Gene Cloning, Expression, and Characterization of a β-Agarase, AgaB34, from Agarivorans albus YKW-34

Fu, Xiao Ting¹², Cheol-Ho Pan¹, Hong Lin¹, and Sang Moo Kim¹*  

¹College of Food Science and Engineering, Ocean University of China, Qingdao 266003, China  
²Faculty of Marine Bioscience and Technology, Kangnung National University, Gangneung 210-702, Korea  
³Natural Products Research Center, KIST Gangneung Institute, Gangneung 210-340, Korea

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A β-agarase gene, agaB34, was functionally cloned from the genomic DNA of a marine bacterium, Agarivorans albus YKW-34. The open reading frame of agaB34 consisted of 1,362 bp encoding 453 amino acids. The deduced amino acid sequence, consisting of a typical N-terminal signal peptide followed by a catalytic domain of glycoside hydrolase family 16 (GH-16) and a carbohydrate-binding module (CBM), showed 37–86% identity to those of agarases belonging to family GH-16. The recombinant enzyme (rAgaB34) with a molecular mass of 49 kDa was produced extracellularly using Escherichia coli DH5α as a host. The purified rAgaB34 was a β-agarase yielding neoagarotetraose (NA4) as the main product. It acted on neoagarohexaose to produce NA4 and neoagarobiose, but it could not further degrade NA4. The maximal activity of rAgaB34 was observed at 30°C and pH 7.0. It was stable over pH 5.0–9.0 and at temperatures up to 50°C. Its specific activity and k₅₀/Kₘ value for agarose were 242 U/mg and 1.7×10⁴ M⁻¹•s⁻¹, respectively. The activity of rAgaB34 was not affected by metal ions commonly existing in seawater. It was resistant to chelating reagents (EDTA, EGTA), reducing reagents (DTT, β-mercaptoethanol), and denaturing reagents (SDS and urea). The E. coli cell harboring the pUC18-derived agarase expression vector was able to efficiently excrete agarase into the culture medium. Hence, this expression system might be used to express secretory proteins.

Keywords: β-Agarase, cloning, Agarivorans albus, neoagarooligosaccharide

Agar, the cell wall matrix of red algae, consists of two different components of agarose and agaroperist [2]. Agarose is a neutral linear polysaccharide composed of alternating residues of 3-O-linked β-D-galactopyranose (G-unit) and 4-O-linked 3,6-anhydro-α-L-galactopyranose (A-unit) [11]. Agarases, catalyzing the hydrolysis of agar, were characterized as α-agarase (E.C. 3.2.1.158) that cleaves α-1,3 linkage to produce agarooligosaccharides [24] and β-agarase (E.C. 3.2.1.81) that cleaves β-1,4 linkage to produce neoagarooligosaccharides [14].

Neoagarooligosaccharides produced by β-agarase have potential applications in food, pharmaceutical, and cosmetic industries owing to their physiological and biological activities. They inhibit the growth of bacteria, slow down the degradation of starch, reduce the caloric value of food, and are used as low-calorie additives to improve food qualities [10]. Neoagarotetraose (NA4) and neoagarohexaose (NA6) showed high antioxidative properties [31] and exhibited as efficient moisturizers on skin [22]. Besides production of oligosaccharides, agarase also acted as excellent tools for preparation of protoplasts from red algae [3] and recovery of DNA from agarose gel [27].

Agarivorans is a recently described genus [15], which was named owning to its agar devouring ability. So far, several β-agarase genes from different genera of bacteria have been cloned and the agarases they encoded have been purified and characterized [5, 6, 13, 21]. There are two reports on agarases from Agarivorans strains [17, 20]. The two agarases from Agarivorans strains had molecular masses of more than 100 kDa, and their amino acid sequences indicated that they belong to glycosidase hydrolase family 50 (GH-50).

Strain YKW-34 was isolated from the gut of turban shell, which was capable of degrading the cell walls of both brown algae Laminaria japonica and red algae Gelidium amansii. It was formerly classified as the genus Vibrio based on phenotypic features [33], but it was identified as Agarivorans albus based on its 16S rRNA gene sequence [8]. In our previous study, an alginate lyase [7] and two agarases (AgaA34 [8] and AgaB34) with excellent properties were found in the culture medium of this strain. In order to clone the genes encoding these enzymes, a functional cloning...
method was adopted to screen the chromosomal gene of this strain. In this study, gene cloning of a family GH-16 agarase (AgaB34), as well as expression, production, and purification of the recombinant enzyme (rAgaB34), is described. AgaB34 was different from other agarases produced by *Agarivorans* strains owing to its smaller molecular mass of 50 kDa and its catalytic domain of GH-16.

**Materials and Methods**

**Bacterial Strains and Culture Conditions**

*Agarivorans albus* YKW-34 was isolated from the gut of turban shell, *Turbinidae batillus cornutus* [33]. The strain was grown at 20°C in marine broth medium (Difco, Detroit, MI, U.S.A.), *Escherichia coli* DH5α (IniRON, Daejeon, Korea) [endAl hsdR17 (r−m− s−) supE44 thi-I recA1 gyrA (Naf) relA1 ΔlacZΔargF]U169 (F800lacZAM15)] was used as the host for the construction of the gene library and expression, and was grown at 37°C in Luria-Bertani (LB) broth supplemented with ampicillin (100 μg/ml) when required.

**Cloning of Agarase Gene**

Chromosomal DNA of *Agarivorans albus* YKW-34 was isolated as described by Wilson [30] and partially digested by Sau3AI. DNA fragments (3.0–8.0 kb) were ligated into the BamHI site of plasmid pUC18 using a T4 DNA ligase (IniRON). The ligation mixture was transformed into *E. coli* DH5α. The clones showing agarase activity were selected on LB agar plates containing 100 μg/ml of ampicillin. The colonies that formed depressions on the agar plate were considered as harboring agarase activity. Plasmids, pUSS1 and pUSS2, purified from two agarase-positive colonies using a plasmid purification kit (DNA-Spin, iNtRON) were sequenced on an ABI DNA polymerase (Solgent). The nucleotide database, and the complete nucleotide sequence of *A. albus* was compared with those in a NCBI site (http://www.ncbi.nlm.nih.gov/BLAST/).

**Bioinformatics Approaches**

The nucleotide sequence of *agaB34* and its deduced protein product were analyzed by different programs. Similarity searches were conducted with BLAST at the NCBI Web site. The free energy of the palindromic sequence structure was calculated by M-Fold (http://frontend.bioinfo.pj.edu/applications/mfold). The predicted signal sequence was identified with SignalP (http://www.cbs.dtu.dk/services/SignalP). The predicted molecular mass and pl value of the mature protein were calculated using Compute pI/Mw (http://us.expasy.org/tools/pi_tool.html). The predicted protein domains were identified with Conserved Domain BLAST (http://www.ncbi.nlm.nih.gov/Structure/ cdd/wrpsb.cgi).

**Extracellular Expression of rAgaB34**

The expression plasmid was constructed based on the plasmid pUSS1, which has a linear size of 5.1 kbp with an insert fragment of 2.4 kbp. There is a BamHI site in the insert fragment and a HindIII site in the multiple cloning site of the plasmid located 30 bp downstream of the 3′ end of the insert fragment. The sequence (~500 bp) between the site of BamHI and the C-terminal of the agarase gene was amplified by PCR from pUSS1 using *Phi* DNA polymerase (Solgent). The F-primer used was 5′-AGATGATGATGAGATTGCAACC-3′ containing a BamHI restriction site (underlined), and the R-primer used was 5′-GAAAGCCTCTGAGTGCTGTCGTCACAGATTGAGAGCCTGG-3′ containing a HindIII restriction site (underlined), a stop codon (double underlined), and the nucleotide sequence encoding 6× His (italic). The amplified product was adenylated using Taq polymerase (Solgent) and ligated into plasmid pGEMT-Easy (Promega). The ligated plasmid, designated as pGBH1, was amplified in DH5α, isolated, and digested with BamHI and HindIII. The fragment (~ 510 bp) was purified and ligated with the 4.2 kbp fragment of pUSS1, which was digested previously by BamHI and HindIII. The constructed plasmid, designated as pUSH1, was sequenced to check any sequence change. pUSH1, which produced a derivative of rAgaB34 with a 6× His tag at its C-terminal, was then transformed into *E. coli* DH5α. The recombinant DH5α was cultured at 37°C in a LB broth supplemented with 100 μg/ml ampicillin. When the OD600 reached 0.5, IPTG was added to the culture medium to give a concentration of 1 mM. After incubation for an additional 6 h at 37°C, the culture fluid was centrifuged at 12,000 × g for 15 min.

**Purification of rAgaB34**

The cell-free supernatant was concentrated by ultrafiltration (10-kDa cutoff membrane; Millipore, Bedford, MA, U.S.A.) and applied to a Ni²⁺ Sepharose 6 FF column (1×10 cm; Amersham, Uppsala, Sweden) pre-equilibrated with buffer A (50 mM Tris-HCl, 0.3 M NaCl, pH 8.0). After washing the column with buffer A supplemented with 10 mM histidine until the OD600 reached zero, the bound protein was eluted with buffer A supplemented with 250 mM histidine at a flow rate of 0.3 ml/min. Main active fractions were pooled and dialyzed against 50 mM Tris-HCl buffer (pH 8.0). The purified agarase was applied to SDS-PAGE on a 10% polyacrylamide gel by the method of Laemmli [16]. Protein bands were detected by staining the electrophorized gel with Coomassie Brilliant Blue R-250. The molecular mass markers (Sigma) consisted of albumin (66 kDa), ovalbumin (45 kDa), glyceraldehyde 3-phosphate dehydrogenase (36 kDa), carbonic anhydrase (29 kDa), and trypsin inhibitor (20.1 kDa).

**Enzyme Assay**

The agarase activity was quantified by spectrophotometric determination of reducing sugars by the Nelson method [19]. After incubating 50 μl of enzyme solution with 200 μl of 1% agarose (Sigma) and 100 μl of 0.1% agarose (Millipore), the amount of reducing sugar was determined by Nelson reagent using bovine serum albumin as the calibration standard. Protein concentration was determined by the Bradford method [4] using bovine serum albumin as the calibration standard.

**Characterization of rAgaB34**

The effects of pH and temperature on the activity of rAgaB34 were assayed at each pH (4.0–12.0) at 40°C and each temperature (10–90°C) at pH 8.0. The pH and thermal stabilities of the agarase were determined by preincubating the enzyme solution at each pH (4.0–12.0) at 40°C and each temperature (10–90°C) at pH 8.0 for 1 h and then measuring the residual enzyme activity. The effects of various metal ions (100 mM) and several other reagents (10 mM) on enzyme activity were determined by adding 100 μl of each reagent to the reaction mixture, respectively. NA6...
and NA4 (Dextra, Reading, U.K.) were used instead of agarose to identify the cleavage pattern of rAgaB34. Thin-layer chromatography was performed to determine the products [1]. The values of [Km] and kcat/Km (catalytic efficiency) were calculated based on Kcat, Vmax, and [E] (agarase concentration) values.

Analysis of Enzymatic Product
Hydrolysis of 1.0% (w/v) agarose by rAgaB34 was carried out at 40°C with shaking at 120 rpm overnight. End products were determined by a matrix-assisted laser desorption-ionization time-of-flight (MALDI-TOF) mass spectrometer (QSTAR Pulsar I; Applied Biosystems, Foster City, CA, U.S.A.) and a carbon-13 nuclear magnetic resonance (13C NMR) spectrometer (JNM-ECP 300M; Jeol, Tokyo, Japan).

Nucleotide Sequence Accession Number
The nucleotide sequence of agaB34 was submitted to the GenBank nucleotide sequence database. The sequence is available in the DDBJ/EMBL/GenBank nucleotide sequence database with the accession number EU200967.

RESULTS
Cloning of the Agarase Gene
About 5000 transformants carrying recombinant plasmids were screened by plate assays, and two agarase-positive E. coli transformants were obtained. pUSS1 and pUSS2, the plasmids purified from these two agarase-positive colonies, had a 2.4-kb and a 3.0-kb Sau3AI fragment, respectively. Both transformants were screened by plate assays, and two agarase-positive colonies were obtained. pUSS1 and pUSS2, the plasmids

Fig. 1. Nucleotide sequence of agaB34 gene and its deduced amino acid sequence.

The ~35 and ~10 regions of a possible promoter sequence and the potential Shine-Dalgaro (SD) sequence are underlined. The putative signal peptide is indicated by dashed underline. Nucleotide sequence based on which the primers were designed is indicated by convergent arrows.

Protein of 430 amino acid sequence with calculated molecular mass of 48,637 Da and pl value of 6.34. The possible cleavage site is between Ala23 and Ala24. Comparison of the N-terminal sequence of the deduced mature protein to those of the native agarases indicated that the gene product is AgaB34. The deduced amino acid sequence of agaB34 showed a high homology to those of several agarases, such as 86% to AgaA from Pseudalderomonas sp. CY24, 85% to AgaD from Vibrio sp. PO-303, 71% to AgaV from Vibrio sp. V134, and 61% to AgaY from Vibrio sp. SY12. The deduced amino acid sequence of agaB34 is indicated by an asterisk. The N-terminal sequence of the deduced mature protein to those of the native agarases indicated that the gene product is AgaB34. The deduced amino acid sequence of agaB34 showed a high homology to those of several agarases, such as 86% to AgaA from Pseudalderomonas sp. CY24, 85% to AgaD from Vibrio sp. PO-303, 71% to AgaV from Vibrio sp. V134, and 61% to AgaY from Vibrio sp. V134.

Conserved domain search with the 453 amino acid sequence showed a high homology to those of several agarases, such as 86% to AgaA from Pseudalderomonas sp. CY24, 85% to AgaD from Vibrio sp. PO-303, 71% to AgaV from Vibrio sp. V134, and 61% to AgaY from Vibrio sp. V134. The possible cleavage site is between Ala23 and Ala24. Comparison of the N-terminal sequence of the deduced mature protein to those of the native agarases indicated that the gene product is AgaB34. The deduced amino acid sequence of agaB34 showed a high homology to those of several agarases, such as 86% to AgaA from Pseudalderomonas sp. CY24, 85% to AgaD from Vibrio sp. PO-303, 71% to AgaV from Vibrio sp. V134, and 61% to AgaY from Vibrio sp. V134.

Expression and Purification of Recombinant AgaB34 (rAgaB34)
Time courses of cell growth of recombinant E. coli DH5α and production of rAgaB34 are shown in Fig. 3. The maximum agarase activity of 1.67 U/ml was detected in
the culture fluid of DH5α after induction for 6 h with IPTG at 37°C. No agarase activity was determined in the intracellular fraction of the cells (Fig. 3), which indicated rAgaB34 is a secretory protein. The rAgaB34 was purified to homogeneity by affinity chromatography. The purification procedures from a 50-ml culture medium are summarized in Table 1. The specific activity of purified rAgaB34 for agarose at 40°C was 242.18 U/mg. Thus, the production of rAgaB34 was about 6.9 mg/l. The molecular mass of the rAgaB34 was estimated to be 49 kDa by SDS-PAGE analysis (Fig. 4), which was coincident with that of the native AgaB34 and that estimated from the gene sequence.

**Characterization of rAgaB34**

The pH and temperature profiles of rAgaB34 are shown in Fig. 5. The agarase exhibited the maximum activity and the culture fluid of DH5α after induction for 6 h with IPTG at 37°C. No agarase activity was determined in the intracellular fraction of the cells (Fig. 3), which indicated rAgaB34 is a secretory protein. The rAgaB34 was purified to homogeneity by affinity chromatography. The purification procedures from a 50-ml culture medium are summarized in Table 1. The specific activity of purified rAgaB34 for agarose at 40°C was 242.18 U/mg. Thus, the production of rAgaB34 was about 6.9 mg/l. The molecular mass of the rAgaB34 was estimated to be 49 kDa by SDS-PAGE analysis (Fig. 4), which was coincident with that of the native AgaB34 and that estimated from the gene sequence.

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**Table 1. Purification of rAgaB34 expressed by E. coli.**

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Total protein (mg)</th>
<th>Total activitya (U)</th>
<th>Specific activity (U/mg)</th>
<th>Yield (%)</th>
<th>Fold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture fluid</td>
<td>5.20</td>
<td>83.73</td>
<td>16.09</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>Ni²⁺ Sepharose 6</td>
<td>0.28</td>
<td>67.08</td>
<td>242.18</td>
<td>80</td>
<td>15</td>
</tr>
</tbody>
</table>

*aAgarase activity was determined by the Nelson method using agarose as the substrate.*
stability at pH 7.0. More than 70% activity was detected after incubation at a wide pH range of 5.0–9.0 for 1 h. The enzyme possessed maximum agarase activity at 30°C and was thermostable up to 50°C. Effects of various reagents on the activity of rAgaB34 were investigated (Fig. 6). Neither significant activation nor inhibition of rAgaB34 was observed by metal ions (Na⁺, K⁺, Mg²⁺, Ca²⁺, and Ba²⁺), chelators (EDTA and EGTA), reducing reagents (β-mercaptoethanol and DTT), and detergents (SDS and urea), whereas the activity of rAgaB34 was inhibited by heavy metal ions (Cu²⁺, Mn²⁺, Zn²⁺, and Al³⁺). For its cleavage pattern, rAgaB34 hydrolyzed NA6 into NA4 and NA2, but NA4 could not be further hydrolyzed into NA2 even after long incubation time (data not shown). The Lineweaver-Burk plot of rAgaB34 acting on agarose is shown in Fig. 7. \( K_m \), \( V_{max} \), \( k_{cat} \), and \( k_{cat}/K_m \) values for agarose were 0.24 mg/ml (2.4×10⁻⁵ M), 50 U/mg, 41 /s, and 1.7×10⁶ /sM, respectively.

### Cleavage Pattern and Enzymatic Product

End products from agarose were determined by MALDI-TOF MS and \(^{13}C\) NMR. As shown in Fig. 8, the dominant peaks with molecular masses of around 676 and 959 Da corresponded most probably to molecules of [M+2Na]⁺ and [M+Na]⁺, respectively. Thus, the enzymatic products were a tetramer (630 Da, \( C_{24}H_{39}O_{19} \)) and a hexamer (936 Da, \( C_{36}H_{56}O_{28} \). The intensity of MALDI-TOF MS indicated that the tetramer was the dominant product (Fig. 8).

A typical pattern for neoagarooligosaccharides was observed according to the NMR spectrum (Fig. 9). The resonances at 96.960 and 92.979 ppm were assigned to be β and α anomic carbons of the G-unit at the reducing end of the neoagarooligosaccharides, respectively. No resonance was observed at around 90.7 ppm, which is the characteristic signal of the A-unit at the reducing end of the agarooligosaccharides. These results indicated that the tetramer and hexamer produced by rAgaB34 were NA4 and NA6, respectively.

### DISCUSSION

A β-agarase gene, AgaB34, was cloned and sequenced from *A. albus* YKW-34. Upon BLAST analysis using the full-
length AgaB34 as a query, four proteins were found to have similarities of more than 50% homologies to AgaB34. These proteins were β-agarases belonging to family GH-16 (http://www.cazy.org/). Other enzymes belonging to this family, such as mannanase, glucosidase, and xylosidase, are also structurally related to AgaB34, but with lower similarity. Thus, AgaB34 belongs to the GH-16 β-agarase subfamily. The crystal structures of two agarases, agarases A and B from Zobellia galactanivorans, belonging to this subfamily were analyzed [13]. They are believed to have a family GH-16 catalytic module. Alignment of amino acid sequences of AgaB34 and the above two agarases indicated that several amino acid residues identified as active sites and calcium-binding sites in agarases A and B from Zobellia galactanivorans were also conserved in AgaB34 (Fig. 2B). These residues include aromatic amino acids (Phe51, Tyr68, Trp72, Trp138, Phe173, Tyr292), negative-charged amino acids (Asp144, Asp149, Glu152, Glu263, Asp291), and a positive-charged amino acid (Arg175). The alignment further identified that AgaB34 belongs to the GH-16 β-agarase subfamily. The products and cleavage pattern of rAgaB34 also agreed well with those of other agarases belonging to this subfamily [5, 6, 21].

Another conserved domain existing in AgaB34 is CBM, which was found in xylanase from Streptomyces olivaceoviridis [9] and ebulan from Sambucus ebulus [23], whose crystal structures were also analyzed. Highly specific ligand binding from Zobellia galactanivorans, belonging to this subfamily were analyzed [13]. They are believed to have a family GH-16 catalytic module. Alignment of amino acid sequences of AgaB34 and the above two agarases indicated that several amino acid residues identified as active sites and calcium-binding sites in agarases A and B from Zobellia galactanivorans were also conserved in AgaB34 (Fig. 2B). These residues include aromatic amino acids (Phe51, Tyr68, Trp72, Trp138, Phe173, Tyr292), negative-charged amino acids (Asp144, Asp149, Glu152, Glu263, Asp291), and a positive-charged amino acid (Arg175). The alignment further identified that AgaB34 belongs to the GH-16 β-agarase subfamily. The products and cleavage pattern of rAgaB34 also agreed well with those of other agarases belonging to this subfamily [5, 6, 21].

Another conserved domain existing in AgaB34 is CBM, which was found in xylanase from Streptomyces olivaceoviridis [9] and ebulan from Sambucus ebulus [23], whose crystal structures were also analyzed. Highly specific ligand binding
was found to occur on the exposed surface of CBM. CBM includes three Q-X-W(F) motifs whose role was found to target the catalytic domain to the substrate [26], and involves some amino acid residues that serve as sugar binding sites [23]. Sequence alignment of AgaB34 and the above two proteins indicated that key amino acid residues of CBM, including those in Q-X-W(F) motifs and some of the sugar binding sites, were highly conserved in AgaB34 (Fig. 2C).

Based on the amino acid similarity, β-agarases were classified into three families of GH-16, GH-50, and GH-86 (http://www.cazy.org/), among which family GH-16 β-agarases are most abundant with members from different microorganisms. However, there is no report of GH-16 agarase from the strain belonging to the Agarivorans genus. Two agarases reported from Agarivorans strains belonged to family GH-50 [17, 20]. Thus, AgaB34 was the first family GH-16 agarase derived from the Agarivorans genus. Because AgaB34 is from an Agarivorans strain, a special agar-devouring strain, it is reasonable to assume that AgaB34 might possess novel properties comparing with other family GH-16 agarases.

rAgaB34 had a molecular mass of 49 kDa and a specific activity of 242 U/mg. The specific activity was higher than those of many reported family GH-16 agarases; 63.6 U/mg of AgaA [5] and 16.4 U/mg of AgaD [6] from Vibrio sp. PO-303, 160 U/mg of AgaA and 100 U/mg of AgaB from Zobellia galactanivorans [13], but lower than 398 U/mg of RagaA7 from the deep-sea novel microorganism Microbulbifer sp. JAMB-A7 [21]. rAgaB34 was stable at pH 5–9 and had maximum activity at pH 7.0. Its pH properties were similar to those of reported agarases [5, 6, 21], with regard to thermal stability, rAgaB34 was stable up to 50°C, whereas reported agarases were stable up to 40°C [20] or 37°C [6]. Only RagaA7 from Microbulbifer sp. JAMB-A7 [21] showed a similar thermal stability with rAgaB34. Reported agarases possessed optimal activity at temperature of 40°C [5, 6] or 50°C [21], which is above the gelling temperature of agar (around 38°C). It is novel that rAgaB34 could hydrolyze the gelated agarose and had the maximal activity at 30°C. Thus, it might be used for industrial production of neogalactooligosaccharide directly from marine algae under economic conditions. The resistance of rAgaB34 against commonly existed metal ions in marine environment and various other chemical reagents also benefits its industrial application. Moreover, the kinetic properties of rAgaB34 were superior to those of other reported agarases. rAgaB34 had a K_m value of 0.24 mg/ml against agarose, which was lower than the 1.1 mg/ml of AgaD from Vibrio sp. PO-303 [6], 2.33 mg/ml of AgaA from Vibrio sp. PO-303 [5], and 3.0 mg/ml of RagaA7 from Microbulbifer sp. JAMB-A7 [21].

The recombinant agarase rAgaB34 was produced extracellularly by E. coli DH5α with a satisfactory yield of 1,670 U/l. When E. coli was used as a host, most recombinant agarases were produced intracellularly with a relatively lower yield. AgaA and AgaB from Zobellia galactanivorans and AgaD from Vibrio sp. PO-303 were produced intracellularly by E. coli with yields of 160 U/l, 800 U/l [13], and 620 U/l [6], respectively. Excretion of rAgaB34 to the culture medium resulted in higher agarase yield and also greatly facilitated purification of this enzyme.

Intracellular production of a protein has certain disadvantages. High-level accumulation of the expressed protein often leads to the formation of inclusion body, which needs to be further solubilized and refolded to obtain the native protein conformation. On the other hand, the intracellular soluble protein may be destroyed as a result of proteolytic degradation. It was reported that the proteins transported outside were at least 10 times more stable than those that remained in the cytoplasm [28]. There have been various attempts to establish a secretory expression system [12, 29, 32] because excreting protein to the medium allows the protein to be obtained in its soluble and perfectly folded form, and to be more easily purified without cell lysis. However, because of the outer membrane barrier, the Gram-negative bacterium E. coli is generally not considered to be an appropriate host for the secretion of proteins into the culture medium. In this study, E. coli cells harboring the pUC18-derived agarase expression vector pUSH1 were able to efficiently excrete agarase into the culture medium. This expression system might be used to express secretory proteins.

The high-level expression of a recombinant agarase and its relatively excellent specific activity, thermal stability, and kinetic properties, as well as the novel thermal activity, enlighten the potential application of this agarase. Moreover, the achievements in secretion of agarase using E. coli provides an attractive approach to the construction of recombinant strains for the large-scale production of secretory proteins.

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