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RESEARCH PAPER

Effect of Zn^{2+} on halohydrin dehalogenase expression and accumulation through multi-parameter correlation research with *Escherichia coli* P84A/MC1061

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ABSTRACT

Using 5 Zn^{2+} supplementation strategies in a 50 L batch bioreactor named FUS-50L(A), possible correlations among Zn^{2+} content and addition timing, physiologic activity (PA), halohydrin dehalogenase (HheC) accumulation of *Escherichia coli* P84A/MC1061 were systematically investigated. First, Zn^{2+} was confirmed as the significant factor, and its optimal concentration for HheC expression was 3.87 mg/L through fermentation experiments in shaking flasks. Second, based on experimental results from the different strategies, it was found that PA, nutrient consumption rate (NCR) and specific growth rate (μ) for *E. coli* P84A/MC1061 were promoted in the log phase (4–8 h) under appropriate Zn^{2+} concentrations in the lag phase and late log phase. Furthermore cell biomass was also increased to a higher level and the maximum HheC activity (i.e. $HheC_{max}$) was increased by 9.80%, and the time to reach $HheC_{max}$ was reduced from 16 to 12 hours. Furthermore, appropriate supplementation of Zn^{2+} caused higher μ for *E. coli* P84A/MC1061, which resulted in more rapid accumulation of increased acetic acid concentrations, leading to higher acetic acid consumption avoiding any negative effects on producing HheC because of carbon source being exhausted prematurely and acetic acid being consumed rapidly.

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Introduction

As a result of the rapid and continuous development of the chemical industry, the demand for treatment of water and soil being polluted by organic halides is becoming more and more serious.[1] Because of incomplete or inefficient degradation pathways, traditional methods such as physical and chemical remediation lead to the generation of reactive intermediates that enhance overall toxicity to the environment.[2] Recently developed biocatalysis methods, in particular the utilization of haloalcohol dehalogenases, represent a very effective approach for the bioremediation of environmental pollutants.[3] There have been numerous studies on halocarbon biodegradation in the fields of microbiology, biochemistry, and microbial ecology,[4-6] which has led to a boom in industrial bioremediation.[7,8]

Haloalcohol dehalogenase, which is also called halohydrin-hydrogen halide catenase,[9] is a key enzyme in the degradation of organic halides in microbial degradation. According to the differences in homology, these enzymes can be classified into 3 types: HheA, HheB, and HheC, respectively. Of these, the most extensive research has been performed on the catalytic mechanism of HheC. This interest is due to the fact that HheC can bind to chloroacetone (a common pollutant), in a relatively short time, while also showing high affinity and stereoselectivity (i.e., R-type) for sidechains adjacent of halide alcohols or epoxides. A sequence homology search and secondary and tertiary structure predictions indicated that the halohydrin dehalogenases are structurally similar to proteins belonging to the family of short-chain dehydrogenases/reductases (SDRs).[10] Moreover, catalytically important serine and tyrosine residues, which are highly conserved in the SDR family, are also present in HheC and other halohydrin dehalogenases. However, the third essential catalytic residue in the SDR family, a lysine, is replaced by an arginine in halohydrin dehalogenases, which differentiates HheC. HheC (molecular mass 112 KDa) has been shown to consist of 4 identical

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subunits, each containing 246 amino acids with a molecular mass of about 28 KDa. The spatial structure of HheC was confirmed in 2001 and 1997.[11,12] Up to date, research on halohydrin dehalogenase mainly focuses on cloning, sequencing, restructuring expression in vitro and purification,[13] and only recently has a report on optimizing the nutritional conditions for HheC production been published.[14] However further research on the influence of trace elements on HheC production have not been reported, and Zn^{2+} in particular is of interest.

In this research, the effects of Zn^{2+} on HheC produced through *E. coli* P84A/MC1061 were investigated using 5 Zn^{2+} supplementation strategies in a 50 L batch bioreactor, followed by determination of optimal concentration and timing for Zn^{2+} supplementation. Moreover, through using the multi-parameter correlation analysis method, the reason for Zn^{2+} affecting the expression and accumulation of HheC was clearly demonstrated.

Results

Experiments confirming significant impact factor (SIF) for optimal metal ion supplementation

The effects of Zn^{2+}, Co^{2+} and Fe^{2+} on the growth of *E. coil* P84A/MC1061 were shown as Fig. 1. It can be seen that adding all 3 ions could promote OD_{600}; Zn^{2+} had the greatest effect on growth, and the best supplemental concentration was 0.60 mg/L. Moreover, adding Zn^{2+} also promotes HheC production, with a supplementary concentration in the medium was 0.80 mg/L showing the best effect (data not shown). Thus, Zn^{2+} was selected as the SIF, and on the basis of above experimental results, 6 supplementations of finer resolution of Zn^{2+} concentration were selected for determining optimal supplementation conditions. The results of these flask tests are represented in Fig. 2, which demonstrates that OD_{600} and enzyme activity showed the greatest improvement when the supplementary concentration of Zn^{2+} was 0.70 mg/L (Total content is 3.87 mg/L, which include 2 parts of Zn^{2+} ions from supplementation and yeast extract FM888), so 3.87 mg/L Zn^{2+} was choosed as the optimal concentration for HheC expression.

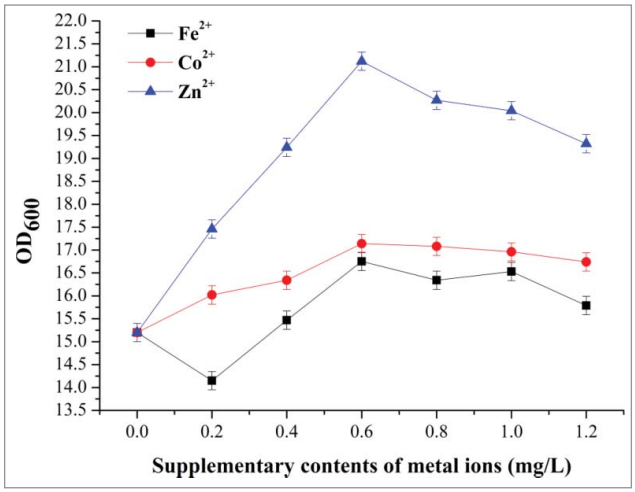

Figure 1. Effect of varied concentrations of Fe^{2+}, Zn^{2+} and Co^{2+} on the growth of *E. coli* P84A/MC1061 in shake flask experiments.

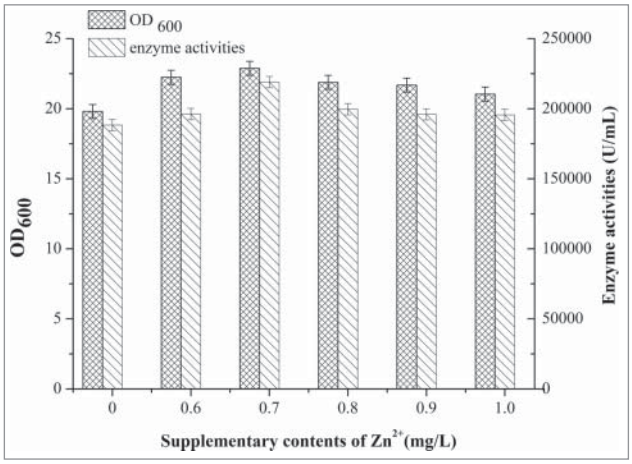

Figure 2. Effect of Zn^{2+} on the growth and HheC production of *E. coli* P84A/MC1061 in shake flask experiments.

Multi-parameter correlation analysis of batch fermentation experiments in a 50 L batch bioreactor supplemented with Zn^{2+}

Aiming at studying the dynamic effect of Zn^{2+} on the growth and metabolic characteristics of *E. coli* P84A/MC1061, batch fermentation studies were conducted in a 50 L bioreactor, and oxygen uptake rate (OUR), carbon dioxide emission rate (CER), respiration quotient (RQ), pH values, and HheC expression were monitored, as shown in Fig. 3, which served as a base-case control (pdr1). This base-case was then compared with the same supplementation at different timings with the optimal zinc concentration as identified above.

Pdr2, which represents supplementation at the start of the fermentation, and in comparison with pdr1, it was obvious to see that the OUR with

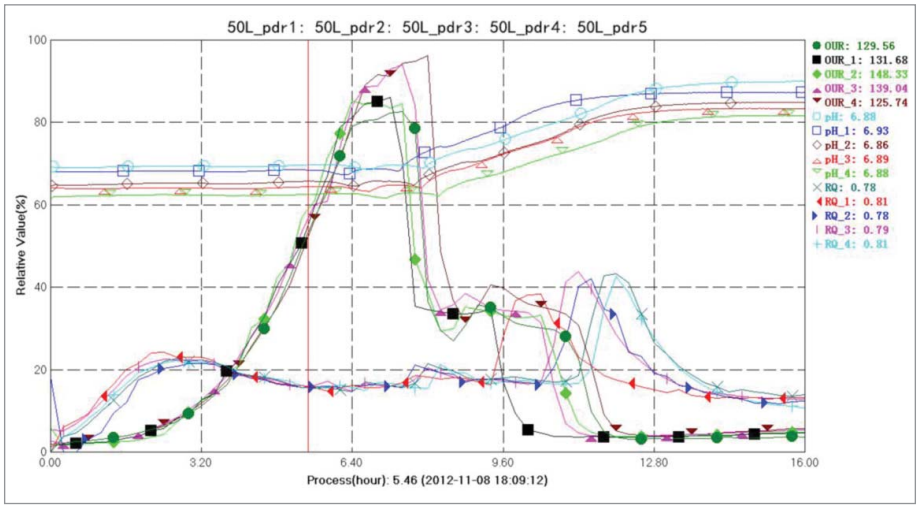

Figure 3. Effect of varied Zn^{2+} supplementation strategies on HheC production in a 50 L batch bioreactor.

supplementation (OUR_{max} = 222.15 mg/L·h) was higher than that of pdr1 (OUR_{max} = 209.51 mg/L·h). It was particularly noteworthy that a period of secondary growth around 10 h in pdr1 and 9 h in pdr2 was observed, while increases in pH values began 4 h earlier in pdr2 compared with pdr1, signifying more exuberant cell metabolism and more rapid consumption rate in pdr2. Similarly, the time to reach maximum RQ in pdr2 (RQ_{max} = 2.15) was approximately 4 h ahead of that of pdr1 as well (RQ_{max} = 1.83).

The growth curves (not shown) of *E. coli* P84A/MC1061 in pdr1 and pdr2 showed that they both had an approximate lag phase (0–4 h), but *E. coli* cells in pdr2 showed a higher μ than that of pdr1 from 4 to 7 h, and it reached maximum biomass in less time. What's more, the $HheC_{max}$ was raised from 90,715 U/mL in pdr1 to 95,590 U/mL in pdr2, representing an increase of 5.40%, and the required time to synthesize $HheC_{max}$ was reduced from 16 to 12 h, as can be seen in Fig. 4.

From Table 2, it can also be seen that the specific enzyme activity (μ_E) of *E. coli* P84A/MC1061 both in pdr1 and pdr2 gradually increased, however, μ_E in pdr2 were obvious higher than that of pdr1. Furthermore, the higher μ_E was, the higher purity of the enzyme was, and thus the purity of the enzyme in pdr2 was higher than that of pdr1.

All the above phenomena illustrate that Zn^{2+} not only promotes the physiologic activity and μ of *E. coli* P84A/MC1061, but also improves the expression of HheC and μ_E (enzyme purity).

The effect of timing of Zn^{2+} supplementation and Zn^{2+} concentration pressure on E. coli *activity, μ and HheC expression*

As Zn^{2+} could improve the expression of HheC under suitable conditions, determination of optimal addition timing of Zn^{2+} for producing increased enzyme protein was another important component of this research work. Thus, 3 additional treatments comprised of partial or whole additions at 0 h and 5 h (pdr3), 0 h and 8 h (pdr4) and 8 h (pdr5), were performed as well (see Table 1). Through analysis of online and offline parameters, it was found that *E. coli* P84A/MC1061 in the 5 batch

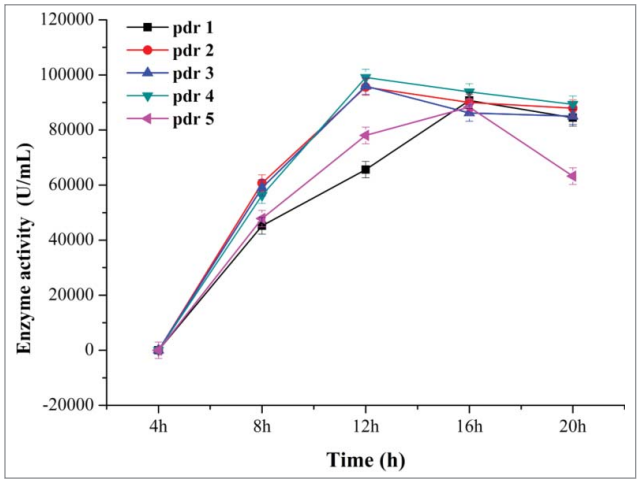

Figure 4. Halohydrin dehalogenase production of *E. coli* P84A/MC1061 grown in a 50 L batch bioreactor on varied Zn^{2+} supplementation strategies.

Table 1. Strategies for Zinc^{2+} supplementation during culture of *E. coli* P84A/MC1061 cells for HheC production in a 50 L batch bioreactor.

	Addition timing/Addition concentration of SIF		
Batch number	0 h	5 h	8 h
Pdr1 (control)	/	/	/
Pdr2	OC	/	/
Pdr3	Half of OC	Half of OC	/
Pdr4	Half of OC	/	Half of OC
Pdr5	/	/	OC

*SIF—the significant impact factor (SIF)
*OC—Optimal concentration of SIF.

bioreactors showed a similar 4 h lag phase in which the nitrogen and carbon source consumption rate were lower (data not shown), while Zn^{2+} which was absorbed into cells in this period, as can be seen in Fig. 5.

Comparing OUR in pdr1 and pdr5, it was shown that after a rise in first 8 hours, the former began to drop while the latter continued to increased and reaching a maximum at 8 h. Meanwhile, comparing OUR in pdr3 and pdr4, both showed a rise in first 5 h, but the former showed increased values after 5 h (i.e., the time of additional of the second half of Zn supplementation). However, OUR in pdr4 had clearly increased after 8 h, and exceeded pdr3 OUR values. All this information indicated that adding appropriate concentrations of Zn could significantly improve the physiologic A of *E. coli* P84A/MC1061.

In addition, the observed increases in pH values in were pdr2, pdr3, pdr4 (almost the same with pdr1) and pdr5, chronologically. This is explained by the fact that the nutrient consumption rate (NCR) of *E. coli* could be obviously affected by the quantity and timing of Zn^{2+}, and within the supplementary concentration range from zero to the optimally-determined value (0.70 mg/L), the increased Zn^{2+} content and earlier addition benefitted NCR of *E. coli*.

Table 2. Specific enzyme activities (μ_E) of *E. coli* P84A/MC1061 grown in a 50 L batch bioreactor in the absence (pdr1) or presence (pdr2) of 3.873 mg/L Zn^{2+}.

	μ_E (U/mg)	
Time (h)	pdr1	pdr2
4	0	0
8	3717.6	4927.1
12	4079.5	5972.6
16	5752.8	6024.1
20	5909.1	6171.5

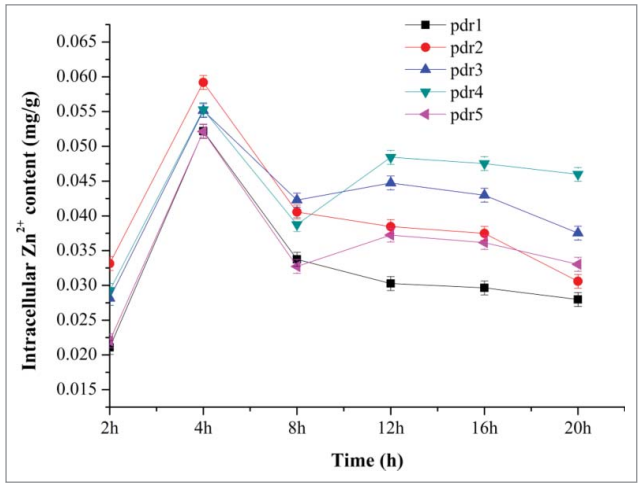

Figure 5. Intracellular Zn^{2+} concentration(IZC) of per gram *E. coli* P84A/MC1061 cells grown in a 50 L batch bioreactor on varied Zn^{2+} supplementation strategies.

From Fig. 4, it was found that the $HheC_{max}$ in pdr4 was higher than other treatments, and compared with pdr1, the $HheC_{max}$ in pdr4 was increased by 9.80%, while enzyme activities in pdr1 and pdr5 were very low. Moreover, the time to obtain $HheC_{max}$ in pdr1 and pdr5 was 4 h slower than the other 3 treatments, which indicated that total optimal content of Zn^{2+}, whether either added initially or stepwise, benefitted synthesis of HheC.

From Fig. 5, it could be obviously seen that intracellular Zn^{2+} concentrations (IZC) in all treatments showed an increasing trend from 2 to 4 h, with negligible cell concentrations 0–2 h. Furthermore, IZC in pdr2 showed the highest levels, and during 4 to 8 h, IZC in pdr1, 2, 4, 5 decreased sharply, yet that of pdr3 dropped relatively slowly. Therefore, within a certain range, there was a positive correlation between Zn^{2+} absorbed amount and Zn^{2+} concentration pressure during lag and log phase(The concentration pressure means cells could not absorb Zn^{2+} ions easily until its content in fermented broth being maintained at a higher level). During 8 to 12 h, IZC in pdr1 and pdr2 kept on going down, and that of other treatments increased gradually, especially that of pdr4, which had a significant raise. After 12 h, IZC in 5 fermentors maintained at relatively stable levels, respectively, and pdr4 got the higher lever.

It was inferred that substantial amounts of Zn^{2+} were absorbed by *E. coli* in the lag phase and used to synthesize and activate enzymes mainly used for anabolism. Subsequently, some unbound Zn^{2+} ions were released into the broth because most of free Zn^{2+} was

no longer in demand for activating anabolic enzymes in the log phase. Moreover, Zn^{2+} contents of initial media in pdr1 and pdr5 were the same (i.e., 3.17 mg/L), but their IZC were not maintained at higher levels in stationary phase, while IZC was increased to a higher level when half of the optimal supplementary content of Zn^{2+} was added to the broth at the beginning of fermentation and late log phase (i.e., pdr4). Importantly, compared with pdr4, the second half of optimal supplementary content of Zn^{2+} being added to the broth at middle of log phase in pdr3 did not change the decreased trend of IZC during 4 to 8 h and its IZC was also at a lower level in stationary phase.

All of the information presented here indicates that extracellular Zn^{2+} content should be maintained at a proper level during lag phase and the late log phase, (i.e., 3.48 to 3.51 mg/L during lag phase and be 3.48 to 3.77 mg/L during late log phase), under which IZC could be maintained at a relatively higher level and HheC could be rapidly and largely synthesized in stationary phase.

Study on the acetic acid accumulation and consumption

Acetic acid is a common metabolite in *E. coli* fermentation. Varma et al., found that acetic acid accumulation was closely related with μ and OUR, namely, OUR increased correspondingly with increasing μ and OUR increasing trend was limited when μ reached to a specific value (0.70), which resulted in acetic acid accumulated.[15]

From Fig. 3, secondary growth phenomenon appeared around 10 h in pdr1 and 9 h in pdr2, and this phenomenon was also present in the other fermentors. Fiseschko and Ritch T (1986) found that after the carbon source was depleted, acetic acid could be used as carbon source by *E. coli*.[16] Thus, it was necessary to detect the content of acetic acid and determine and relationship between the acetic acid accumulation and the secondary growth, and the results are shown in Fig. 6.

Figure 6 showed that the time necessary to reach the highest concentration of acetic acid in pdr1 and pdr2 were respectively about 10 and 9 h, which is when the secondary growth phenomenon began to appear. What's more, it was found that glycerol in pdr1 and pdr2 were respectively exhausted at about 10 and 9 h (data not shown), while acetic acid contents

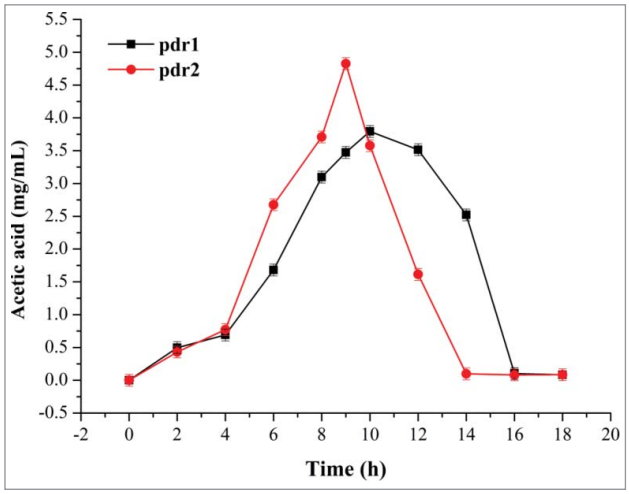

Figure 6. Acetic acid concentration in fermented broth produced by *E. coli* P84A/MC1061 grown in a 50 L batch bioreactor on varied Zn^{2+} supplementation strategies.

decreased progressively with the secondary growth carrying on. On the other hand, the content of acetic acid accumulation in pdr2 was higher than that of pdr1 from 4 to 9 h which was caused by the higher specific growth rate in pdr2, but was lower than that of pdr1 after 10 h which was due to the fact that Zn^{2+} addition would result in a higher NCR.

According to the experimental results quantifying specific growth rate (μ, data not shown) and acetic acid, it was found that acetic acid began to accumulate rapidly when μ was above 0.32, furthermore, the higher μ increased, the larger content of acetic acid accumulated. This suggests that acetic acid accumulation was a direct result of the higher μ, for example, the acetic acid content in pdr2 increased from around 0.50 mg/mL to about 5.00 mg/mL(the maximum content) in the shortest period because of its maximum μ compared with other experimental batches. Meanwhile, the appropriate concentration of Zn^{2+} has been observed to cause higher μ which resulted in higher acetic acid accumulation, and the performance that higher acetic acid consumption rate coupling with larger acetic acid accumulation was also detected in HheC fermentation by *E. coli* P84A/MC1061, and the phenomenon of secondary growth was directly appeared in the OUR and CER curves (Fig. 3) resulted from acetic acid consumed as carbon source because of nutrients in the initial medium being almost exhausted, what's more, the negative effect on stability of *E. coli* plasmid caused by acetic acid accumulation might be avoided.[17] Thus, addition of appropriate concentrations of Zn^{2+} to the fermented broth at the

optimal timing (pdr 2 in Table 2) was very important for producing HheC effectively.

Discussion

From the experimental data, it was seen that the rapid expression of HheC were occurred in the late log phase. That is, substantial synthesis of HheC required a certain degree of biomass accumulation. However HheC expression were no longer accurately positive related with *E. coli* P84A/MC1061 growth when the biomass was above a value. So the expression and accumulation of HheC were a partial growth-associated process.

There were many reports about biologic functions of Zn^{2+}. From the beginning of 1960s, many scientific studies have proven that Zn^{2+} plays an important role in more than 120 kinds of enzymes, but also Zn^{2+} were indispensable in growth, development and differentiation to normal cells of all creatures.[18,19]

Combined our research conclusion above with references, it was inferred that Zn^{2+} influences *E. coli* P84A/MC1061 growth, expression and accumulation of HheC.

One possible explanation for this is that DNA polymerase and RNA polymerase were zinc metalloenzymes,[20–22] furthermore, Zn^{2+} played a very important role in cell differentiation and gene expression,[18,23] and therefore, changing the content of Zn^{2+} would probably influence transcription, translation and protein synthesis of *E. coli* P84A/MC1061. Furthermore, Zn^{2+} is an an activator of alcohol dehydrogenase and lactate dehydrogenase in the glycolysis process,[24,25] therefore, the glycolysis metabolic output of *E. coli* P84A/MC1061 would be promoted through the activating effect of Zn^{2+} absorbed from the broth, which maintained at a appropriate Zn^{2+} concentration pressure during lag phase and late log phase increased physiologic activity to a higher level resulting in a the higher metabolic output.

Moreover, Zn^{2+} and nitrogen metabolism in *E. coli* are closely related. Specifically, Zn^{2+} content can regulate the activity of nitrate reductase,[26] and nitrate reductase activity would affect the ability of nitrogen utilization and absorption.[27] Thus, nutrient consumption rates for *E. coli* may be promoted by the positive regulation of Zn^{2+}, in the condition of that a proper Zn^{2+} concentration pressure was maintained both in the lag and late log phase.

What's more, HheC had 3 important catalytic residues: Tyr 145, Ser132 and Arg149,[10] and Zn^{2+} can form complex with tryptophan, which is closely relation with serine,[28] affecting the transformation process. Thus, Zn^{2+} may influence the structure or enzyme active center of HheC, that is, Zn^{2+} may affect the catalytic activity of HheC.

Last but not least, intracellular Zn^{2+} content is responsible for the concentration changes of certain metal ions (for example: manganese, iron and copper ions),[29] thus, epicyte-mediated by metal ions changes, and the structure or the enzyme activity involved in nucleic acid metabolism is affected.[30]

This study not only laid the foundation for the expression and accumulation of HheC, but also played a great role in guiding industrial scale production of HheC by *E. coli* P84A/MC1061. Based on our research and previous references, we should further focus our future studies on investigating the separation and purification of HheC, moreover, the molecular biology studies on inclusion bodies, DNA polymerase and RNA polymerase during the process of the HheC expression should be also conducted.

This work clearly demonstrates that Zn^{2+} has striking effects on not only physiologic activity, but also expression and accumulation of HheC produced by *E. coli* P84A/MC1061. From above research, it was found that physiologic activity, nutrient availability, and specific growth rate could be relatively promoted in the log phase under proper Zn^{2+} concentration pressures maintained both in the lag and late log phase, under which the biomass could be also increased to a higher level benefited for the rapid expression and large accumulation of HheC mainly in stationary phase.

Furthermore, appropriate concentrations of Zn^{2+} supplementation caused higher μ of *E. coli* P84A/MC1061, which resulted in more rapid and larger acetic acid accumulation, leading to higher acetic acid consumption rate, thus avoiding negative effects on plasmid stability of *E. coli* P84A/MC1061.

Materials and methods

Strain and culture conditions

E. coli P84A/MC1061 was provided by the culture preservation center of the Angel Yeast Co. Ltd and was maintained on Luria-Bertani (LB) agar slants [(g/L) tryptone 10, yeast extract (Model FM888 produced by Angel Yeast Co. Ltd, 90 percent of molecular

weight range from 400 to 1000 Dalton, 40 percent of solubility, 4.50–5.50 percent of amino nitrogen) 5, NaCl 5 and agar 20] at 4°C with subculture each month. For cultivation in 250 mL Erlenmeyer flasks, 25 ml of liquid medium [(g/L) glycerin 4, yeast extract (FM888) 50, NaCl 2.00, KH_2PO_4 6.50, K_2HPO_4 6.50, Na_2HPO_4 3.50, $MgSO_4{\cdot}7H_2O$ 1.50] were inoculated and incubated at 28°C in a rotatory shaker (ZHWY-211D, Shanghai Zhicheng, China) at 200 rpm for 20 h to prepare the inocula. Subsequently, (2% v/v) inoculum was added aseptically into each 250 mL flask containing 25 mL liquid medium and fermented at 28°C. When the OD_{600} of the cultures in the flasks reached to 2, 0.50 mL L (+)-arabinose solution was added to the fermented broth, as the inducer.

Shaking flask experiments

Some trace elements have an important influence on the fermentation process for producing HheC by *E. coli* P84A/MC1061.[14,31] Based on our previous research, Zn^{2+}, Co^{2+} and Fe^{2+} were selected, and their supplementary concentrations in the media were as followed (mg/L): 0, 0.20, 0.40, 0.60, 0.80, 1.00 and 1.20. After preliminary experiments, the significant impact factor (SIF) was selected, and then finer concentration gradients (e.g. 0, 0.60, 0.70, 0.80, 0.90 and 1.00 mg/L) were selected to determine optimal concentration. Every concentration of different trace elements was prepared 3 flasks.

Batch fermentation in 50 L reactor

To explore the effect of timing for supplementation, and study how optimal supplementation concentrations influence the growth and metabolism of *E. coli* P84A/MC1061 in a batch bioreactor, 5 supplementation strategies were tested (named as pdr1, pdr2, pdr3, pdr4 and pdr5) as Table 1. Pdr is from a corresponding author's name, Dong-Rui Pan. pdr1 is the control; pdr2 is added the optimal Zn^{2+} concentration in 0 h; pdr3 is added half of the optimal Zn^{2+} concentration in 0 h, the other half in 5 h; pdr4 is added half of the optimal Zn^{2+} concentration in 0 h, the other half in 8 h; pdr5 is added the optimal Zn^{2+} concentration in 8 h.

Fermentation was performed in a 50L batch reactor equipped with online analysis software (Biostar 5.5; Guoqiang Bioengineering Equipment Co., Ltd, China) and an off-gas analyzer (PAS7000; Hartmann & Braun, Germany). 2% (v/v) inoculum was added into the reactor, which contained 30 L of liquid medium [(g/L) glycerin 12, yeast extract (FM888) 50, NaCl 2.00, KH_2PO_4 6.50, K_2HPO_4 6.50, Na_2HPO_4 3.50, $MgSO_4{\cdot}7H_2O$ 1.50] prepared in high purity water. The batch reactor was operated under conditions of 30°C, 0.035 MPa, a compression-ventilation ratio (VVM) of 1.70 and above 30% dissolved oxygen (DO), 200 rpm in lag phase, 200–450 rpm in log phase and stationary phase. The pH was not controlled during the entire fermentation process. To obtain the cells, the cultures were harvested after 22 h, washed with high-purity water, and then centrifuged at 5,000 rpm for 10 min. Thereafter, the bacteria were treated ultrasonically, and the supernatant was analyzed to determine enzyme activity and concentration of zinc ions ($ZnSO_4$). L (+)-arabinose was purchased from Sigma-Aldrich (USA) and dissolved in distilled water to make solution with 0.05 g/ml concentration, which was used to induce *E. coli* express HheC.

Fermentation analysis

2.50 g of cells were harvested by centrifugation (5000 rpm, 10 min) and resuspended in 25 mL 50 mM Tris-EDTA buffer. After sonication (time, 14 min; puml, 5 s; Amp, 40%), the supernate was obtained by centrifugation (5000 rpm, 10 min), and used as the enzyme solution for detection. Based on the references,[32,33] an effective method for detecting the activity of HheC was established, HheC was determined using the chromogenic substrate 2-bromoethyl alcohol by monitoring the absorbance change at 460 nm in 50 mM Tris-SO_4 buffer at 30 °C on a spectrophotometer. 3 mL 2-bromoethyl alcohol was preheated and kept at 30 °C for 10 min, then add 100 μL enzyme solution to this reaction mixture and let the both reacting for 10 min. The calculating formula is as follows:

$$\text{Enzyme activity}(\text{U}/\text{mL}) = \text{c} \times \frac{3.10}{0.10} \times \text{n} \times 1000 \div \text{t} \tag{2.1}$$

c- bromide ion concentration in sample (mmol/L);
t -reaction time (min);
3.10-reaction total volume (mL);
0.10- sampling volume (mL);
n - dilution ratio

The specific enzyme activity (μ_E) was determined using the method of Liu et al.[34]

To determine reactor cell concentration, a 10 mL sample of the fermentation broth, diluted to the appropriate extent with distilled water, was used to determine biomass by spectrophotometry at 600 nm, without dry cell weight calibration.

Zinc ion concentrations were determined using atomic absorption spectrometry,[35] and acetic acid content was determined using the HPLC method of Andersson et al.[36] Determination of oxygen uptake rate (OUR), carbon dioxide emission rate (CER), respiratory quotient (RQ) and pH in the bioreactor were determined by the online analysis software Biostar 5.5 and an off-gas analyzer.

Disclosure of potential of interest

No potential conflicts of interest were disclosed.

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Notes on contributors

XL, DRP and YDL drafted the manuscript. DRP and XDS performed fermentation. XDS and YDL performed all experiment parameters detection. XL, DRP, YLT and YDL performed analysis. XL conceived of the study. ZHL participated in the design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

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