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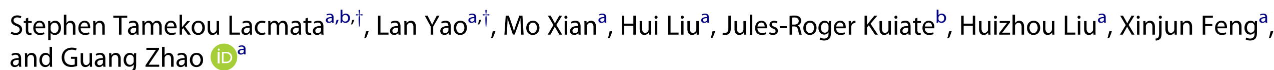
RESEARCH PAPER

A novel autolysis system controlled by magnesium and its application to poly (3-hydroxypropionate) production in engineered *Escherichia coli*



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ABSTRACT
The release of intracellular products, especially polyhydroxyalkanoates, is still a great challenge in industry. To solve this bottleneck, a novel autolysis system strictly controlled with magnesium was constructed and applied to poly(3-hydroxypropionate) production in engineered *Escherichia coli*. The autolysis system was constructed by inserting the 5′untranslated region (5′UTR) behind promoter P*mgtA* with lysis genes (*S, R*, and *Rz*, from *E. coli*) overexpressed. The autolysis system functioned well (lysis efficiency of more than 90%) in the P3HP producer with double plasmids containing lysis genes and P3HP biosynthesis genes, whereas the P3HP production was reduced due to plasmid losses. After the autolysis genes and P3HP biosynthesis genes were integrated into one plasmid, the P3HP content of 72.7% (2.4 times of the control) and the plasmid stability of 79.8 ± 3.1% were achieved in strain Q2646 with promoter P*mgtA*-UTR. However, the strain Q2647 with promoter P*mgtA* could not accumulate P3HP because of rapid cell lysis. The novel autolysis system activated in Mg^{2+}-depleted conditions proves to be feasible for polyhydroxyalkanoates production, which may have great application potential for other intracellular products.

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Introduction

The release of intracellular products from microbial cells is an essential process for industrial production of bio-based chemicals. Organic solvent, alkali or detergent and ultrasonication are widely used for cell disruption and intracellular substances extraction in industry.[1] However, these extraction processes usually account for as high as 70∼80% of the total production cost [2] and the cell disruption is a significant cost factor in industry. To develop a cost-effective recovery process for the intracellular products, many attempts have previously been made to disrupt the cells by constructing autolysis systems. Liu et al. developed an auto-inducible lysis system in *E. coli* using the lysis genes from *Salmonella* phage P22 and promoter P*nrsB*, which activated by addition of nickel.[3] Zhang et al. constructed an autolysis *E. coli* system regulated by Mg^{2+} concentration, which consisted of the lysis genes from λ bacteriophage and promoter P*mgt* from *Salmonella typhimurium*.[4] Hajnal et al. designed a synthetic ribosome binding site for the autolysis of *E. coli* and *Halomonas campaniensis* under environmental stresses.[5]

Compared with the autolysis systems induced by external chemical or physical stimuli such as IPTG, L-arabinose, nickel and heat shock, the autolysis system controlled by magnesium depletion is more feasible in microbial production due to zero-added chemicals and no additional energy consumption. However, the previous study on the auto-inducible lysis systems controlled by magnesium suggested that the lysis genes (*S*, *R*, and *Rz*) could still be activated at Mg^{2+} concentration of 50 μM or so.[4] Because the lysis proteins can degrade the peptidoglycan and cause cell wall disruption,[6,7] the lysis genes must be strictly regulated, otherwise even low level of protein expression can seriously influence the cell growth and the biosynthesis of intracellular products. Therefore, the

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regulation of the lysis genes in the present Mg^{2+}- controlled system should be further improved.

P3HP, a novel polyhydroxyalkanoates, is regarded as a promising alternative to the fossil fuel-based materials. The microbial production of P3HP has been achieved by genetically engineered strains so far.[8,9] However, few efforts have been devoted to the application of the autolysis system to P3HP fermentation. To overcome the present problems, we designed and constructed a new autolysis system by introducing the *mgtA*-UTR as a Mg^{2+}-responsive riboswitch. Furthermore, the effects of this novel autolysis system on the production of P3HP were investigated in the present paper.

Results and Discussion

Construction of optimized autolysis E. coli *system*

To verify the function of the lysis genes, the strain Q2537 was constructed by transforming the pY01 into *E. coli* DH5*α*, which contained the lysis genes (*S*, *R*, and *Rz*) controlled by L-arabinose promoter P_{BAD}. The bacterial-growth curves of Q2537 and *E. coli* DH5*α* containing empty plasmid pBAD18 were shown in Fig. 1. After the expression of lysis genes were induced by L-arabinose, the OD_{600} of Q2537 fell sharply from 0.61 to 0.19 within 1 h. Meanwhile, the cell growth of uninduced Q2537 showed the same increasing trend as the control strain *E. coli* DH5*α*. The results suggested that the lysis genes were efficient on the cell disruption.

The effects of the lysis systems controlled by P_{BAD}, P*mgtA* and P*mgtA* -UTR in the strains Q2537, Q2549, and Q2582 were further analyzed. The corresponding lysis efficiencies were 91.5 ± 1.0%, 96.5 ± 1.5%, and 97.0 ± 1.0%, respectively (Fig. 2). The results showed that both promoter P*mgtA* and P*mgtA* -UTR could successfully regulate the expression of lysis genes under Mg^{2+} limited condition. The *mgtA* 5′-UTR functions as a Mg^{2+}-responsive riboswitch in *Salmonella* and *E. coli*.[10,11] This *mgtA* riboswitch can cause premature termination of the *mgtA* transcription at high external Mg^{2+} levels.[10,12] When the Mg^{2+} concentration reduced to μM level,[13] the *mgtA* transcription reactivated and caused cell lysis. Thus, the tight control of the lysis genes can be achieved by inserting the 5′-UTR behind promoter *mgtA*.

The lysis system was also integrated into the *Escherichia coli* chromosome. Unexpectedly, the lysis efficiency of the resulting strain was just 10.1 ± 1.2%. It was speculated that P*mgtA* was too weak to induce the expression of the lysis genes after integrated into the chromosome. So, promotors P_{lac1-6} and P_{T7} were also tested. However, the lysis efficiencies were still quite low as 12 ± 0.7% and 18 ± 1.8%. The results suggested that the expression of the lysis genes was not enough to disrupt the cells in a short time after the lysis genes were integrated into the chromosome.

The expression of the lysis genes (*S*, *R*, and *Rz*) in *E. coli* is lethal due to the degradation of the peptidoglycan of the cell wall. Even low level of lysis genes expression is likely to influence the biosynthesis of intracellular products. Therefore the effects of autolysis systems with P*mgtA* and P*mgtA* -UTR on P3HP production should be further investigated.

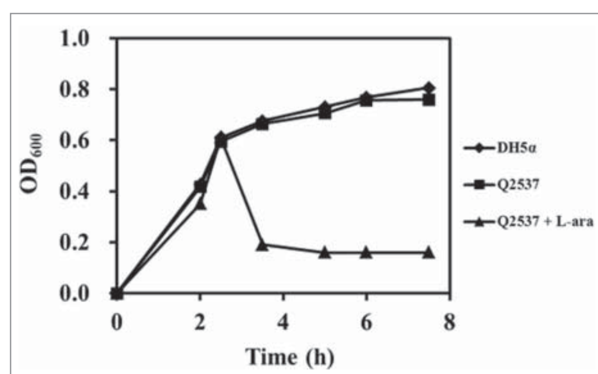

Figure 1. Effects of lysis genes expression on the growth of *E. coli*. *E. coli* DH5*α* with empty plasmid pBAD18-kan and Q2537 with lysis genes were used as the control strains. Q2537 with lysis genes was induced with L-arabinose at 0.6∼0.8 OD_{600}.

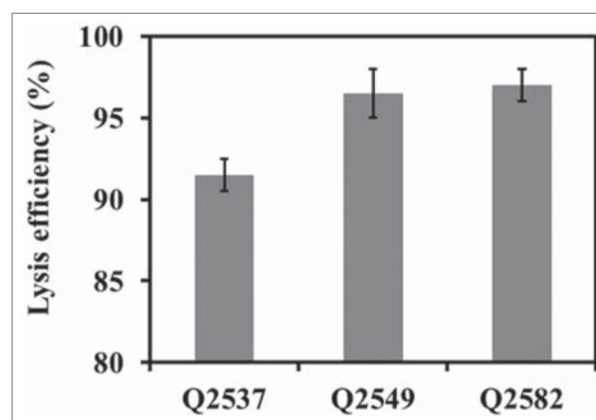

Figure 2. The Alysis efficiencies of different lysis systems controlled by P_{BAD}, P*mgtA* and P*mgtA* -UTR in the strains Q2537, Q2549, and Q2582.

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Table 1. P3HP production, contents and plasmids stability of different strains with double plasmids.

Strains	CDW(g/L)	P3HP (g/L)	P3HP content (%)	Plasmids stability (%)		
				pWQ02	pY02	pY03
Q1638	6.6 ± 0.11	2.1 ± 0.01	31.8	83.1 ± 3.2	—	—
Q2572	5.4 ± 0.16	1.7 ± 0.03	31.4	30.3 ± 0.5	—	20.7 ± 1.2
Q2588	5.2 ± 0.21	1.4 ± 0.04	26.9	40.0 ± 2.2	17.1 ± 3.2	—

(Q1638 without lysis system was used as the control. P3HP content was calculated using the ratio of P3HP weight to cell dry weight.)

P3HP production by E. coli *with double plasmids containing autolysis genes and P3HP biosynthesis genes*

To test the influence of the lysis systems on P3HP accumulation, the strains Q2588 (containing plasmids pY02 and pWQ02) and Q2572 (containing plasmids pY03 and pWQ02) were used for P3HP production in shake flask fermentation. As shown in Table 1, P3HP production of Q2572 was decreased in contrast with the control strain Q1638. Both the P3HP production and content of strain Q2588 were much lower than the control. Furthermore, the lysis efficiencies of the strains Q2588 and Q2572 were also tested. The lysis efficiencies of higher than 90% were achieved through the double plasmids system containing autolysis genes and P3HP biosynthesis genes. Therefore, the autolysis system functioned well in the P3HP producer with double plasmids system, whereas the P3HP production was reduced.

Introduction of exogenous plasmid can increase metabolic burden and lead to plasmid losses during fermentation. To study whether this potential cause existed in this system, plasmid stability was tested. Comparing with Q1638, the stabilities of the plasmid pWQ02 in Q2572 and Q2588 were reduced dramatically from 83.1% to 30.3 ± 0.5% and 40.0 ± 2.2%. And the stabilities of the plasmids pY02 and pY03 were just 17.1 ± 3.2% and 20.7 ± 1.2%. Therefore, the strains containing 2 plasmids system were not stable enough and needed to be improved for the P3HP production.

P3HP production by E. coli *with single plasmid containing autolysis genes and P3HP biosynthesis genes*

Due to the large losses of the plasmids during the fermentation with the double plasmids strains, the autolysis systems containing P*mgtA* and P*mgtA*-UTR were respectively integrated into the plasmid pWQ02 originally containing the P3HP biosynthesis genes (Fig. 3). The resulting plasmids pY04 and pY05 were separately transformed into the strain Q1463[14] to generate Q2647 and Q2646. The resulting strains containing single plasmid were tested for P3HP accumulation.

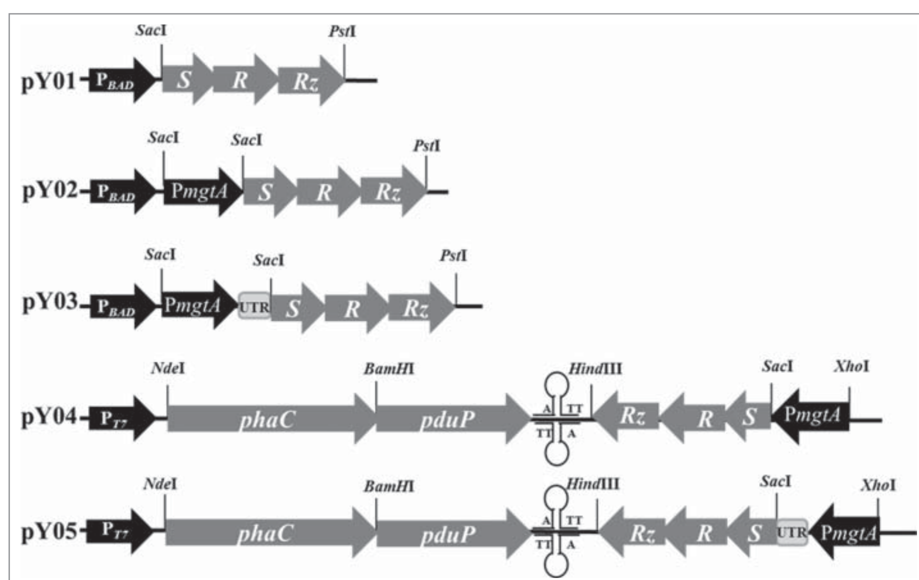

Figure 3. The backbones of plasmids constructed in this study.

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Table 2. P3HP production, contents and plasmids stability of different strains with single plasmid.

Strains	CDW(g/L)	P3HP (g/L)	P3HP content (%)	Plasmids stability (%)
Q1638	6.6 ± 0.51	2.0 ± 0.02	30.3	84.1 ± 2
Q2646	2.2 ± 0.04	1.6 ± 0.01	72.7	79.8 ± 3.1
Q2647	1.8 ± 0.02	0	0	8.4 ± 0.9

(Q1638 without lysis system was used as the control. P3HP content was calculated using the ratio of P3HP weight to cell dry weight.)

The P3HP production of 1.6 ± 0.01 g P3HP l^{-1} was achieved in the recombinant strain Q2646 with the P3HP content of 72.7%, which was 2.4 times of the control (Table 2). Furthermore, the plasmid stability was 79.8 ± 3.1%, which is similar to the control. However, the P3HP did not accumulate in the recombinant strain Q2647 during the fermentation (Table 2) and the cell lysis happened at early stage. The results showed that strictly controlled autolysis system was obtained in strain Q2646 with promoter P*mgtA*-UTR, while the autolysis system controlled by promoter P*mgtA* in Q2647 relatively expressed too early to synthesize the P3HP under the fermentation conditions.

Table 3. Bacterial strains and plasmids used in this study.

Strains and plasmids	Relevant properties	Source or reference
Strains		
Escherichia coli DH5α	F^-,*φ80dlacZ△M15,△(lacZYA-argF)U169, deoR, recA1, endA1, hsdR17(rk-,mk+)*	Invitrogen
Escherichia coli BL21(DE3)	F^-, *ompT*, *hsd*SB (rB^-mB^-), *ga, dcm,* *λ(DE3)*	Invitrogen
Q1463	△*prpR*::lacI P_{T7} *gdrAB* P_{T7} *dhaB*123	Ref. 14
Q1638	Q1463 carrying pWQ02	Ref. 14
Q2537	*Escherichia coli* DH5α carrying pY01	This study
Q2549	*Escherichia coli* DH5α carrying pY02	This study
Q2572	Q1638 carrying pY03	This study
Q2582	*Escherichia coli* DH5α carrying pY03	This study
Q2588	Q1638 carrying pY02	This study
Q2646	Q1463 carrying pY05	This study
Q2647	Q1463 carrying pY04	This study
Plasmids		
pBAD18	*Km*^{R}, cloning vector, pBR322 origin, araBAD promotor	NBRP-*E. coli* at NIG
pET21a	*Amp*^{R}, pBR322 origin, lacI P_{T7}	Novagen
pACYCDuet-1	*Cm*^{R}, p15A origin, lacI P_{T7}	Novagen
pWQ02	*Amp*^{R}, pET21a carrying polyhydroxyalkanoate synthase *phaC* and propionaldehyde dehydrogenase *pduP*	Ref. 15
pWQ04	*Cm*^{R}, pACYCDuet-1 carrying glycerol dehydratase *dhaB123* and its reactivating factor *gdrAB*	Ref. 15
pY01	*Km*^{R}, pBAD18 carrying lysis genes *S, R,* and *Rz*	This study
pY02	*Km*^{R}, pBAD18 carrying lysis genes *S, R,* and *Rz* and promoter P*mgtA*	This study
pY03	*Km*^{R}, pBAD18 carrying lysis genes *S, R,* and *Rz*, and promoter P*mgtA*-UTR	This study
pY04	*Amp*^{R}, pET21a carrying *phaC, pduP*, lysis genes *S, R,* and *Rz, and* promoter P*mgtA*	This study
pY05	*Amp*^{R}, pET21a carrying *phaC, pduP*, lysis genes *S, R,* and *Rz*, and promoter P*mgtA*-UTR	This study

Conclusions

A novel autolysis system strictly controlled by magnesium was constructed by inserting the 5′-UTR behind promoter P*mgtA*. Products like P3HP can accumulated continuously in the cells and released automatically by this autolysis system. The P3HP content reached 72.7% in engineered *E. coli* with promoter P*mgtA*-UTR, which is 2.4 times of the control strain. The novel autolysis system activated in Mg^{2+}-depleted conditions proves to be feasible for polyhydroxyalkanoates production, which may have great application potential for other intracellular products.

Materials and methods

Bacterial strains and growth conditions

E. coli DH5*α* was used for plasmid cloning and maintenance. Luria-Bertani medium (LB medium), and N-minimal medium (M9 medium) (per liter: 15.2 g $Na_2HPO_4·12H_2O$, 3.0 g $KH_2PO_4·3H_2O$, 0.5 g NaCl, 1.0 g NH_4Cl, 20 g glucose, and 1 ml trace elements stock solution (per liter: 3.7 g $(NH_4)_6Mo_7O_{24}·4H_2O$, 24.7 g H_3BO_3, 15.8 g $MnCl_2·4H_2O$, 2.9 g $ZnSO_4·7H_2O$, 2.5 g $CuSO_4·5H_2O$), pH 7.0) were used for bacterial culture and verification of lysis efficiency. For P3HP fermentation, a modified minimal medium containing the following components (per liter): 1.5 g KH_2PO_4, 3 g $(NH_4)_2SO_4$, 1.9 g KCl, 1.09 g citric acid, 1.14 g sodium citrate, 0.138 g $FeSO_4·7H_2O$, 3 g glucose, 20 g glycerol, and 1ml trace elements solution was used. 50 *μ*g/ml kanamycin or 100 *μ*g/ml ampicillin was added to keep the recombinant plasmid when necessary. The strains were cultivated at 37°C with 180 rpm unless specific.

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Construction of recombinant plasmids and strains

The lysis genes *S, R,* and *Rz* were amplified from the genome of *E.coli* BL21 (DE3) by PCR with the primers 5′-CGAGCTCAAGGAGATATAATGCCAGAAAAAC ATGACCT-3′ and 5′-AAAACTGCAGCTATCTGCAC TGCTCATTAAT-3′, then cloned into pBAD18 vector between *Sac*I and *Pst*I sites to get plasmid pY01. The single P*mgtA* gene was amplified with primers 5′-CGA GCTCCTTTCGTTATTCAGCACCCG-3′ and 5′-CGA GCTCGCGATATAATACCTGCTGGC-3′, the P*mgtA*-UTR was amplified with primers 5′-CGAGCTC CTTTCGTTATTCAGCACCCG-3′ and 5′-CGAGCT-CAA GGAGTCCCTCCGCACTGT-3′, then cloned into *Sac*I site of pY01 to obtain pY02 and pY03, respectively. The fragment P*mgtA* -*SRRz* was amplified from pY02 and P*mgtA*-UTR -*SRRz* was amplified from pY03, with the same primers 5′- CCGCTCG AGCTTTCGTTATT-CAGCACCCG-3′ and 5′- CCCAAGCTTAAAAGCCTC CGGT CGGAGGCTTTTCTATCTGCACTGCTCAT-TAAT-3′, then cloned into *Xho*I and *Hind*III sites of pWQ02[15] to get pY04 and pY05, respectively. The backbone of the plasmids constructed in this study was shown in Fig. 3. All the plasmids were verified by PCR and gene sequencing.

The plasmids pY01, pY02, and pY03 were transformed into *E. coli* DH5α to generate the strains Q2537, Q2549, and Q2582. The pY02 and pY03 were also transformed into Q1638 to obtain the P3HP producing strains Q2588 and Q2572, respectively. The plasmids pY04 and pY05 were transformed into the strain Q1463 to get the P3HP producing strains Q2647 and Q2646, respectively. Q1463 and Q1638 were constructed in our previous study.[14] All the recombinant strains and plasmids are listed in Table 3.

Detection of the lysis efficiency

Single colony of each strain was cultivated overnight in LB medium to get seed solution, then inoculated at 10% into M9 medium, 10 mM $MgSO_4$ was added to prevent earlier cell lysis. The control strains were *E. coli* DH5α with empty vector pBAD18 and Q2537 without induction. To activate promotor P_{BAD}, 0.1% L-arabinose was added into Q2537 broth cells culture at OD_{600} 0.6~0.8 under 30°C. To evaluate the activity of P*mgtA*, the cell growth (OD_{600}) was monitored at different time intervals.

The lysis efficiency was detected by plate colony-counting methods. 1 ml of the cell-culture LB medium was centrifuged and washed twice with sterile water, then re-suspended with M9 medium without Mg^{2+}. The cell suspensions were separated equally into 2 tubes. One tube, as control group, was supplemented with 10 mM $MgSO_4$ (L- arabinose instead of $MgSO_4$ was added for Q2537). After 1 h of incubation at 37°C, the cells suspension was diluted 10^{-6} times and spreaded on the LB agar plates with or without Mg^{2+} respectively. The monoclonal cells on each plate were counted after cultured overnight. The lysis efficiency was analyzed by comparing the colony-forming units (CFU) on Mg^{2+}-depletion LB agar plates and CFU on LB agar plates containing Mg^{2+}. The lysis efficiencies of other strains were tested using the same method as described above.

Flask fermentation for P3HP production

Shake flask fermentation was performed in a 500mL baffled-flask containing 100 mL modified minimal medium at 37°C shaking at 200 rpm, the initial concentration of $MgSO_4$ in the medium was 10mM. Gene expression of cultures were induced with 0.05mM isopropyl-β-d-thiogalactoside at OD_{600} 0.6~0.8. After induction, the cultures were further incubated at 30°C for 48 h. The cells were harvested by centrifugation and washed with cool absolute ethanol, then dried in an oven at 80°C. P3HP was extracted from the cells with hot chloroform in a Soxhlet apparatus, the content was calculated as described in our pervious paper.[14]

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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References

[1] Numanoglu Y, Sungur S. β- Galactosidase from *Kluyveromyces lactis* cell disruption and enzyme immobilization

using a cellulose-gelatin carrier system. Process Biochem 2004; 39(6):703-9; https://doi.org/10.1016/S0032-9592(03)00183-3

[2] Chisti Y. Strategies in downstream processing. In: Subramanian G editor. Bioseparation and Bioprocessing: A Handbook. New York: Wiley-VCH Press; 1998:3-30.

[3] Liu X, Curtiss R. Nickel-inducible lysis system in *Synechocystis sp* PCC 6803. Proc Natl Acad Sci USA 2009; 106:21550-4; PMID:19995962; https://doi.org/10.1073/pnas.0911953106

[4] Zhang X, Pan Z, Fang Q, Zheng J, Hu M, Jiao X. An autoinducible *Escherichia coli* lysis system controlled by magnesium. J Microbiol Methods 2009; 79:199-204; PMID: 19748529; https://doi.org/10.1016/j.mimet.2009.09.001

[5] Hajnal I, Chen XB, Chen GQ. A novel cell autolysis system for cost-competitive downstream processing. Appl Microbiol Biot 2016; 100(21):9103-10; https://doi.org/10.1007/s00253-016-7669-3

[6] São-José C, Nascimento J, Parreira R, Santos MA. Release of progeny phages from infected cells. In: McGrath S, van Sinderen D editors. Bacteriophages: Genetics and Molecular Biology. Norfolk (UK): Caister Academic Press; 2007. p.307-34.

[7] Loessner MJ. Bacteriophage endolysins-current state of research and applications. Curr Opin Microbiol 2005; 8 (4):480-7; PMID:15979390; https://doi.org/10.1016/j.mib.2005.06.002

[8] Andreesen B, Taylor NL, Steinbuchel A. Poly(3-Hydroxypropionate): a Promising Alternative to Fossil Fuel-Based Materials. Appl Environ Microb 2014; 80 (21):6574-82; https://doi.org/10.1128/AEM.02361-14

[9] Feng XJ, Xian M, Liu W, Xu C, Zhang HB, Zhao G. Biosynthesis of poly(3-hydroxypropionate) from glycerol using engineered *Klebsiella pneumoniae* strain without vitamin B_{12}. Bioengineered 2015; 6 (2):77-81; PMID:25621933; https://doi.org/10.1080/21655979.2015.1011027

[10] Groisman EA, Cromie MJ, Shi Y, Latifi T. A Mg^{2+}-responding RNA that controls the expression of a Mg^{2+} transporter. Cold Spring Harb Symp Quant Biol 2006; 71:251-8; PMID:17381304; https://doi.org/10.1101/sqb.2006.71.005

[11] Cromie MJ, Shi Y, Latifi T, Groisman EA. An RNA sensor for intracellular Mg(2+). Cell 2006; 125:71-84; PMID:16615891; https://doi.org/10.1016/j.cell.2006.01.043

[12] Spinelli SV, Pontel LB, García Véscovi E, Soncini FC. Regulation of magnesium homeostasis in *Salmonella*: Mg^{2+} targets the *mgtA* transcript for degradation by RNase E. FEMS Microbiol Lett 2008; 280:226-34; PMID:18248433; https://doi.org/10.1111/j.1574-6968.2008.01065.x

[13] Kato A, Tanabe H, Utsumi R. Molecular characterization of the PhoP-PhoQ two-component system in *Escherichia coli* K-12: identification of extracellular Mg^{2+}-responsive promoters. J Bacteriol 1999; 17:5516-20.

[14] Gao Y, Liu C, Ding Y, Sun C, Zhang R, Xian M, Zhao G. Development of Genetically stable *Escherichia coli* strains for Poly(3-Hydroxypropionate) production. Plos One 2014; 9(5):e97845; PMID:24837211; https://doi.org/10.1371/journal.pone.0097845

[15] Wang Q, Yang P, Liu CS, Xue YC, Xian M, Zhao G. Biosynthesis of poly(3-hydroxypropionate) from glycerol by recombinant *Escherichia coli*. Bioresour Technol 2013; 131:548-51; PMID:23414748; https://doi.org/10.1016/j.biortech.2013.01.096

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