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Effects of novel auto-inducible medium on growth, activity and CO_2 capture capacity of *Escherichia coli* expressing carbonic anhydrase

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ABSTRACT

A glucose-based auto-inducible medium (glucose-AIM) has been developed to enhance both growth and expression of lac operon-linked carbonic anhydrase (CA) expression in a recombinant strain of *Escherichia coli*. When the *E. coli* expressing CA was grown on various media, the glucose-based auto-inducible medium (glucose AIM) resulted in a CA activity of 1022 mU $OD_{600\ nm}^{-1}$ mL^{-1} at 24 h and a specific growth rate of 0.082 h^{-1}. The CA activity was four to fourteen times higher than those by LB-IPTG. The *E. coli* expressing CA grown on the glucose-AIM showed highest activity at pH 8.5 while it kept high stability up to 40 °C and an inlet CO_2 concentration of 6%. These findings indicate that the glucose-AIM would be a cost-effective medium to support high cell growth, CA activity and stability for effective CO_2 capture.

1. Introduction

Carbonic anhydrase (CA)-driven carbon capture has been studied as an alternative to conventional absorption and adsorption methods for carbon capture (Kanth et al., 2013; Olajire, 2010). This enzyme catalyzes the hydration of carbon dioxide into bicarbonate and a proton in aqueous solution. Recently *Escherichia coli* expressing CA at its outer membrane has shown high potential to effectively capture and sequester carbon dioxide from flue gas and biogas by its hydration action (Fan et al., 2011).

An economical medium for the *E. coli* expressing CA should be developed in order to make practical the CO_2 capture processes that employ them. To date Luria-Bertani medium with isopropyl-β-D-1-thiogalactopyranoside as an inducer (LB-IPTG) has been widely used for expression of enzymes from recombinant bacteria including *E. coli* expressing CA (Fan et al., 2011; Jo et al., 2013; Patel et al., 2013), however, this requires additional chemicals, cost, and careful planning which limited the use of recombinant bacteria in scaled processes (Marbach and Bettenbrock, 2012; Martin et al., 2008).

To circumvent the above drawbacks of LB-IPTG media, the present paper discusses the development of an economical medium for *E. coli* expressing CA to replace LB-IPTG and LB-AIM. The proposed medium for the *E. coli* expressing CA was a glucose-based auto-inducible medium (glucose AIM) that was reformulated from the previous LB-AIM (please see Table 1). It was specifically designed for lower medium cost, high *E. coli* growth, and high expression of recombinant cell membrane-displayed CA. To the best of our knowledge, there have been no other studies to use glucose-AIM for growth and enzyme (carbonic anhydrase) expression in recombinant *E. coli*. The cell growth and CA activity upon inoculation in three different media (LB-IPTG, LB-AIM, and glucose AIM) were comparatively investigated. Furthermore, the CA stability at various conditions such as pH, temperature and inlet CO_2 concentration was investigated.

2. Materials and methods

2.1. Bacteria transformation and expression of carbonic anhydrase

Chemically competent *Escherichia coli* BL21 STAR™ (DE3) cells were transformed by heat shock with the plasmid pETN-CA for surface display of recombinant carbonic anhydrase (Fan et al., 2011). The pETN-CA plasmid carrying a C-terminal His_6-tagged CA from *Helicobacter pylori* fused to the C-terminus of an N-terminal domain of the ice nucleation protein (INP) from *Pseudomonas syringae* for surface-anchoring on the outer membrane of *E. coli* was provided by Professor S.T. Yang (Fan et al., 2011). Transformants were selected on LB agar plate (1% tryptone, 0.5% yeast extract, 1% NaCl, 2% agar) containing 100 μg/mL ampicillin. *E. coli* cells were grown in LB medium (1% tryptone, 0.5% yeast extract, 1% NaCl) with 100 μg/mL of ampicillin. When induced with isopropyl-β-D-1-thiogalactopyranoside (IPTG), *E. coli* strains harboring pETN-CA were grown to OD_{600} = 0.5 in 250 mL of LB medium at 37 °C, then cooled to 25 °C and induced with 0.2 mM IPTG and 0.5 mM $ZnSO_4$.

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Table 1
Composition of three media used for carbonic anhydrase expression in the *E. coil.*

g L^{-1}	LB with IPTG	LB AIM	Glucose AIM
NaCl	10	10	0.5
Tryptone	10	10	–
Yeast extract	5	5	0.125
Ammonium sulfate	–	3.3	1.65
Potassium phosphate, monobasic	–	6.8	6.8
Sodium phosphate, dibasic	–	7.1	7.1
Glucose	–	0.5	1.0
Lactose	–	2.0	4.0
Magnesium sulfate	–	0.15	0.15

The *E. coli* displaying CA was harvested by centrifugation (5000 *g* for 5 min) at 4 °C, resuspended in PBS buffer (8 g of NaCl; 0.2 g of KCl; 1.44 g of Na_2HPO_4; 0.24 g of KH_2PO_4, pH 7.4) containing a protease inhibitor cocktail, and lysed with a Qsonica sonicator. After centrifugation (20,000 × *g* for 15 min), the supernatant was boiled with SDS-PAGE loading buffer directly, and then was loaded to 12% SDS-PAGE. After being separated by electrophoresis, proteins were then transferred to a PVDF membrane. Thereafter, Western Blotting was performed using mouse monoclonal anti-His tag (Lamda) as primary antibody and antimouse antibody conjugated with HRP (Southern Biotech) as secondary antibody to verify the presence of N-CA in the protein sample.

2.2. Effect of different medium on cell growth and CA activity

The LB-IPTG, LB-AIM and glucose AIM were formulated according to protocol (Table 1), sterilized by autoclave, and supplemented with ampicillin at 50 μg mL^{-1} to prevent plasmid loss and 1 mM $ZnSO_4$ to enhance expression of CA. The glucose AIM was prepared based on the composition in Table 1 by excluding tryptone, keeping only slight amount of yeast extract (0.125 g L^{-1} compared with 5 g L^{-1} in LB-AIM; 2.5% of yeast extract concentration in LB-AIM) and increasing glucose and lactose concentration by factor of 2 compared with LB-AIM. The *E. coli* grown in these three media was cultivated under continuous shaking (200 rpm) while the temperature, pH and dissolved oxygen concentration were controlled to 25 °C, pH 7 and 1–1.5 mg O_2 L^{-1}, respectively. IPTG was introduced to the LB-IPTG culture after 2 h of incubation for a final concentration of 50 mM. The growth and activity of *E. coli* expressing CA on LB-IPTG, LB-AIM and glucose AIM were comparatively investigated by monitoring OD_{600} and CA activity using the Wilbur–Anderson reaction.

2.3. Effect of pH, and temperature on CA activity

The *E. coli* expressing CA was grown on glucose-AIM at pH 7 for 24 h, and was harvested to examine CA stability at various pHs and temperatures. The cells were incubated at various pHs (7–10) or temperatures (25–50 °C) for 4 h to determine the residual CA activity (%).

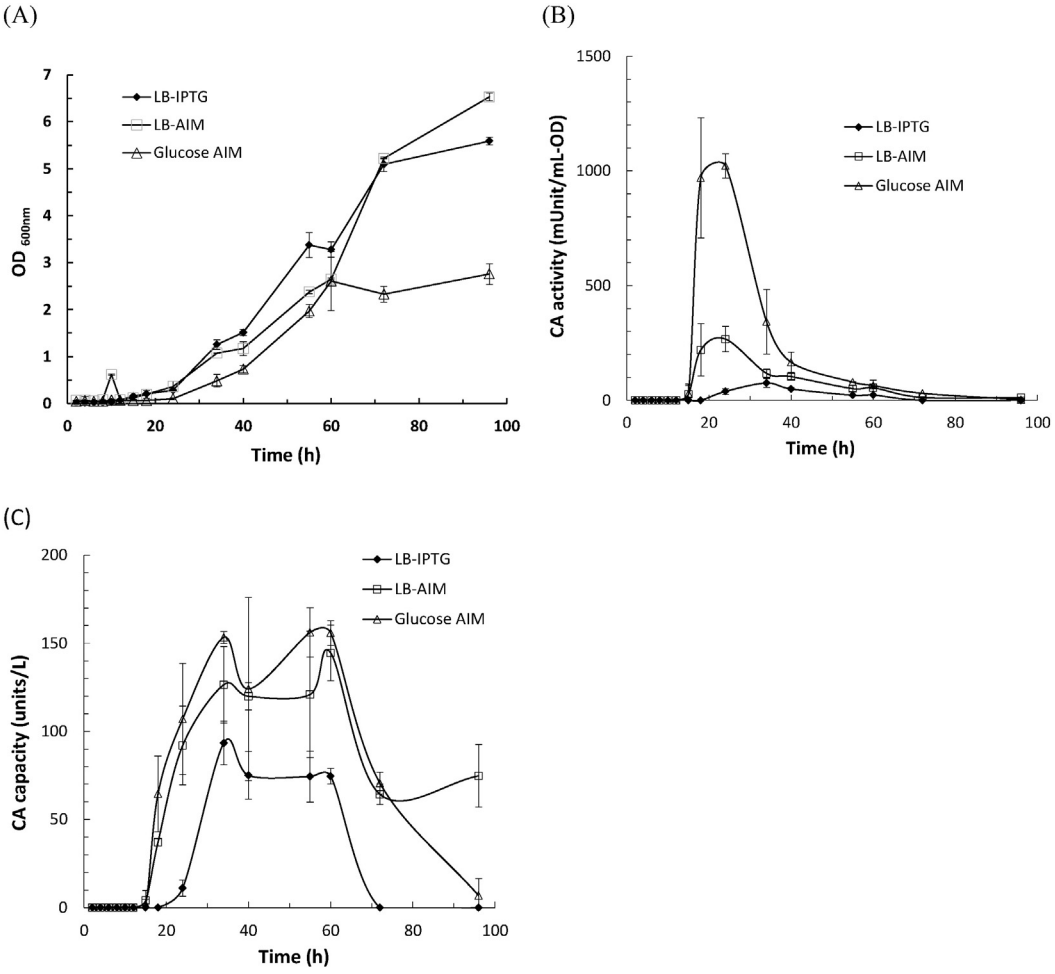

Fig. 1. Cell growth (A), CA activity (B), and CA capacity (C) in LB-IPTG, LB-AIM, and glucose AIM. Conditions: culture volume, 0.25 L in 0.5 L flask; culture pH, 7; and culture temperature, 25 °C. The error bars were calculated from the triplicate measurement.