

The Surface Display of the Alginate Lyase on the Cells of *Yarrowia lipolytica* for Hydrolysis of Alginate

Guanglei Liu · Lixi Yue · Zhe Chi · Wengong Yu ·
Zhenming Chi · Catherine Madzak

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Abstract The alginate lyase structural gene (*AlyVI* gene) was amplified from plasmid pET24-ALYVI carrying the alginate lyase gene from the marine bacterium *Vibrio* sp. QY101 which is a pathogen of *Laminaria* sp. When the gene was cloned into the multiple cloning site of the surface display vector pINA1317-YICWP110 and expressed in cells of *Yarrowia lipolytica*, the cells displaying the alginate lyase could form clear zone on the plate containing sodium alginate, indicating that they had high alginate lyase activity. The cells displaying alginate lyase can be used to hydrolyze poly- β -D-mannuronate (M) and poly- α -L-guluronate (G) and sodium alginate to produce different lengths of oligosaccharides (more than pentasaccharides). This is the first report that the yeast cells displaying alginate lyase were used to produce different lengths of oligosaccharides from alginate.

Keywords Alginate lyase gene · Alginate · Marine bacterium · Yeast surface display · Oligosaccharides

Introduction

Alginate produced by brown seaweeds and certain bacteria is an anionic heteropolysaccharide which is composed of

β -D-mannuronate (M) and α -L-guluronate (G) (Yoon et al. 2000). Because of the high viscosity and gelling properties of alginate with calcium ions, it is widely used in the food, pharmaceutical, and medical industries. The alginate hydrolysates and their derivatives exhibit many important bioactivities, such as stimulating human keratinocytes, accelerating plant root growth, increasing a growth rate of *Bifidobacterium* sp, producing protoplasts from brown sea algae, and enhancing penicillin production from cultures of *Penicillium chrysogenum*. Alginate oligosaccharides produced by alginate lyase can cause cytotoxic cytokine production in human mononuclear cells. The low-molecular-weight alginate derivatives also show antioxidation and prevention of cardiovascular and cerebrovascular diseases.

Alginate lyase splits the glycoside linkages of alginate chains via β -elimination to produce oligosaccharides (Yoon et al. 2000). Many marine bacteria, such as *Pseudoalteromonas elyakovii* IAM (Sawabe et al. 2001), *Sphingomonas* sp. A1 (Miyake et al. 2003), *Klebsiella pneumoniae* (Caswell et al. 1989), *Vibrio* sp. QY101 (Han et al. 2004), *Vibrio* sp. YKW-34 (Fu et al. 2007), and *Pseudomonas* sp. OS-ALG-9 (Fujiyama et al. 1995), have been found to produce a large amount of alginate lyases. So far, approximately 20 genes encoding alginate lyases from marine bacteria have been cloned and sequenced. A1-IV from *Sphingomonas* sp. A1 (Miyake et al. 2003), alginate lyases from *K. pneumoniae* (Caswell et al. 1989), *Pseudomonas* sp. OS-ALG-9 (Fujiyama et al. 1995), *Vibrio* sp. QY101 (Han et al. 2004), *Corynebacterium* strain ALY-1 (Matsubara et al. 2000), *Deleya marina* (Kraiwananapong et al. 1999), *P. elyakovii* IAM (Sawabe et al. 2001; Ma et al. 2008), and *Streptomyces* sp. ALG-5 (Kim et al. 2008) were successfully expressed in *Escherichia coli* cells. It has been reported that the marine bacterium *Vibrio* sp. QY101 which was isolated from a decaying thallus of *Laminaria* sp. is a pathogen of it

G. Liu · L. Yue · Z. Chi · W. Yu · Z. M. Chi (✉)
Unesco Chinese Center of Marine Biotechnology,
Ocean University of China,
Yushan Road, No. 5,
Qingdao, China
e-mail: zhenming@sdu.edu.cn

C. Madzak
UMR1238 Microbiologie et Génétique Moléculaire,
INRA/CNRS/INAPG, CBAI, BP 01,
78850 Thiverval-Grignon, France

(Han et al. 2004). Therefore, it is not feasible to produce alginate lyase on large scale using the pathogenic bacterium.

In recent years, yeast surface display techniques have received increasing attention as they have many applications in biotechnological and industrial fields, such as cell adhesion, molecular recognition, immobilized biocatalysis, bioconversion, bioremediation, change of cell function, signal transduction, biosensor, live vaccine development and ultra-high-throughput screening for the identification of novel biocatalysts (Yue et al. 2008; Zhu et al. 2006; Ni et al. 2008). Alkaline protease, inulinase, amylases, cellulases, xylanases, hemolysin, and other proteins have been successfully immobilized on yeast cells and their potential applications have been evaluated (Yue et al. 2008; Zhu et al. 2006; Ueda and Tanaka 2000; Ni et al. 2008). However, alginate lyase has not been displayed on yeast cells for hydrolysis of alginate so far. A surface display vector pINA1317-YICWP110 for protein display on the yeast *Yarrowia lipolytica* was constructed in our laboratory (Yue et al. 2008). The vector has many advantages over other surface display vectors. It uses a strong recombinant growth-phase-dependent promoter, hp4d (Madzak et al. 2004). The vector carries zeta elements, which allow them to integrate genomic DNA in *Y. lipolytica* strains which are generally regarded as safe. Furthermore, only a “yeast expression cassette” is used for the transformation of the recipient strain. The resulting strain is devoid of bacterial DNA, retaining generally regarded as safe status and avoiding the spread of antibiotic resistance genes in the environment.

Because the alginate-lyase-producing marine bacteria reported so far are the pathogen to *Laminaria* sp, it is not feasible to use it for production of the enzyme by the bacteria. The enzyme displayed on the cells of *Y. lipolytica* can be reused and regenerated and is “naturally” immobilized on the cell surface. Therefore, in this study, the alginate lyase gene from the marine bacterium *Vibrio* sp. QY101 was cloned into pINA1317-YICWP110 and expressed in cells of *Y. lipolytica*. The expressed alginate lyase was displayed on the yeast cells. The yeast cells displaying the alginate lyase were used to hydrolyze alginate.

Materials and Methods

Strains and media The yeast *Y. lipolytica* Po1h (*Mata*, *ura3-302*, *xpr2-322*, *xpr1-2*; phenotype: *Ura*⁻, *AEP*, *AXP*, *Suc*⁺; Madzak et al. 2004) was used as a host for cell surface display. The yeast strain was grown in yeast–polypeptone–dextrose; 1.0% yeast extract, 2.0% bacto-peptone, 2.0% glucose). The yeast transformants were selected on YNB-N5000 (0.17% yeast nitrogen base without amino acids and

ammonium sulfate, 1.0% glucose, 0.5% ammonium sulfate). In order to produce displayed protein by yeast transformants, the PPB medium was used (Jolivald et al. 2005). The *E. coli* strain used in this study for plasmid recovery and cloning experiments was DH5 α [*F*⁻ *endA1* *hsdR17(rK-/mK+)* *supE44* *thi-1 λ* ⁻ *recA1* *gyr96 Δ lacU169(ϕ 80lacZ Δ M15)*] and was grown in Luria broth (LB). The *E. coli* transformants were grown in LB medium with 30 μ g/ml of kanamycin.

Plasmids pET24-ALYVI (Kan^r; Han et al. 2004) carrying the alginate lyase gene from the marine bacterium *Vibrio* sp. QY101 which was isolated from a decaying thallus of *Laminaria* sp. was used as the template for amplification of the alginate lyase gene by polymerase chain reaction (PCR). The surface display vector pINA1317-YICWP110 which contains the C-terminal end of *YICWPI* from *Y. lipolytica* was constructed in this laboratory (Yue et al. 2008). pMD19-T was purchased from TaKaRa (Japan).

Isolation of DNA, restriction digestions, and transformation DNA manipulations were carried out using standard methods (Sambrook et al. 1989). Bacterial plasmid DNA was purified using Perfectprep plasmid minikit (Eppendorf). Restriction endonuclease digestions and DNA ligations were performed according to the manufacturer’s recommendations. *E. coli* was transformed with plasmid DNA according to Sambrook et al. (1989). Transformants were plated out onto LB medium containing 30 μ g/ml of kanamycin. *Y. lipolytica* was transformed according to the methods as described by Xuan et al. (1988).

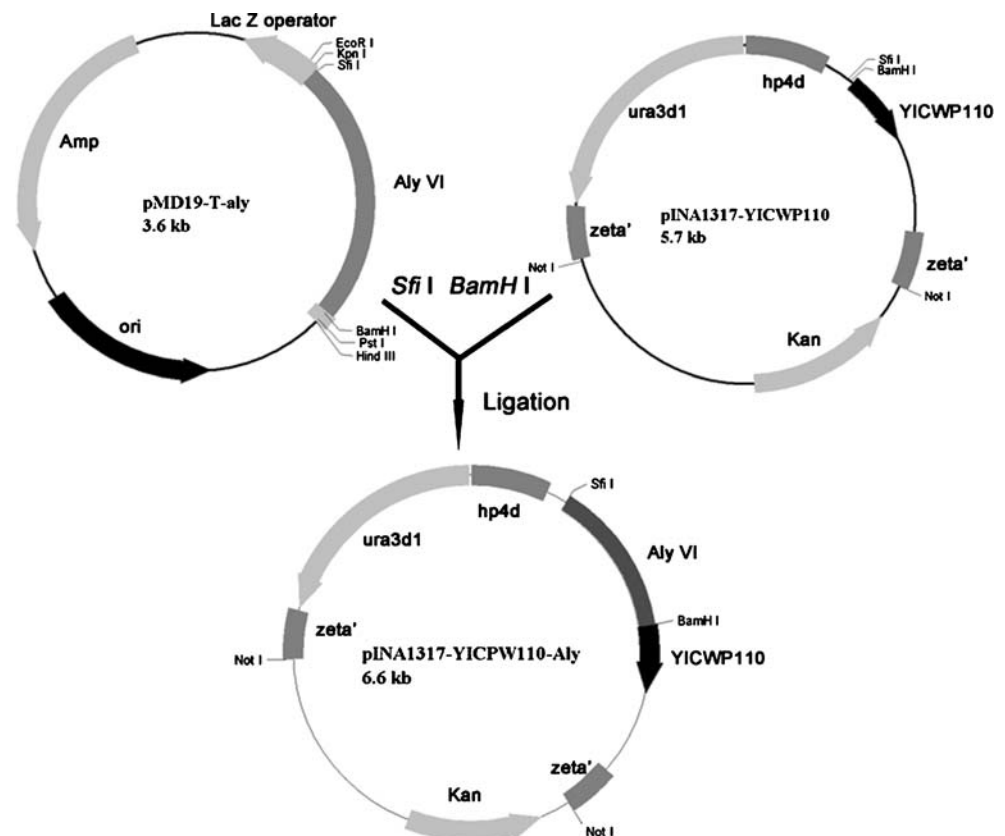
DNA sequence and computer analysis BLAST and ORF Finder programs at the National Center for Biotechnology Information were used for the nucleotide sequence analysis, deduction of the amino acid sequence, and database searches.

Amplification of the alginate lyase gene from pET24-ALYVI (Kan^r) The primers for amplification of the gene encoding the alginate lyase were designed according to *ALYVI* gene (accession number: AY221030) and the multiple cloning site of the surface display vector pINA1317-YICWP110 (Yue et al. 2008). The forward primer Aly1 was 3′-GGCCGTTCTGGCCGAACAAGCAGAGCTTAA AAC-5′ (the bases underlined encode *Sfi*I restriction site) and the reverse primers (Aly2 and Aly3) were 3′-CGGCGC GGATCCTCTATGATCAATATTGATCTTCG-5′ (the bases underlined encode *Bam*HI restriction site) and 3′-GGATCC GTGATGATGGTGGTG TCTATGATCAATATGAT-5′ (the bases underlined encode *Bam*HI restriction site and the bases boxed encode 6-His), respectively. The gene encoding the alginate lyase was amplified by PCR

using pET24-ALYVI as a template. The reaction system (50 μ l) was composed of 5.0 μ l of TaKaRa 10 \times Ex Taq buffer, 4.0 μ l of TaKaRa 5 mM dNTP, 2.0 μ l (20 μ M) of Aly1, 2.0 μ l (20 μ M) of Aly2 or Aly3, 0.3 μ l of ExTaq DNA polymerase (5 U/ μ l), 2.0 μ l (10.0 ng/ml) of the template DNA, and 35.0 μ l of H₂O. The conditions for the PCR amplification were as follows: initial denaturation at 94°C for 5 min, denaturation at 94°C for 45 s, annealing temperature at 62°C for 45 s, extension at 72°C for 1 min, and final extension at 72°C for 8 min. PCR was run for 30 cycles and the PCR cycler was GeneAmp PCR System 2400 (PerkinElmer, Waltham, MA, USA). PCR products were separated in agarose gel and recovered using TaKaRa Agarose Gel DNA Fragment Recovery Kit Ver.2.0. The recovered PCR products were ligated into pMD19-T and transformed into the competent cells of *E. coli* DH5 α . The transformants were selected on the plates with kanamycin. The plasmids were extracted by using the methods as described by Sambrook et al. (1989). The cloned DNA fragments inserted on the vector were sequenced by Shanghai Sangon Company. After alignment, it was found that the sequence of the cloned DNA fragments was completely identical to the DNA sequence of the *ALYVI* gene (accession number: AY221030).

Alginate lyase display on cells of *Y. lipolytica* The gene amplification by PCR, the gene expression, and the lyase display on the yeast cells of *Y. lipolytica* were performed as described by Yue et al. (2008). The pMD19-T carrying the cloned *ALYVI* gene was digested with *Sfi*I at 50°C overnight then with *Bam*HI at 37°C for 3 h. The digests containing the *ALYVI* gene were separated in agarose gel and recovered using TaKaRa Agarose Gel DNA Fragment Recovery Kit Ver.2.0. The recovered digest products were ligated into the surface display vector pINA1317-YICWP110 digested with the same enzymes (Yue et al. 2008) and the resulting plasmid carrying *ALYVI* gene was designated as pINA1317-YICWP110-ALY (Fig. 1). The plasmid was transformed into the competent cells of *E. coli* DH5 α . The transformants were selected on the plates with kanamycin. The plasmids were extracted by using the methods as described by Sambrook et al. (1989). pINA1317-YICWP110-ALY extracted from *E. coli* transformants were digested with *Not*I. The fragments carrying YICWP110-ALY were separated in agarose gel and recovered using TaKaRa Agarose Gel DNA Purification Kit Ver.2.0. The recovered fragments were transformed into *Y. lipolytica* Po1h by lithium acetate method (Xuan et al. 1988). The transformants were spread on the YNB plates

Fig. 1 Construction of the plasmid pINA1317-YICWP110-ALY for alginate lyase surface display on *Y. lipolytica* cells



and grown at 28°C for 96 h. *Y. lipolytica* Po1h only carrying fragment YICWP110 was used as a control.

Integration of the *ALYVI* gene into genomic DNA in the positive transformants was confirmed as described below. The positive transformants carrying *ALYVI* gene were grown in PPB liquid medium for 120 h. The alginate lyase activity of different positive transformants was determined as described below. The cells of *Y. lipolytica* Po1h only carrying yeast cassette without *ALYVI* gene were used as a control.

Determination of alginate lyase activity The cells of the positive transformants carrying *ALYVI* gene and *Y. lipolytica* Po1h only carrying yeast cassette without *ALYVI* gene were grown in PPB medium for 120 h, respectively. The cultures were washed three times with phosphate-buffered saline (PBS) buffer (pH 7.2) by centrifugation at $6,000\times g$ and 4°C for 5 min. The pellets were resuspended in PBS buffer (pH 7.2). Half of milliliter of the cell suspension was mixed with 0.5 ml of 0.3% sodium alginate solution (dissolved in PBS buffer pH 7.2) and the mixture was incubated at 30°C for 30 min. After that, the reaction was stopped immediately in boiling water for 5 min. After cooling, the mixture was centrifuged at $14,000\times g$ for 10 min and optical density value of the supernatant obtained was determined with spectrophotometer at 235 nm (Li et al. 2003). The reaction mixtures which contained the same cell suspension inactivated at 100°C for 5 min were used as a control. One unit

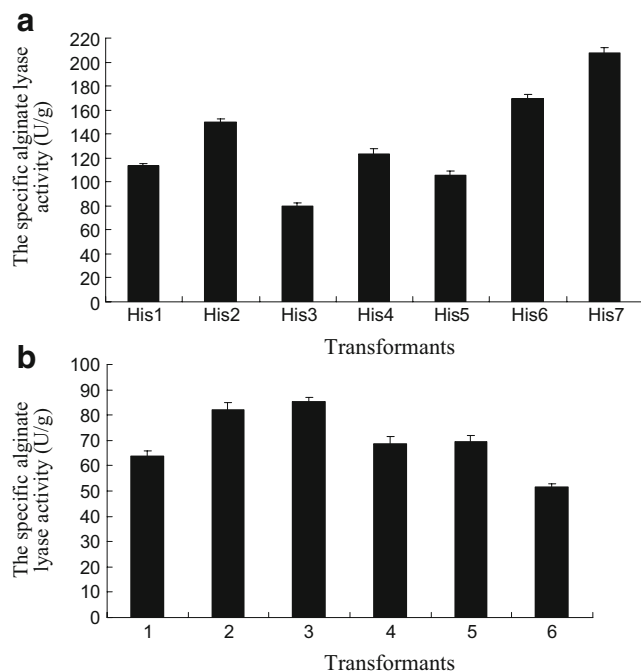


Fig. 2 The specific alginate lyase activities of the yeast transformants carrying *ALYVI* genes with the bases encoding 6× His tag (a) and without the bases encoding 6× His tag (b), respectively. All the data are given as means±SD, $n=3$

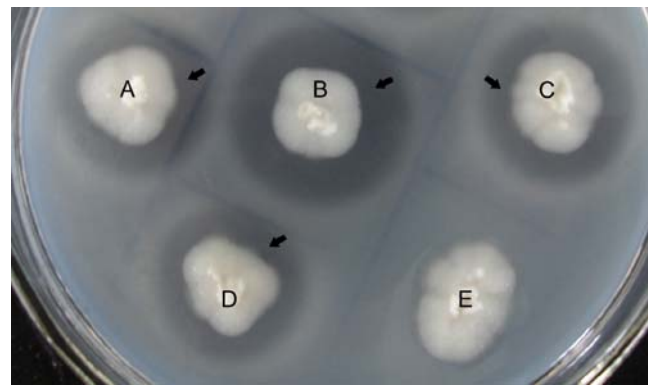


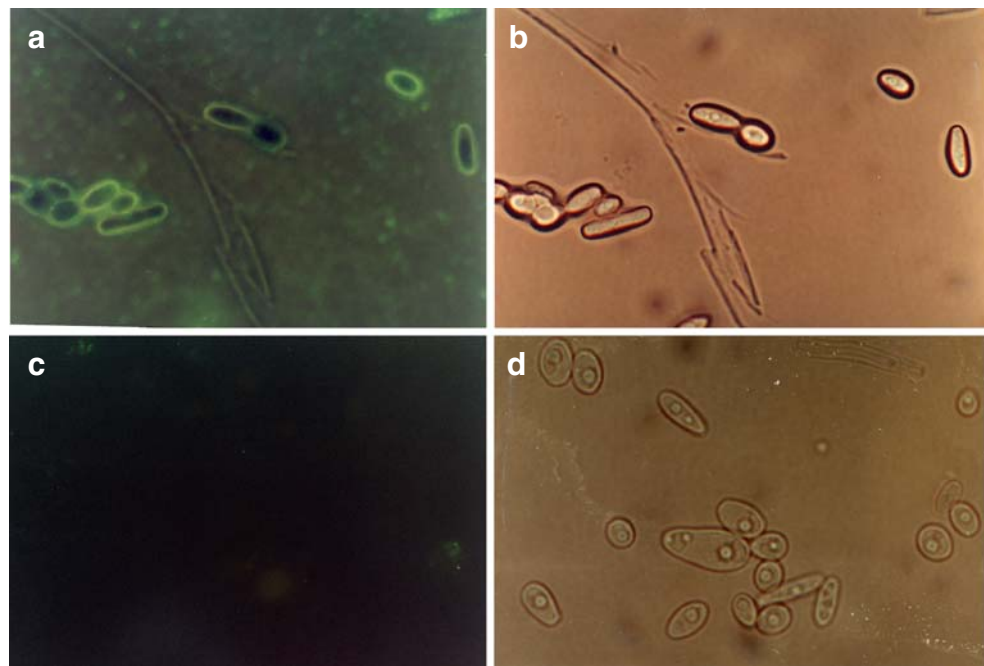
Fig. 3 The clear zones (A–D) formed on the plates with sodium alginate by the transformants carrying *ALYVI* gene and no clear zone (E) formed by the control cells only carrying YICWP-110 without *ALYVI* gene. The clear zones (A and D) were formed by the transformants carrying *ALYVI* gene without the bases encoding 6× His tag while the clear zones (B and C) were formed by the transformants carrying *ALYVI* gene with the bases encoding 6× His tag

of alginate lyase activity was defined as the increase of 0.01 absorbance unit at 235 nm. Cell dry weight of the cultures was determined as described by Gao et al. (2007). The specific alginate lyase activity was defined as units per gram of cell dry weight. Alginate lyase activity was also estimated using the plate which contained 0.5% sodium alginate. Different colonies of the transformants were transferred to the plates and incubated at 28°C for 5 days. Then, 70% ethanol solution was added to the plates and the colonies were submerged for 30 min. Finally, the clear zones around the colonies were observed and photographed.

Effects of pH and temperature on the displayed alginate lyase activity The effect of pH on the alginate lyase activity was determined by incubating the yeast cells displaying alginate lyase between pH 4.0 and 9.0 using the standard assay condition. The buffers used were 0.1 M acetate buffer (pH 4.0–6.0) and 0.1 M Tris–HCl buffer (pH 7.0–9.0). The optimal temperature for activity of the alginate lyase was determined at temperatures of 16°C, 30°C, 40°C, and 50°C in 0.1 M PBS buffer (pH 7.3).

Immunofluorescence microscopy The immunofluorescence microscopy was conducted as described by Adams et al. (1998) using the 6× His monoclonal antibody produced by Clontech (USA) as primary antibody, and immunoglobulin G/fluorescein isothiocyanate (IgG/FITC) as secondary antibody produced by ZSGB-BIO (China). *Y. lipolytica* Po1h cells carrying YICWP110-ALY were grown in PPB liquid medium for 120 h. The cells were collected and washed three times by centrifugation with PBS buffer. The pellets were suspended in 3.7% formaldehyde and incubated overnight at 28°C with shaking. Then, the yeast cells were

Fig. 4 Immunofluorescent labeling of transformed *Y. lipolytica* cells using 6× His monoclonal antibody as primary antibody and IgG/FITC as secondary antibody. Microphotographs were taken under visible light (**b** and **d**), and immunofluorescence microphotographs were taken under emission at 550 nm (**a** and **c**). **c** and **d**: *Y. lipolytica* control cells harboring YICWP110; **a** and **b**: *Y. lipolytica* cells harboring YICWP110-ALY. Magnification 40×10



labeled using the primary and secondary antibodies mentioned above.

Confirmation of integration of the target gene into *Y. lipolytica* genome To get the evidence that DNA fragments carrying YICWP110-ALY have been integrated into *Y. lipolytica* genome, genomic DNAs from the corresponding transformants were extracted and used as templates for PCR. The forward primer and the reverse primer used for this checking were Aly1 and Aly2 as described above, respectively. PCR amplification was performed as described above. The sizes of the PCR products were estimated using the Automated Gel Documentation and Analysis System (Gene-Genius, USA).

Depolymerization of sodium alginate, polyM, and polyG by the displayed alginate lyase The mixture consisting of 0.3% (w/v) sodium alginate or 0.3% (w/v) polyG or 0.3%

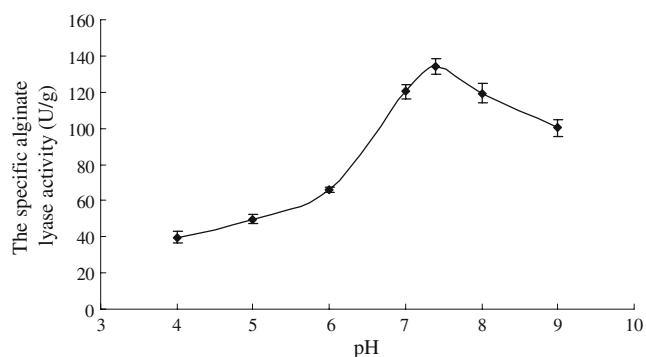


Fig. 5 Effects of different pHs on activity of the alginate lyase displayed on the yeast cells. All the data are given as means±SD, $n=3$

(w/v) polyM, 0.1 M PBS buffer (pH 7.3), and 200 μ l of the yeast cells displaying the alginate lyase was incubated at 30°C for 4 h. The alginate hydrolysates released were determined as described above.

Thin-layer chromatography The alginate-depolymerization products were analyzed by thin-layer chromatography (TLC) using a solvent system of 1-butanol–acetate acid–water (3:2:3, v/v). The products were visualized by heating the TLC plate at 130°C for 2 min after spraying with 10% (v/v) sulfuric acid in ethanol (Hashimoto et al. 2000).

Results and discussion

Effects of 6×His Tag on the activity of alginate lyase immobilized on the yeast cells The ALYV I gene encoding

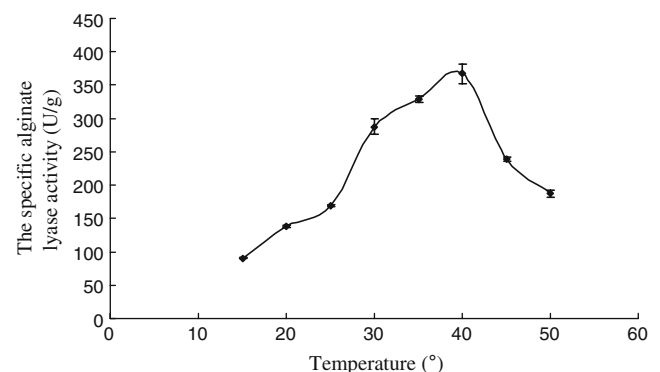


Fig. 6 Effects of different temperatures on activity of the alginate lyase displayed on the yeast cells. All the data are given as means±SD, $n=3$

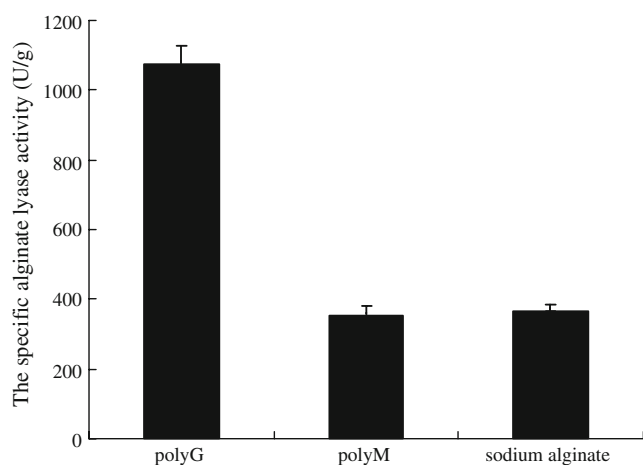


Fig. 7 Hydrolysis of polyM, polyG, and sodium alginate. All the data are given as means \pm SD, $n=3$

alginate lyase from the marine bacterium *Vibrio* sp. QY101 was amplified from the plasmid pET24-ALYVI (Kan^r) carrying the alginate lyase gene with the reverse primers with the bases encoding 6 \times His tag and without the bases encoding 6 \times His tag. Construction of the plasmid pINA1317-YICWP110-ALY for the alginate lyase surface display on *Y. lipolytica* cells is shown in Fig. 1. After the genes were cloned into the surface display plasmid pINA1317-YICWP110, they were expressed in the yeast cells of *Y. lipolytica* and the alginate lyase was displayed on the yeast cells. We found that the activity of the alginate lyase with 6 \times His tag was much higher than that of the alginate lyase without 6 \times His tag. For example, the transformant His7 displaying the alginate lyase with 6 \times His tag had the specific activity of over 208.0 U/g of cell dry weight (Fig. 2a) while the transformant 3 displaying the alginate lyase without 6 \times His tag had the specific activity of less than 85.4 U/g of cell dry weight (Fig. 2b). The colonies carrying the alginate lyase with 6 \times His tag also could form bigger clear zones on the plates than those carrying the alginate lyase without 6 \times His tag (Fig. 3). Thus, the transformant His7 displaying the alginate lyase with 6 \times His tag was used for the subsequent studies. Strauss and Gotz (1996) also reported that the activity of surface-immobilized lipase varied with the spacer length. The activity increases from 0.8 to 83 units per milligram lipase as the spacer length varies from 10 to 92 amino acids. They thought that the length of the cell-wall-spanning region of the carrier protein must exceed a critical length to allow efficient folding of the passenger protein. Therefore, 6 \times His tag as the spacer of the displayed alginate lyase on the yeast cells may also play positive role in the efficient folding of the alginate lyase on the yeast cells, resulting in the increased alginate lyase activity (Figs. 2 and 3). However, it is still unknown if the longer His tag could further increase alginate lyase activity.

In order to confirm the presence of the 6 \times His-alginate lyase-YICWP110 fusion protein on the yeast cell surface, immunofluorescence labeling of the yeast cells was performed with 6 \times His monoclonal antibody as primary antibody and IgG/FITC as secondary antibody. YICWP110-ALY-carrying *Y. lipolytica* cells were grown in PPB medium for 120 h. The *Y. lipolytica* cells displaying 6 \times His-alginate lyase-YICWP110 fusion protein were labeled by IgG/FITC, and green fluorescence on the yeast cells was observed, while no fluorescence was observed on control YICWP110-carrying *Y. lipolytica* cells (Fig. 4). These results clearly demonstrated that the 6 \times His-alginate lyase-YICWP110 fusion protein was displayed on the cell surface, allowing its recognition by the antibodies. It can also be noticed from the results in Fig. 4 that 100% of the observed cells displayed alginate lyase. In our previous studies (Yue et al. 2008; Ni et al. 2008), it was also found that 100% of the *Y. lipolytica* cells displayed enhanced green fluorescent protein, hemolysin, and alkaline protease using the same glycosylphosphatidyl inositol-anchor-fusion expression system and the *Y. lipolytica* cells displaying hemolysin and alkaline protease exhibited hemolytic activity toward erythrocytes from flounder and all the proteins tested, respectively.

After the fragments without the signal sequence of *ALYVI* gene were amplified from genomic DNA in *Y. lipolytica* carrying the alginate lyase gene by PCR, the

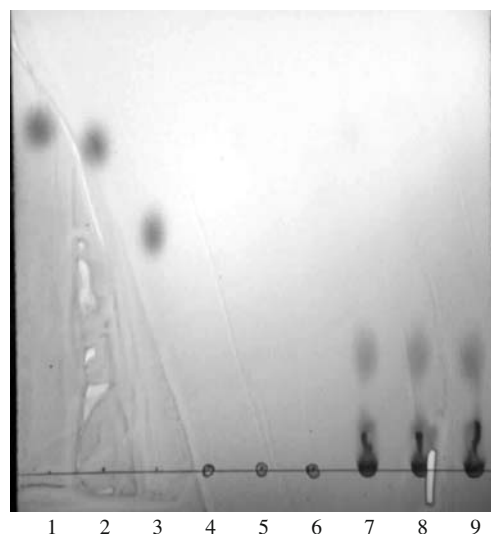


Fig. 8 Thin-layer chromatogram of the end products of alginate, polyM, and polyG after hydrolysis with the displayed alginate lyase. Lane 1 glucose; lane 2: maltose; lane 3: raffinose; lane 4: unhydrolyzed polyM (1.0%); lane 5: unhydrolyzed polyG (1.0%); lane 6: unhydrolyzed alginate (1.0%); lane 7: hydrolyzed polyM (1.0%); lane 8: hydrolyzed polyG (1.0%) and 9: hydrolyzed alginate (1.0%). The end products of the hydrolysis were analyzed by using TLC plate as described in “Materials and Methods.” The products were visualized by heating the TLC plate after spraying with 10% (v/v) sulfuric acid in ethanol

expected size of PCR product of 1,210 bp in size was observed (data not shown), suggesting that *ALYVI* gene indeed has been integrated into the genomic DNAs in *Y. lipolytica*.

Optimal pH and temperature of the alginate lyase displayed on the yeast cells The alginate lyase activity was measured at various pHs in buffers with the same ionic concentrations. The maximum activity (363 U/g) of the alginate lyase displayed on the yeast cells was observed at pH 7.3 (Fig. 5). However, the optimal pH of the purified recombinant alginate lyase from *E. coli* BL21 (DE3) cells containing the plasmid carrying the alginate lyase gene and the purified native alginate lyase produced by *Vibrio* sp. QY101 was found to be 7.5, respectively (Han et al. 2004; Li et al. 2003). The maximum activity of the displayed alginate lyase, 360 U/g, was observed at 40°C during the tested temperature ranging from 15°C to 50°C (Fig. 6). The optimal temperature of the purified recombinant alginate lyase from *E. coli* BL21 (DE3) cells containing plasmid was also 40°C (Han et al. 2004). This indicates that the optimal temperature and pH of the alginate lyase immobilized on the yeast cells were the same as those of the recombinant alginate lyase produced by *E. coli* cells carrying pET24-alyVI. In contrast, the optimal temperature of the purified alginate lyase from *Vibrio* sp. QY101 was 30°C (Li et al. 2003). However, the optimal temperature of the alkaline protease was decreased after it was immobilized on the yeast cells by the same way compared to that of the free alkaline protease purified from *Aureobasidium pullulans* HN2.3 (Ni et al. 2008).

Hydrolysis of alginate, polyM, and polyG by the displayed alginate lyase Based on the substrate specificity, alginate lyases have been classified as poly(M) lyases (EC 4.2.2.3) and poly(G) lyases (EC 4.2.2.11), which preferentially degrade the poly(M) block and poly(G) block, respectively. Therefore, sodium alginate, polyM, and polyG were digested by the displayed alginate lyase and the alginate hydrolysates released from sodium alginate, polyM, and polyG were determined as described in “Materials and methods,” respectively. The results in Fig. 7 show that polyM can be much more easily hydrolyzed by the displayed alginate lyase than polyG and sodium alginate. This means that the displayed alginate lyase is poly(M) lyases. Alginate lyases from herbivorous marine mollusks such as abalone and turban shell are also poly(M) lyases (Yamamoto et al. 2008). A1-IV and A1-IV from *Sphingomonas* sp. prefer polyM (Hashimoto et al. 2005). However, recently, bifunctional alginate lyases which act on both poly(M) and poly(G) blocks have also been isolated. For example, the extracellular alginate lyase produced by *P. elyakovii* and the recombinant *E. coli* is capable of degrading all block

structures derived from sodium alginate and produces a series of tri to octaoligouronates (Sawabe et al. 2001; Ma et al. 2008). In contrast, A1-II from *Sphingomonas* sp. prefers polyG rather than polyM and releases tri and tetrasaccharides (Yoon et al. 2000). The alginate lyase produced by *Streptomyces* sp. ALG-5 appears to be polyguronate lyase degrading polyG block preferentially than polyM block (Kim et al. 2008).

The hydrolysis products of alginate, polyM, and polyG by the displayed alginate lyase were analyzed by TLC. All the alginate, polyM, and polyG could be converted into different lengths of oligosaccharides (more than pentasaccharides) by the displayed alginate lyase (Fig. 8). This characteristic may find highly potential applications in seaweed genetics, food, and pharmaceutical industries. Alginate that was purified from the fermentation solution of marine bacterium *Vibrio* sp. 510 under specific reaction conditions was hydrolyzed by the native alginate lyase and seven oligosaccharides, including di, tri, and tetrasaccharides, were detected (Zhang et al. 2004). All the alginate, polyM, and polyG can be converted into oligosaccharides with more than tetrasaccharides by the purified recombinant alginate lyase from *E. coli* (Ma et al. 2008). The degraded products by alginate lyase produced by *Streptomyces* sp. ALG-5 were found to be di, tri, tetra, and pentasaccharides (Kim et al. 2008).

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