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Antibacterial activity of a cell wall hydrolase from *Lactobacillus paracasei* NRRL B-50314 produced by recombinant *Bacillus megaterium*

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Abstract The cell-free supernatant (CFS) from *Lactobacillus paracasei* NRRL B-50314 culture has been previously reported as containing antibacterial activity against a wide variety of Gram-positive bacteria. The CFS protein gel slice corresponding to antibacterial activities was subjected to trypsin digestion and ion trap MASS (Gel/LC–MS/MS) analysis. BlastP search of the resulted IQAVISIAEQQIGKP sequence led to a hypothetical cell-wall associated hydrolase (designated as CWH here) from *Lactobacillus paracasei* ATCC 25302. Further analyses of CWH revealed that the IQAVISIAEQQIGKP belongs to a highly conserved region of the NlpC/P60 superfamily. The *L. paracasei* NRRL B-50314 CWH gene, cloned in pStrepHIS1525CWH$_{477}$, was introduced into *Bacillus megaterium* MS 941. The production of CWH$_{477}$ protein was induced by xylose. The CWH$_{477}$ protein was purified by using NiNTA column, and elution fraction E2 showed highest antibacterial activity. This study and bioinformatics analyses suggested that the antibacterial activity of CWH could originate from its cell wall degrading enzymatic function.

Keywords *Lactobacillus paracasei* · Antibacterial protein · Cell-wall associated hydrolase · *Bacillus megaterium*

Introduction

Misuse or overdose of conventional chemically synthesized or semi-synthetic antibiotics can lead to residues accumulated in the environment and speed up the emergence of resistant strains to currently available drugs [3]. In clinical medicine, bacterial infections caused by multi-drug-resistant strains can be life-threatening. In the biofuel fermentation industry, serious bacterial contamination of the fermentation tanks, where fermenting yeasts grow in nutrient broth and ferment sugars to ethanol, can lead to reduced ethanol production. Practical measures of restraining contamination, including downtime, cleaning and decontamination of the production facilities, often increase overall operational cost [4]. Applying antimicrobial agents in commercial facilities could be effective in preventing undesired bacteria from growing and competing for nutrients with fermenting yeasts, but consequently, this strategy might contribute to the emergence of multi-drug resistant strains [2]. Thus, new antibacterial agents are needed to curb the bacterial contamination and multi-drug resistance. This is of particular significance for the ethanol industry since the major fermentation byproduct dried distillers grains with solubles (DDGS) has been sold as animal feed, and spreading of multi-drug resistance infection would be a threat to animal health. Biodegradable agents with bactericidal activities are desired alternatives to synthetic antibiotics, thus, need to be explored in preventing and controlling bacterial infections.

Recently we reported the characterization of cell free supernatant (CFS) by a novel isolate *Lactobacillus*

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paracasei NRRL B-50314 that showed antibacterial activities against *S. aureus* MSSR, MRSA, Hetero VISA and Homo VISA strains [7, 8]. In the present study, we cloned a gene encoding a hypothetical cell-wall associated hydrolase (designated as CWH in this study) and expressed the CWH gene in *Bacillus megaterium*. The antibacterial function of the full length CWH and several truncated recombinant CWH proteins were assessed.

Materials and methods

Bacterial strains and growth conditions

L. paracasei NRRL B-50314 strain [7, 8] was maintained on MRS plates (Becton–Dickinson, Sparks, MD) under anaerobic conditions (BBL GasPak anaerobic system, Becton–Dickinson, Franklin Lakes, NJ) and a single colony was inoculated and grown in MRS broth at 30 °C without shaking. *B. megaterium* strain MS941 was obtained from Molecular Biotechnology (MoBiTec GmbH, Germany). *E. coli* Top10 and *B. megaterium* strain MS941 were grown at 37 °C on LB supplemented with tetracycline (LB^{Tc}; Tc 10 μg/ml final concentration) either on agar plates or in LB broth (Becton–Dickinson, Sparks, MD) with shaking at 220 rpm.

Mass spectrometry analyses of antibacterial protein

The antibacterial properties of CFS from *L. paracasei* NRRL B-50314 were characterized previously [7, 8]. A total of 50 ml CFS from NRRL B-50314 overnight culture was collected by centrifugation (3,795*g*, 20 min), then separated using Amicon Ultra-15 Centrifugal Filter Units (4,000 g, 10 min, 25 °C, 100 kDa NMML, www.Millipore.com), the filtrate (45 ml) was concentrated 100 times by acetone precipitation. Briefly, to 3 ml of CFS, 12 ml of cold acetone (−20 °C) was added, the mixture was incubated overnight at −20 °C, then centrifuged at 4 °C, 10, 968 g for 15 min. The resulting pellet was dried in room temperature for 10 min, then washed with 500 μl ddH_2O, centrifuged for 10 min at 4 °C, and dried under vacuum centrifugation at 35 °C for 5 min. The pellet was resuspended in PBS buffer, and 10 μg of proteins were used for SDS-PAGE analyses. Duplicate samples were subjected to Tris–HCl SDS-PAGE (BioRad, Hercules, CA). One gel was used for antibacterial gel overlay assay [8], a second gel was used for protein sequencing analyses (see below). After electrophoresis, the second gel was rinsed 10 min with ddH_2O, and the gel piece corresponding to the antibacterial activities was excised and shipped to the Wistar Proteomics Facility (http://www.wistar.org, Philadelphia, PA 19104) for trypsin digestion and ion trap mass spectrometry (Gel/LC–MS/MS) sequence analyses.

Table 1 PCR and sequencing primers used in this study

LpacaseiCWH 108-5′	TTATGAAGTGCTGGCGACTG
LpacaseiCWH 2188-3′	CTCGCGAGAATGTTCCAGTT
LpacaseiCWH *Aat*ll-3′	GACGTCGTTCAAAAAGGCAT
LpacaseiCWH *Aat*ll-5′	GAACGACGTCGGTGTTAACA
LpacaseiCWH 450*Kas*I-5′	GGCGCCATGGTAGATGCAA AGAAA
LpacaseiCWHStop*Sph*I-3′	GCATGCTTACCGCCACCAATG-TAGA
LpacaseiCWH 1745*Sph*I-3′	ACACGCATGCCGTTCAAAAAGG
LpacaseiCWH 1460*Kas*I-5′	GGCGCCTTTGATGCAACTGA-GGAACA
LpacaseiCWH 256-5′	AAAAGTTGACCGAAACGGTG
LpacaseiCWH 1155-3′	GTAACAGGAGCTGGAGCTGG
LpacaseiCWH 140-5′	CTTCAACGGGGACAGTCAAT
LpacaseiCWH 1046-3′	GCTGTTCCTGAGTTGCATCA
LpacaseiCWH1895*Bam*HI-3′	GGATCCTTACCGCCACCAAT GTAGA
SplipA forward	CTATCTGTTTTAGCCGCTC
pStrepHIS1525-5′	CGCTGATTCTATCTGTTTTAG CCGC
pStrepHIS1525-3′	AGTGATGGTGATGGTGATGC

Plasmid construction

PCR primers used in this study (Table 1) were synthesized by Integrated DNA Technologies, Inc. (Coralville, Iowa). Genomic DNA was isolated from *L. paracasei* NRRL B-50314 as described previously [9]. Primers *Lpcasei* CWH108-5′ and *Lpcasei* CWH 2188-3′ were used for genomic PCR. The resulting 2 kb PCR fragment including both 5′ and 3′ flanking sequences of the cell wall-associated hydrolase (CWH) gene was cloned in pCR2.1-TOPO vector (Invitrogen, Life technologies) to produce pCR2.1-CWHg. The internal *Kas*I site of the pCR2.1-CWHg clone was mutated to GACGTC by overlapping PCR using *Lpcasei* CWH108-5′/*Lpacasei*CWH*Aat*II-*3′* and *Lpacasei*CWH*Aat*II-*5′/Lpcasei* CWH 2188-3′ primer pairs. The resulting PCR fragment, containing a new *Aat*II site that replaced the *Kas*I site, was cloned into pCR2.1-TOPO to produce pCR2.1-CWHa. A new *Kas*I site was introduced in pCR2.1-CWH_a immediately upstream of ATG start codon using primer *Lpacasei*CWH450*Kas*I-*5′* and a *Sph*I site was introduced at position 1745 using primer *Lpacasei*CWH-1745Stop*Sph*I-*3′*. The resulting construct was designated as pCR2.1-CWHf, and the CWH sequence was confirmed by DNA sequencing using M13 and reverse primers. The 1.4 Kb *Kas*I/*Sph*I fragment from pCR2.1-CWHf was cloned

into pStrepHIS1525 shuttle vector (MoBiTec GmbH, Germany www.mobitec.com) at *Kas*I/*Sph*I sites, resulting-in the plasmid pStrepHIS1525-CWH_{477} (the subscript number indicated the number of amino acids translated from corresponding construct). The entire CWH sequence was confirmed by sequencing using primers pStrepHIS1525-5′, pStrepHIS1525-3′, SplipA forward, and gene specific primers *Lpacasei*CWH 256-5′, *Lpacasei*CWH 450*Kas*I-5′, LpacaseiCWH 1460*Kas*I-5′, *Lpacasei*CWH1895*Bam*HI-3′, *Lpacasei*CWH 1155-3′, and *Lpacasei*CWH 1745*Sph*I-3′ (Table 1). The plasmid pStrepHIS1525-CWH_{477} was used to transform protoplasts of *B. megaterium* strain MS941.

Additional clones containing partial CWH gene sequences were constructed and sub-cloned into pStrepHIS1525 shuttle vector at *Kas*I/*Sph*I sites as described below. Error-prone PCR [6] were carried out to introduce random mutations near the C-terminal region of CWH by using pCR2.1-CWHf as template and *Lpacasei*CWH1460*Kas*I-*5′*and *Lpacasei*CWHStop*Sph*I-*3′* primers. The resulting PCR fragments were cloned into pCR2.1-TOPO vector. Individual clones were sequenced and the mutant CWH_{207}, CWH_{182} and CWH_{102} were selected. Then *Kas*I and *Sph*I fragments from CWH_{207}, CWH_{182}, and CWH_{102} were transferred into *Kas*I/*Sph*I sites of pStrepHIS1525 vector. CWH B-1_{115} was constructed by standard PCR of pCR2.1-CWHg clone using *Lpacasei*CWH 256-5′ and *Lpacasei*CWH 1745 *Sph*I-3′ primers and double digested with *Kas*I (internal *Kas*I site) and *Sph*I, into *Kas*I/*Sph*I sites of pStrepHIS1525 vector. After transfer into pStrepHIS 1525 vector, these clones were confirmed by sequencing using pStrepHIS1525-5′, pStrepHIS1525-3′ and SplipA forward primers.

Transformation of *B. megaterium* protoplasts

The genus *Bacillus* is one of the most developed Gram-positive bacteria for biotechnology applications. *B. megaterium* has been used recently for heterologous expression of stable and high-yield proteins attributed to its plasmid stability and exclusive production of one major protease. The *B. megaterium* MS941 is an ideal host for production of recombinant extracellular proteins since the major extracellular protease gene was deleted [16]. Protoplasts of *B. megaterium* MS941 (MoBiTec GmbH, Germany) were used for transformation following vendor's instructions. Briefly, 5 µg of pStrepHIS1525CWH plasmid DNA was mixed with 500 µl of protoplasts in SMMP buffer (components are listed below) and 1,500 µl PEGS (40 % w/v of PEG6000 in 1X SMM buffer containing 0.5 M sucrose, 20 mM maleic acid, 20 mM $MgCl_2$, pH 6.5). The SMMP buffer was made from mixing equal volume of 2× SMM with 2× AB3 (antibiotic medium No. 3, DIFCO). The protoplasts-DNA solution was incubated at room temperature for 2 min and then 5,000 µl SMMP was added and mixed gently, followed by centrifugation at 3,000 rpm for 10 min at room temperature. The protoplasts pellet was resuspended with 500 µl SMMP and the mixture was incubated at 37 °C by shaking at 100 rpm for 90 min. Then 200 µl of the regenerated cells were mixed with 2.5 ml CR5-top agar (500 ml containing 51.50 g sucrose, 3.25 g MOPS, 0.33 g NaOH, 2.0 g agar, 0.1 g casamino acids, 5.0 g yeast extract, 0.115 g K_2SO_4, 4.60 g $MgCl_2$ $6H_2O$, 0.023 g KH_2PO_4 and 1.012 g $CaCl_2$ $2H_2O$). The mixture was poured on pre-warmed LB plate with tetracycline (10 µg/ml). The plates were incubated at 37 °C overnight and individual colony was picked for further analyses.

Xylose induced expression of recombinant protein

The pStrepHIS1525 protein expression is controlled by *xyl*A promoter, therefore the production of recombinant protein can be induced by addition of xylose in growth media [14]. A single colony of *B. megaterium* strain MS941 carrying either pStrepHIS1525CWH_{477} or pStrepHIS1525 (or other construct) was inoculated into 2 ml LB^{Tc} from LB^{Tc} plate, and grow overnight at 37 °C with shaking at 220 rpm. The 2 ml fresh overnight culture was inoculated into 30 ml LB^{Tc}, grown at 37 °C with shaking at 220 rpm. The recombinant protein expression was induced by addition of D-xylose (final concentration 0.5 %) to the culture media when the OD_{600} reached 0.3. Then two 3 ml samples were withdrawn at 0, 2, 4 and 6 h after xylose induction. Supernatants of these samples were used for acetone precipitation as described above. The protein pellet was resuspended in PBS buffer, protein concentrations were determined by using Bio-Rad protein assay and 10 µg of proteins were used for SDS-PAGE analyses.

To purify the recombinant protein, the xylose induced CWH10 culture supernatant was concentrated (approximately 100 fold) using Amicon Ultra-15 Centrifugal Filter Units (10 kDa NMWL), http://www.Millipore.com). About 190 µl NiNTA agarose (Qiagen www.qiagen.com)) was added to 500 µl concentrated CHW10 supernatant and the mixture was incubated at 4 °C by rocking for 1 h, then loaded onto gravity column. The NiNTA agarose with bound protein was washed four times with 1.5 ml NiNTA wash buffer (50 mM NaH_2PO_4, 300 mM NaCl, 20 mM imidizole). Proteins were eluted with 2 ml NiNTA elution buffer (50 mM NaH_2PO_4, 300 mM NaCL, 250 mM imidizole). The flow thru, washes and four elution fractions of 0.5 ml each were collected and subjected to SDS-PAGE analyses and antibacterial assays as described previously

Table 2 Antibacterial Assay using *Lactococcus lactis* LM 0230 as target cells

Samples from purification steps	Sample A_{530}	Control A^{a}_{530}	% inhibition
Crude supernatant	0.115 ± 0.030	0.239 ± 0.009	52.0
Amicon Ultra-15 concentrated	0.178 ± 0.016	0.239 ± 0.009	90.8
NiNTA Flow Thru	0.176 ± 0.009	0.239 ± 0.009	25.3
Wash	0.264 ± 0.014	0.239 ± 0.009	26.1
NiNTA Elution1	0.192 ± 0.028	0.643 ± 0.026	58.9
NiNTA Elution 2	0.414 ± 0.009	0.643 ± 0.026	70.1
NiNTA Elution 3	0.569 ± 0.008	0.643 ± 0.026	35.6
NiNTA Elution 4	0.022 ± 0.004	0.643 ± 0.026	11.4

The assay was done in a 96 well assay plate, each well containing 280 µl MRS media, 5 µl fresh *L. lactis* LM 0230 overnight culture, and 15 µl of sterile filtered sample or control. Amicon Ultra-15 concentrated shows the highest inhibition and NiNTA Elution 2 shows the second highest inhibition (the highest among all elution fractions)

[a] The controls were 15 µl MRS media for first four steps, and 15 µl NiNTA elution buffer for NiNTA elution steps E1–E4

[7, 8]. The antibacterial assay is also described briefly in footnote of Table 2.

Results

Sequence of the antibacterial CFS protein

The antibacterial activities against a layer of *Lactococcus lactis* LM2030 as target cells were readily detected in crude CFS [7, 8]. Protein sequence analyses of the gel slice corresponding to antibacterial activity position resulted in the identification of a IQAVISIAEQQIGKP segment (Fig. 1).

Cell wall-associated hydrolase

Blastp search (http://blast.ncbi.nlm.nih.gov/) using the above amino acid sequence identified a hypothetical cell-wall associated hydrolase (CWH). The top two matches are the hypothetical cell wall-associated hydrolase YP_805583.1 (492 amino acids) from *Lactobacillus casei* ATCC 334 (Fig. 1), and the homolog EEI69340.1 (497 amino acids) from *Lactobacillus paracasei* ATCC 25302. The translated CWH sequences contain the IQAVISIAEQQIGKP segment, and this segment is located near the C-terminus of the hypothetical cell wall-associated hydrolase.

Further bioinformatics analyses revealed that the IQAVISIAEQQIGKP segment belongs to a highly conserved region of the NlpC/P60 superfamily; this group of proteins was named after new lipoproteinC from *E. coli* and a 60 kDa extracellular protein of *Listeria monocytogenes* [1].

Cloning and analyses of the CWH gene

PCR primers (Table 1) were designed based on the cell wall-associated hydrolase gene sequence of *L. paracasei* ATCC25302, and the corresponding CWH from *L. paracasei* B-50314 was cloned from genomic PCR. The full length CWH gene was sequenced and deposited in GenBank (accession number: KJ194043). Based on the sequencing result, the CWH from *L. paracasei* B-50314 encodes a protein with 493 amino acids.

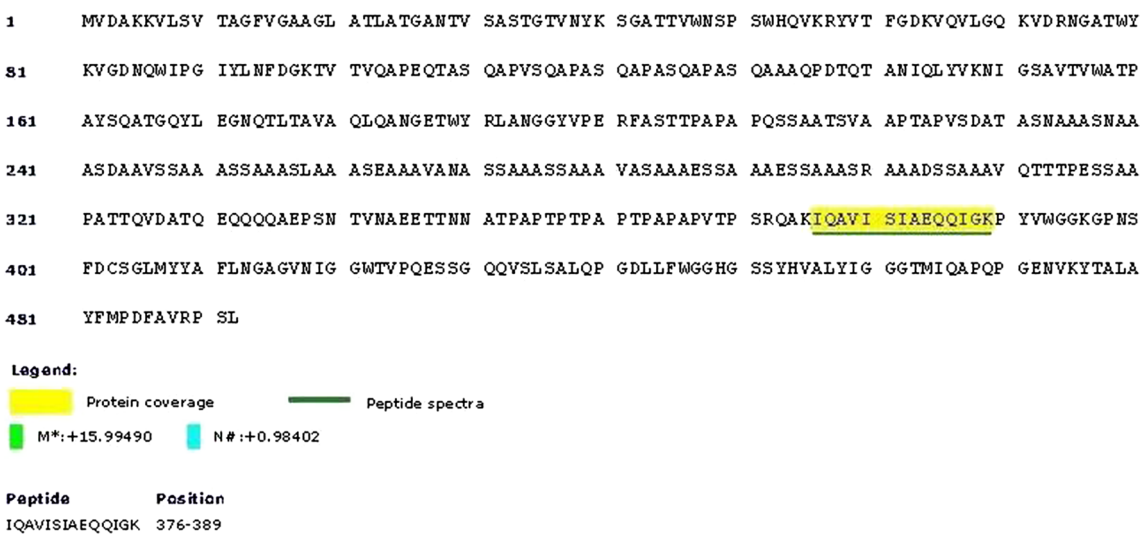

Fig. 1 Amino acid sequence of an antibacterial protein and bioinformatics analyses revealed that these sequences are located in a highly conserved motif of a Cell Wall associated Hydrolysase, a transmembrane protein of 492 amino acids, from *L. casei* ATCC 334

Bioinformatics analyses using SDSC Biology Workbench (http://workbench.sdsc.edu) of four related CWHs indicated that the CWH of *L. paracasei* B-50314 is phylogenetically closer to the CWH of *L. paracasei* ATCC 25302 than that of *L. casei* ATCC 334 (Data not shown).

Expression of CWH in *B. megaterium*

Previous studies demonstrated that bacterial proteins can be expressed in the *B. megaterium* pStrepHIS1525 vector expression system, in which the xylose inducible promoter and a signal peptide were built into express and secrete the target protein in media [11]. Aiming to test the functionality of the CWH gene, the pStrepHIS1525-CWH_{477} construct was introduced in *B. megaterium* to generate a recombinant *B. megaterium* CWH10 line. The calculated recombinant CWH is 47.9 kDa. SDS-PAGE showed that a protein around 48 kDa was detected in CWH10 after xylose induction (positioned by an arrow in Fig. 2, lane 2) and absent without xylose (Fig. 2, lane 1).

To reach the maximum production, a time course study for xylose induced expression of CWH was conducted. Proteins collected from CFS of the CWH10 cultures at 2, 4 and 6 h after addition of xylose were analyzed. Maximum CWH expression was achieved within 4 h of induction and the recombinant CWH level remained constant after 6 h of induction (Fig. 3).

Antibacterial activity analyses of the CWH_{477}

The xylose induced recombinant CWH contains six His residues translated from the vector sequence at the 3′ end, thus allowing the binding of the recombinant CWH to a NiNTA column. The purified recombinant protein was eluted in four fractions from the affinity column and subjected to an antibacterial activity assay as described previously [7, 8]. The antibacterial activity against *Lactococcus lactis* LM2030 as target cell was detected from the purified recombinant CWH477. Among the four elution fractions tested, E1 and E2 showed 58.9 and 70.1 % of inhibitions respectively, while E4 showed only 11.4 % of inhibition (Table 2). These results demonstrated that fraction E2 has the highest antibacterial activity and the E2 activity was further confirmed by forming a clear zone in a plate overlay assay as described previously [7, 8] using a layer of *Lactococcus lactis* LM2030 (collected at log phase) as target cells (data not shown). These results demonstrated that the recombinant CWH477 does contain antibacterial activities.

Analyses of truncated CWH derivatives

It was hypothesed that the conserved NlpC/P60 motif of CWH might play a significant role in antibacterial function.

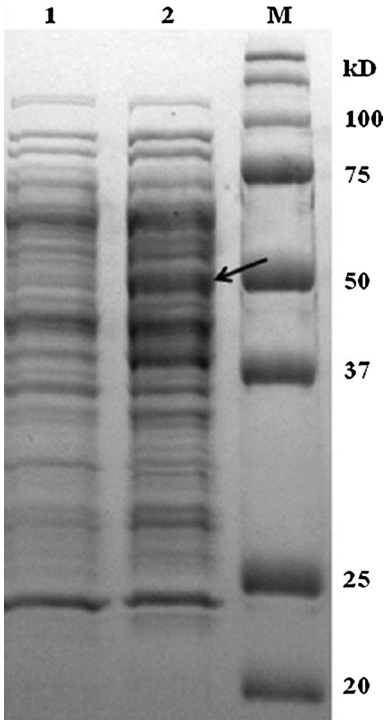

Fig. 2 SDS-PAGE of total cellular protein from recombinant *B. megaterium* carrying pStrepHIS1525 CWH_{477} (CWH10). *Lane 1* proteins extracted from CWH10 without xylose induction; *Lane 2*, proteins extracted from CWH10 2 h after xylose induction, the production of recombinant CWH_{477} protein is shown by the *arrow*. A total of 10 μg of proteins were loaded in each lane. *Lane M*, the molecular weight marker (10 μg of Precision Plus Protein Standard from Bio-Rad). The gel was stained with *Coommassie blue*

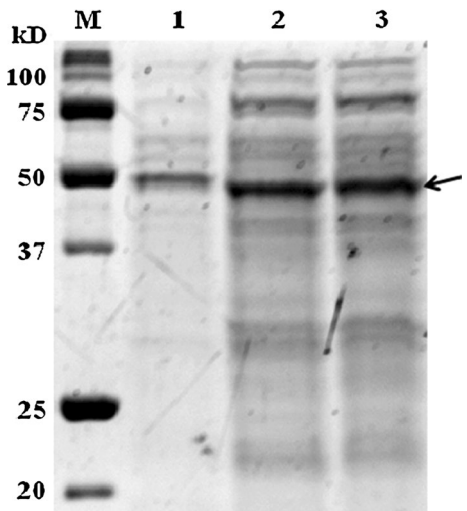

Fig. 3 Time course analyses of xylose induced and secreted proteins of recombinant *B. megaterium* carrying pStrepHIS1525 CWH_{477}. The supernatant of CWH10 cultures were collected 2 h (*Lane 1*), 4 h (*Lane 2*) and 6 h (*Lane 3*) after xylose induction. The supernatants were concentrated by cold acetone precipitation. About 10 μg protein samples were subjected to SDS-PAGE analyses

Fig. 4 Alignment of four truncated recombinant CWH proteins along with the control vector sequences used in this study. The partial and/or mutated CWH protein sequences were translated from the recombinant DNA constructs in pStrepHIS1525 vectors. *Blue* highlighted sequences are signal peptide sequences from the shuttle vector, signal peptide cleavage site (AGA) is highlighted *pink*, CWH conserved motif is highlighted in *green*. Both CWH_{115} (line 4) and vector control (line 5) do not contain the conserved IQAVISIAEQQIGKP sequence, CWH_{102} (line 1) contains only the IQAVISIAE sequence (color figure online)

Thus, several truncated CWH clones were constructed either with or without the conserved NlpC/P60 motif. The CWH_{207}, CWH_{182}, and CWH_{102} in pStrepHIS1525 vector carrying specific mutations were constructed (Data not shown) and introduced in *B. megaterium.* Based on recombinant protein sequences, which were analyzed by using the SDSC Biology Workbench (http://workbench.sdsc.edu/), the anticipated proteins secreted by *B. megaterium* CWH_{207}, CWH_{182}, and CWH_{102} contained 179, 154, and 74 amino acids respectively after cleavage of the 28 amino acid signal peptide (highlighted blue in Fig. 4). The corresponding proteins were produced in recombinant *B. megaterium* (Data not shown). The CWH_{207}, CWH_{182} clones produced smaller proteins including the IQAVISIAEQQIGKP (highlighted green in Fig. 4) motif while CWH_{102} clone ended with IQAVISIAE. However, antibacterial activities were not detected from these purified recombinant proteins, neither from crude CFS of the recombinant CWH_{207}, CWH_{182}, and CWH_{102} clones in *B. megaterium* (data not shown).

The recombinant protein CWH_{115} from clone CWH B-1_{115}, covering the 5′ region of CWH near the internal *Kas*I site, did not contain the IQAVISIAEQQIGKP motif (Fig. 4). Activity assay indicated that the CWH_{115} did not have antibacterial function either. These results suggested that the antibacterial activity of CWH is not solely residing in the conserved IQAVISIAEQQIGKP sequence.

Discussion

In this study, we reported a novel cell wall associated hydroloase CWH from *L. paracasei* B-50314 that was produced and secreted into the culture broth. The CWH gene was cloned and introduced in *B. megaterium* and the recombinant CWH protein was produced, secreted into the cultural media after xylose induction. The purified CWH_{477} protein showed antibacterial activity, which likely due to the cell wall degrading enzymatic action of CWH.

There are 12 and 13 putative peptidoglycan hydrolases in the genomes of *Lactobacillus plantarum* [15] and *Lactobacillus casei* BL23 [13], respectively. Most of the hydrolases might be redundant in diversified functions including

cell morphology, cell wall formation, biofilm formation and autolysis [5], and the lysis of other competitive bacterial species (a function relevantly to this work). There have been studies about how bacterial cells control the cellular activities of hydrolases, in addition to a series studies about cell autolysis with increased hydrolase activities. However, the overall regulatory process is largely unknown due to the complexity and redundancy nature of these enzymes.

Albeit other putative peptidoglycan hydrolases exist in the *L. paracasei* genome, to the best of our knowledge, there has been no studies in the genome of *L. paracasei* linking peptidoglycan hydrolases to antibacterial functions yet. One case of a bacteriophage cell wall hydrolase was reported capable of rapid killing of *Streptococcus pneumonia* [10].

It is interesting that thus far, by using bioinformatics search tools, only one cell wall associated hydrolase gene was found in the genome. This unique cell wall associated hydrolase belongs to the NIpC/P60 superfamily. Proteins of the NIpC/P60 superfamily, including endopeptidases, amidases and acyltransferases, were identified by genome analyses and most of the members are hypothetical proteins. Until recently, a few proteins belonging to the superfamily were characterized with diversified roles in cell growth, development, reproduction, cell wall integrity, and virulence [1, 12, 17].

In this study, the antibacterial activity was detected with the recombinant CWH_{477}, not in any smaller truncated recombinant CWH proteins. The results suggested that the intact CWH protein, likely through correct folding, plays an important role for the hydrolase activity and antibacterial function. The function of this CWH might involve hydrolyzing the target bacterial cell wall. The result of this study can be used for further understanding and applications of the CWH in controlling growth of other bacterial species.

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