MICROBIOLOGY, Dec. 2015. Vol. **9**(4), p. 3255-3263

Fed-Batch Fermentation and Downstream Processing for Large-Scale Production of Recombinant *Pf*-Lactate Dehydrogenase and Its Application in Malaria Diagnosis and as a Platform for Screening of Antimalarial Chemotherapeutic Agent

Saurabh Shrivastava[1], Anil K Singh[1], Kshitij chandel[2], Ritu Gill[3] and N. Gopalan[1]*

[1]Bio-process Scale up Facility, Defence Research and Development Establishment, Ministry of Defence (Govt. of India), Gwalior - 474 002, India.
[2]Vector Management Division, Defence Research and Development Establishment, Ministry of Defence (Govt. of India), Gwalior, - 474 002, India.
[3]Centre for Biotechnology, MD University, Rohtak-124001, India.
[4]Madhav Institute of Technology and Science, Gwalior, India.

(Received: 10 May 2015; accepted: 20 July 2015)

The potential of malaria parasite (*Plasmodium falciparum*) generating resistance to currently used antimalarial drugs is a major curse for malaria patients. The multiple targets on which antimalarial drugs act hamper the occurrence of such resistance. As an essential metabolic enzyme involved in energy production in the parasite, the lactate dehydrogenase of *P. falciparum* (*Pf*LDH) provides an alternative target for parasite killing. In the present study, we have efficiently optimized the fed-batch fermentation and downstream processing for high yield production of recombinant *Pf*LDH using *Escherichia coli*. The fed-batch operation gives the freedom of manipulating the process via substrate feed rate. The r*Pf*LDH was produced in previously optimized media (Modified Terrific Broth) by fed-batch fermentation using 5 L bioreactor. Fed-batch fermentation resulted in a wet weight of 98.6 g/L and dry cell biomass 24.2 g/L. With the improved downstream process, purified r*Pf*LDH had a yield of 461.20 mg/L. Expression and purification were optimized and the expressed recombinant *Pf*LDH was verified by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting. The recombinant *Pf*LDH was purified for uniform measurement of enzymatic activity and immobilized on 96-well plates for detection of *Pf*LDH-targeted inhibitors. Hematin (HT) and Gossypol (GP) three well-known inhibitors of *Pf*LDH, were chosen as positive inhibitors for evaluating the feasibility of the in-vitro colorimetric enzyme assay. The fed-batch fermentation and downstream processing methods optimized in this study have enormous application for high yield production of recombinant pLDH in a cost effective manner using bacterial system. The large scale production of recombinant *Pf*LDH is useful in the diagnosis during the incidence of malarial infection and also it can be used as economic potential target site for the screening of chemotherapeutic agents for the development of novel antimalarial drugs.

Keywords: *Pf*LDH, Fed-batch fermentation, Malaria diagnosis, Antimalarial

Malaria is a major public health problem. An estimated 3.3 billion people were at risk of malaria. There were an estimated 216 million episodes of malaria in 2010. There were an estimated 655 000 (537 000 – 907 000) malaria deaths in 2010. Approximately 86% of malaria deaths globally were of children under 5 years of age. Malaria caused by five species of parasites of the genus

* To whom all correspondence should be addressed.
Mob.: +91-9179936322;
E-mail: natagopalan@hotmail.com

Plasmodium that affect humans (*P. falciparum, P. vivax, P. ovale, P. malariae* and *P. knowlesi*[16, 17]), malaria due to *P. falciparum* is the most deadly[1]. According to the WHO (2010), global eradication of malaria is unachievable, because of the emergence of drug resistance in *P. falciparum*. The emergence of drug resistance means that many antimalarial drugs and prophylactics have become less effective.

Drug resistance has emerged as one of the greatest challenges facing malaria control today. There is a need of unique target site against which the antimalarials could be screen. *Pf*LDH, as an essential metabolic enzyme responsible for energy production in the parasite, *Pf*LDH catalyzes dehydrogenation of lactate and generates pyruvate by using NAD^+ as a cofactor. The inhibition of *Pf*LDH in the parasite leads to parasite death, suggesting a potential antimalaria target[3, 9-15]. The suitability of *P. falciparum* lactate dehydrogenase (*Pf*LDH) as an antimalarial target was further confirmed with the elucidation of the crystal structure of the enzyme which revealed the inserted amino acids formed a cleft adjacent to the active site that might be suitable for binding inhibitors[10]. Further apart from antimalarial target, pLDH is a well known diagnostic target owing to presence of unique epitopes as compared to human lactate dehydrogenase (LDH) isoforms[18, 19].

The efficacy of conventional antimalarial drugs and insecticides in controlling falciparum malaria outbreaks is declining with increasing resistance of parasites and their vectors[4-8]. The renewed efforts are required to develop novel and affordable antimalarials to defeat the detrimental effects of drug resistance, especially in emergent countries. In the present study, we produced r*Pf*LDH in large-scale and the activity of r*Pf*LDH was established by using Western blotting, ELISA, enzymatic assay and drug sensitivity assay/drug screening. This suggested that the produced *Pf*LDH would potentially be of value in detecting *Plasmodium* as well as for further drug screening to develop the novel antimalarial drugs. Hence the study has been done to develop the fed-batch fermentation process and the downstream processing for the large-scale production of r*Pf*LDH.

MATERIAL AND METHODS

Micro-organism

The micro-organism used in the present study was *E. coli* SG13009 harbouring recombinant plasmid pQE-30Xa containing *Plasmodium falciparum* Lactate Dehydrogenase (pQE-30Xa-*Pf*LDH) gene[20]. Stock cultures of the micro-organisms were maintained in 30% (v/v) glycerol at -80ºC.

Media and Solutions

The combination of medium ingredients has a profound influence on the metabolic pathways running in the microorganism which regulates the production of numerous metabolites. Since, the small scale expression study by optimizing the best suitable growth medium and different culture condition has been done [Unpublished data]. As a consequence, the modified Terrific Broth with the optimized formulation [Terrific broth, (Hi-media, Mumbai, India) containing 8 ml/L glycerol, 2X-M9 salts (Difco, USA), 2X-Yeast extract, 1.2 ml/L of $Mgcl_2$ and 1 ml/L trace metal solution] was selected as a best suitable growth medium (pH7.2) and 1mM IPTG was used to induce the expression of r*Pf*LDH in the present study.

Fed-batch fermentation

The fed-batch fermentation was carried out in a fully automated bench top fermentor (Bioflo 3000; New Brunswick Scientific, Edison, NJ, U.S.A.) equipped with a 5 litre working volume glass vessel and stainless steel headplate. The fermentor was attached with a computer which interfaced with Biocommand Plus software (New Brunswick Scientific). The software was used for data acquisition and operation of the fermentor in fed-batch mode as per the instructions manual from the manufacturer. The calibration of pH probe was done before the sterilization of medium using standard buffer (pH 9, 7 and 4) as per manufacturer instruction manual. DO probe (Mettler-Toledo, Geissen, Germany) was calibrated after polarizing and before the addition of inoculum as per the manufacturer instruction manual. The culture medium (4.5 litres), pH probe and DO probe was inserted to the vessel and sterilized by exposure at 121ºC, 15 lbf/in^2 for 15 min. A pre-inoculation sterility check was done by holding the medium

for a minimum period of 15 h after the sterilization. During the time the turbidity, pH, agitation, temperature was monitored continuously and the DO probe was polarized in the mean time. The antibiotic (100 µg of ampicillin/ml) was added into the fermentation vessel by injection through injection port. The primary seed culture was grown in a 200 ml shake flask containing 13.5 ml modified terrific broth with antibiotic (100 µg of ampicillin/ml) by inoculating the 1.5 ml frozen glycerol stock of the clone expressing r*Pf*LDH. The culture was incubated at 37ºC at 200 rpm in shaker incubator (New Brunswick Scientific) for 8 h. Further, the secondary seed culture was cultured in a 2.5 L shake flask containing 250ml of modified terrific broth with antibiotic (100 µg of ampicillin/ml) by inoculating the 15 ml primary seed culture and the culture was incubated at 37ºC at 200rpm for overnight. The 5%(v/v) of the overnight grown secondary seed culture was inoculated through the inoculation port of the head plate into the fermentor containing pre-sterilized 4.5L modified terrific broth with antibiotic (100 µg of ampicillin/ml), the A_{600} was recorded and the initial fermentation condition was recorded as follows- Initial culture medium volume 4.75 L, temperature 37ºC, airflow rate 3.0 L/min and agitation 100 rpm. The DO was controlled in a range of 30–70% either by agitation (100-500rpm), airflow (2.0-6.0L/min) or enriched airflow with pure oxygen (5-10%). Temperature was maintained (initially temperature: 37ºC and post-induction temperature 22ºC) by circulating water with chiller system (Zulabo, USA). The pH was maintained at 7.2 ±0.2 using 10% (v/v) liquid ammonia and 0.5 N hydrochloric acid as required and the foaming was controlled by the addition of active silicon polymer antifoam A emulsion (A-6457, Sigma). The culture was induced with 1 mM IPTG at OD 0.862. After depletion of the initial nutrients in the batch medium, as indicated by an increase in the DO concentration, constant or intermittent feeding was carried out under nutrient-limiting conditions. The batch was terminated after 8h of post induction. The fermentation culture was centrifuged (Sorvell-RC5C) at 8000 ×g for 15 minutes at 4ºC. The centrifuged cell biomass was washed with cell wash buffer (10mM Tris, 100mM NaCl and 10mM EDTA; pH 8.0) and stored at -80ºC until the further use of downstream processing.

Biomass analysis

The cell density/ Wet cell weight (WCW) and dry cell weight (DCW) analyses were carried out by manual sampling of the fermentor culture through sampling port. The A_{600} of the culture was measured using a spectrophotometer (Thermo, Electron corp., USA) after each 30 minute time interval. The 2 ml of culture were centrifuged at 10,000×g for 5 min. The supernatant was completely removed and the remaining pellet was dried into an oven at 100ºC for overnight.

Purification of rPfLDH using IMAC affinity chromatography

The r*Pf*LDH protein was purified using Ni-NTA affinity column (Qiagen, Germany) chromatography using AKTA explorer as per the standard protocol of the manufacturer. The 2.5 g of fermented cell washed pallet was suspended in 50 ml pre-chilled cell lysis buffer (20 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole, 1 mM PMSF, pH 8.0) and lysed by sonication (10 × 30 s pulses with 45 s interval) for 15 min using high gain probe set at 40% frequency of sonicator (Sonics Inc., Plainview, NJ, U.S.A.) The lysate was centrifuged at 12,000g for 15 min and the resulting supernatant (5 ml) was loaded onto pre-pack chromatography column containing Ni-NTA slurry (Qiagen) which was pre-equilibrated with lysis buffer. The column was then washed with a step gradient of 20-150 mM imidazole (in buffer containing 50 mM NaH_2PO_4, 300 mM NaCl, pH 8.0). This was followed by elution of r*Pf*LDH with 350 mM imidazole containing elution buffer (50 mM NaH_2PO_4, 300 mM NaCl, pH 8.0). The eluted protein was collected as 1 ml fractions and individually analyzed on SDS-PAGE.

Dialysis and concentration using MWCO

The purified eluted fractions containing the highest amount of recombinant protein were pooled and concentrated using centrifugal ultra filtration devices (10KDa, MWCO). The concentrated purified r*Pf*LDH was further analyzed *via* SDS-PAGE and protein concentration was estimated using Pierce BCA kit (Pierce, USA) following the manufacturer's instructions.

Analysis of Purified rPfLDH via SDS-PAGE and Western blotting

The analysis of purified r*Pf*LDH was performed to check the authenticity of the protein. Samples were analysed over 10% SDS-PAGE [21].

Gels were stained with Coomassie R-250 Brilliant Blue stain. To confirm the identity of r*Pf*LDH, the purified protein was separated by 10% SDS-PAGE under reducing conditions. The unstained separated proteins were transferred electrophoretically on to polyvinyl difloride (PVDF) membrane (Millipore, USA) using semidry transfer unit (Biorad, USA). The membrane was blocked with 2% (w/v) BSA in PBS and incubated at 4ºC overnight. Blocked membrane was washed three times each time for 5 min with PBS with 0.05% (v/v) Tween 20 and incubated with HRP conjugated anti-His antibodies in PBS at 1:2000 dilution at 37ºC for 1 h. The membrane was washed again as above. The blot was developed with DAB-H_2O_2 chromogen-substrate mixture.

Evaluation of purified rPfLDH for diagnostic potential

To evaluate the diagnostic potential of purified r*Pf*LDH which we had produced by fed-batch fermentation, we performed the indirect ELISA by coating the 100ng/well of the purified r*Pf*LDH concentration onto MaxiSorp Immuno plates (Nunc, Denmark). The wells were reacted in triplicates with fifty four previously tested [through microscopic examination (Normalstaining/ fluroscence) and BinaxNOW malaria ICT strip test (Binax, Inc, USA)] different human clinical sera samples (49 positive and 5 negative; obtained from ISPAT general hospital, Rourkela-769 005) at 1:500 dilution for 60 min at 37°C along with healthy sample as negative control. The samples were collected with the complete patient history (Table 1). The detailed procedure described earlier [HRP ii, Data not shown]. The samples with twice the OD values of the negative control plus 2 standard deviation (SD) value were considered to be positive.

Kinetic analysis and Screening of antimalarial chemotherapeutic agents using purified rPfLDH as a platform

The spread of resistance to antimalarial drugs over the past few decades has led to an intensification of new platform to allow screening of antimalarial drugs. In order to evaluate the kinetics activity and the potential as a platform for screening the antimalarial chemotherapeutic agents of the *purified rPfLDH* what we have produced by the fed-batch fermentation, we performed the enzyme kinetic assay in 250µl reaction volume using the enzyme concentration 3.00nM as described previously[20]. The Michaelis constants for substrates (pyruvate, lactate) and cofactors (NADH, NAD^+, $APAD^+$) were determined from double reciprocal Lineweaver-Burk plots of initial rate measurements at varied concentrations. 8 experimental points had been taken to determine the *K*m value. Catalytic rate constants (*k*cat) and the catalytic efficiency (*k*cat/*K*m) were calculated. In order to establish our purified r*Pf*LDH as a platform for screening the antimalarial chemotherapeutic agents we selected the three well known *Pf*LDH-targeted inhibitors i.e., GP and HT as positive inhibitors. The dissociation constant (*K*i) for all the three were determined from double reciprocal plots by linear regression analysis[11, 22]. Stock solution (0.1M) of gossypol was prepared in dimethylsulphoxide (DMSO). Final concentrations of DMSO during initial rate measurements did not exceed 0.5%. All experiments were repeated five times to obtain conclusive results.

RESULTS AND DISCUSSION

Fed-batch fermentation

The potential requirement of this protein in larger quantity led to its production in fermenters. Since the primary goal of any fermentation research is, to develop a cultivation method that allows the cost effective production and maximization of the yield of desired product. The composition of the growth medium is crucial for enhancing the desired product formation as well as acetate reduction. The *E. coli* produces the acetate under oxygen-limiting conditions[2] or in the presence of excessive glucose under aerobic conditions, when carbon flux in the central metabolic pathway exceeds its biosynthetic demand and the capacity for energy generation within the cell. Hence, we used the Modified terrific broth supplemented with glycerol in the present study. The fermenter provides the opportunity to control multiple factors to maintain an optimal culture environment for cellmass accumulation and protein production[23]. In the present study, the r*Pf*LDH was produced by fed-batch fermentation process. The method utilized for nutrient feeding and to maintain the demand of oxygen is very crucial in fed-batch fermentation, as it affects both

Table 1. Comparative evaluation of rPfLDH based IgM ELISA with other standard tests

S. No.	Sample ID	Sex / Age	Days of Infe-ction	Microscopy	*Pf*LDH Ag detection BinaxNow Malaria ICT	*Pf*LDH Ag detection SD Malaria Ag *Pf* ELISA	Anti-*Pf*LDH IgM detection by ELISA using r*Pf*LDH
1.	1(K)	M/50	10	*P.falciparum* (+)	*P.falciparum* (+)	*P.falciparum* (+)	+
2.	2(O)	M/19	5	*P.falciparum & vivex* (+)	*P.falciparum & vivex* (+)	*P.falciparum* (+)	+
3.	3(8H)	F/50	15	*P.falciparum* (+)	*P.falciparum* (+)	*P.falciparum* (+)	+
4.	4(24E)	M/70	4	*P.falciparum* (+)	*P.falciparum* (+)	*P.falciparum* (+)	+
5.	5(26E)	M/16	5	*P.falciparum* (+)	*P.falciparum* (+)	*P.falciparum* (+)	+
6.	6(30E)	M/56	15	*P.falciparum* (+)	*P.falciparum* (+)	*P.falciparum* (+)	+
7.	7(52)	F/42	10	*P.falciparum* (+)	*P.falciparum* (+)	*P.falciparum* (+)	+
8.	9(M)	M/50	7	*P.falciparum & vivex* (+)	*P.falciparum & vivex* (+)	*P.falciparum & vivex* (+)	+
9.	10(34)	F/65	5	*P.falciparum & vivex* (+)	*P.falciparum & vivex* (+)	*P.falciparum*	-
10.	11(27)	F/47	8	*P.falciparum* (+)	*P.falciparum* (+)	*P.falciparum* (+)	+
11.	12(20)	F/50	8	*P.falciparum* (+)	*P.falciparum* (+)	*P.falciparum* (+)	+
12.	13(19)	M/29	10	*P.falciparum* (+)	*P.falciparum* (+)	*P.falciparum* (+)	+
13.	14(11E)	M/15	10	*P.falciparum* (+)	*P.falciparum* (+)	*P.falciparum* (+)	+
14.	15(5RH)	M/40	15	*P.falciparum* (+)	*P.falciparum* (+)	*P.falciparum* (+)	+
15.	16(25E)	F/20	10	*P.falciparum* (+)	*P.falciparum* (+)	*P.falciparum* (+)	+
16.	17(4E)	M/27	5	*P.falciparum* (+)	*P.falciparum* (+)	*P.falciparum* (-)	+
17.	18(31)	M/60	12	*P.falciparum* (+)	*P.falciparum* (+)	*P.falciparum* (+)	+
18.	19(13RH)	F/15	6	*P.falciparum* (+)	*P.falciparum* (+)	*P.falciparum* (+)	+
19.	20(14RH)	M/18	9	*P.falciparum* (+)	*P.falciparum* (+)	*P.falciparum* (+)	+
20.	21(9E)	M/40	5	*P.falciparum & vivex* (+)	*P.falciparum & vivex* (+)	*P.falciparum & vivex* (+)	+
21.	22(14E)	F/50	7	*P.falciparum* (+)	*P.falciparum* (+)	*P.falciparum* (+)	+
22.	23(27E)	F/23	5	*P.falciparum* (+)	*P.falciparum* (+)	*P.falciparum* (+)	+
23.	24(26E)	F/60	7	*P.falciparum* (+)	*P.falciparum* (+)	*P.falciparum* (+)	+
24.	25(40E)	M/32	15	*P.falciparum* (+)	*P.falciparum* (+)	*P.falciparum* (+)	+
25.	26(10E)	M/55	10	*P.falciparum* (+)	*P.falciparum* (+)	*P.falciparum* (+)	+
26.	27(D)	M/20	11	*P.falciparum* (+)	*P.falciparum* (+)	*P.falciparum* (+)	+
27.	28(2E)	F/22	5	*P.falciparum* (+)	*P.falciparum* (+)	*P.falciparum* (+)	+
28.	29(65)	M/8	7	*P.falciparum & vivex* (+)	*P.falciparum & vivex* (+)	*P.falciparum & vivex* (+)	+
29.	30(66)	M/8	7	*P.falciparum* (+)	*P.falciparum* (+)	*P.falciparum* (+)	+
30.	31(9E)	M/42	10	*P.falciparum* (+)	*P.falciparum* (+)	*P.falciparum* (+)	+
31.	32(16)	F/25	7	*P.falciparum* (+)	*P.falciparum* (+)	*P.falciparum* (+)	+
32.	33(F)	F/65	8	*P.falciparum* (+)	*P.falciparum* (+)	*P.falciparum* (+)	+
33.	34(4E)	M/7	7	*P.falciparum* (+)	*P.falciparum* (+)	*P.falciparum* (+)	+
34.	35(1E)	F/35	8	*P.falciparum* (+)	*P.falciparum* (+)	*P.falciparum* (+)	+
35.	36(G)	M/25	7	*P.falciparum* (+)	*P.falciparum* (+)	*P.falciparum* (+)	+
36.	37(32)	M/60	13	*P.falciparum* (+)	*P.falciparum* (+)	*P.falciparum* (+)	+
37.	38(17E)	M/49	15	*P.falciparum* (+)	*P.falciparum* (+)	*P.falciparum* (+)	+
38.	39(F)	M/35	15	*P.falciparum* (+)	*P.falciparum* (+)	*P.falciparum* (+)	+
39.	40(6)	F/68	7	*P.falciparum & vivex* (+)	*P.falciparum & vivex* (+)	*P.falciparum & vivex* (+)	+
40.	41(I)	M/10	6	*P.falciparum & vivex* (+)	*P.falciparum & vivex* (+)	*P.falciparum* (-)	-
41.	42(75)	F/32	10	*P.falciparum & vivex* (+)	*P.falciparum & vivex* (+)	*P.falciparum & vivex* (+)	+
42.	43(76)	M/26	10	*P.falciparum* (+)	*P.falciparum* (+)	*P.falciparum* (+)	+
43.	44(1E)	M/5	10	*P.falciparum* (+)	*P.falciparum* (+)	*P.falciparum* (+)	+

the cell density and cell productivity. During the fermentation process, the nutrient become exhausted and the demand of oxygen also increased. Constant or intermittent feeding is carried out under nutrient-limiting conditions and the DO was maintained in a range of 30–70% either by agitation (100-500rpm), airflow (3.0-6.0L/min) or enriched airflow with pure oxygen (5-10%) (figure 1). The pH (7.2) was maintained constant throughout the process by injecting either 10% ammonia or 1M HCl as required; since, the pH begins to rise mainly as a result of increase in the

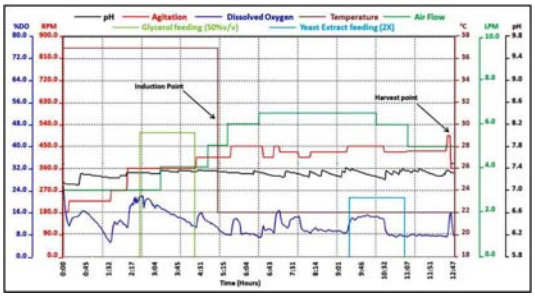


Fig. 1.

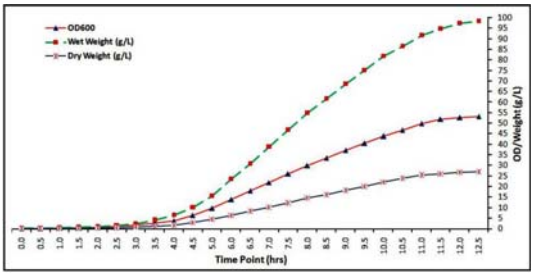
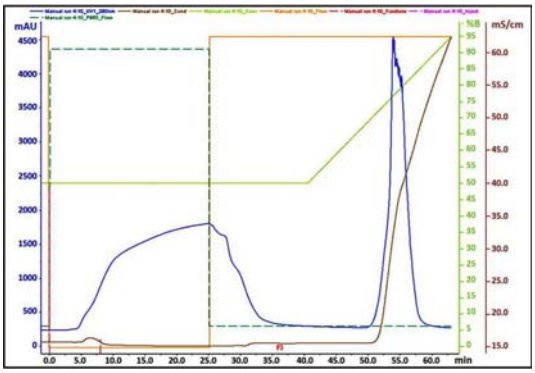


Fig. 2.



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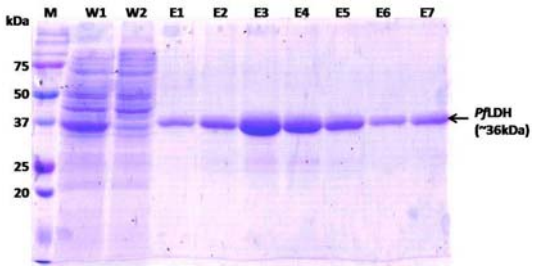

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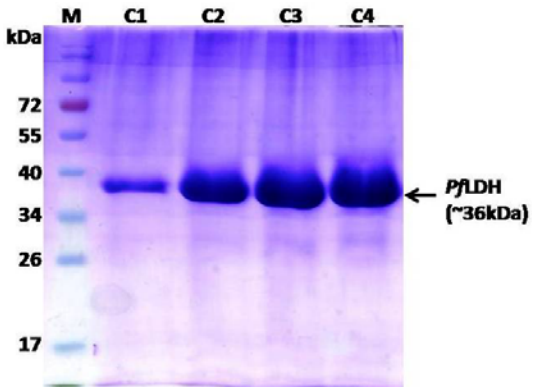

Fig. 5.

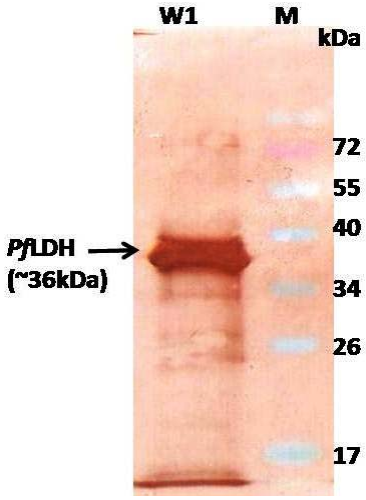

Fig. 6.

concentrations of ammonium ions excreted/ secreted by the cells, when the carbon source is exhausted. The temperature was maintained (initial temperature: 37ºC and post-induction temperature 22ºC) by circulating water with chiller system, to get the r*Pf*LDH biologically active. The culture was induced with 1mM IPTG at OD 0.862 (figure 2). The batch was run for further 8 h after post induction (Total time 12.5 h). With increase in the biomass, the oxygen demand also increased in fed-batch fermentation process. At the time of harvesting, this fed-batch process yielded OD of approximately 53 (figure 2), wet and dry cell weights of approx. 98.6 and 26.2 g/L respectively (figure 2), which were comparable with our previous study [HRP-II reference]. Once feeding is initiated and *E. coli* enters into exponential phase, the feed is consumed more or less in an exponential manner. But the feeding rate has to be controlled so that it does not exceed the nutrient demand or feed consumption rate. This is done by maintaining the pH and DO at about their set values. A fall in pH and DO is an indication of substrate overdosing. Rise in pH and DO values indicate that the carbon source or one of the substrates is limiting and hence feed is required. The maximum amount of r*Pf*LDH was attained after 8 h post induction. The real-time profile of fed-batch fermentation for the production of r*Pf*LDH is shown in Figure 1.

Biomass analysis

Analysis of dry cell weight with respect to A_{600} revealed that the relationship between both the parameters was linear, confirming that A_{600} accurately measured cell density. At the time of harvesting the batch the OD was 53, the wet cell weight (WCW) of 98.6 g/L and dry cell weight (DCW) of 26.2 g/L at end of the batch (figure 2). By the biomass analysis we correlated that the 1 OD_{600} was corresponds to 1.86 g/L of wet cell weight and 0.49 g/L of dry cell weight.

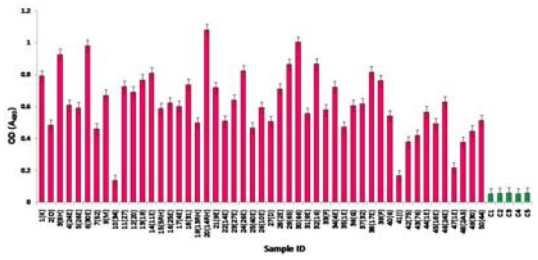

Fig. 7

Purification of rPfLDH using IMAC affinity chromatography

The purification of r*Pf*LDH was done by IMAC affinity chromatography using AKTA explorer system. The soluble fraction of r*Pf*LDH was purified using Ni-NTA column in this study. The purification was done as described in material and methods. The r*Pf*LDH sample was bound with the pre-equilibrated column and gave a peak between 125-180 min during elution (Figure 3). The different fractions of wash and elutes were analyzed over SDS-PAGE, a single band corresponding to ~36kDa was observed (Figure 4).

Dialysis and concentration using MWCO

The concentration was increased using centrifugal ultra filtration devices (10KDa, MWCO). The concentrated purified r*Pf*LDH was analyzed over SDS-PAGE (Figure 5) and protein concentration was found 351mg/L.

Western blot analysis

Western blotting using anti-HIS monoclonal antibodies confirmed the authenticity of the purified protein. The purified recombinant *Pf*LDH gave a positive signal with anti-HIS monoclonal antibodies but fraction from un-induced cell lysate did not show any signal as seen in Figure 6.

Evaluation of purified rPfLDH for diagnostic potential

Evaluation of diagnostic potential of recombinant *Pf*LDH to detect the *P. falciparum* IgM antibodies was carried out through Indirect ELISA. We have taken fifty four previously tested [through microscopic examination (Normal staining/fluroscence) and BinaxNOW malaria ICT strip test (Binax, Inc, USA)] human clinical sera samples (49 positive and 5 negative; obtained from ISPAT general hospital, Rourkela-769 005). We were found all the forty nine samples to be positive for IgM antibodies and no significant OD in healthy sera samples by indirect ELISA (Figure 7). These results of ELISA have confirmed the immunoreactivity of this recombinant *Pf*LDH as it selectively reacts only with positive sera samples and not with the negative ones.

Kinetic analysis and Screening of antimalarial chemotherapeutic agents using purified rPfLDH as a platform

CONCLUSION

Our study confirming that the r*Pf*LDH produced by the above process is a usable diagnostic potential as well as a target for screening and designing novel antimalarial drugs. However, the present study is a preliminary. As our main objective was focused on high yield production and purification of recombinant *Pf*LDH. Further evaluation was beyond the scope of this study.

ACKNOWLEDGEMENTS

The authors thankful to Director, Defence Research & Development Establishment (DRDE), Ministry of Defence, Government of India, Jhansi Road, Gwalior for his constant support and providing necessary facilities for this study. The authors are thankful to PVL Rao, Director, DRDO-BU centre, Coimbatore and Dr. Prativa K Behra, ISPAT General Hospital, Rourkela for the clinical samples.

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