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ORIGINAL PAPER

A new cuticle scale hydrolysing protease from *Beauveria brongniartii*

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Abstract From a screening for the production of new proteases specific for cuticle scales, *Beauveria brongniartii* was selected producing an alkaline Ca^{++} dependent protease. The purified had a molecular weight of 27 kDa and a pI value of 8.0. Substrate specificities of model substrates (wool with partially removed cuticles treated with SDS) were analyzed by protein release, dissolved organic carbon (DOC) and nitrogen analysis. The C/N ratio of released material turned out to be a good parameter to determine the site of action of proteases on fibres. Compared to other enzymes, the fungal protease preferentially hydrolyzed cuticle scales and has thus a potential for anti-shrinking pre-treatment of wool fabrics.

Keywords Wool · Cuticle · Protease · *Beauveria brongniartii*

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Introduction

Wool contributes to the world textile fibre production with only 1%, however, due to its special garment-physiological properties and the vast possibilities of processing and finishing methods it is an important raw material for the textile industry (Zahn et al. 1997). Almost all types of woven and knitted wool products will shrink in absence of any preventative treatments. Felting is the common term for the progressive fibre entanglement of wool products subjected to mechanical action (mainly during laundering). Primarily responsible for felting is the cuticle or scale cell structure when the overlapping cells are tightly stuck to each other and to the underlying cortex (Simpson and Crawshaw 2002). Antifelting treatments are usually based on partial removal, softening or uniform coating of the cuticle scales. For ecological reasons, work is directed to new antifelting processes to avoid the use of chlorine or chlorine compounds used e.g. in the Chlorine/Hercosett process. Furthermore, treatments retaining the natural properties of wool would be preferable compared to coating with synthetic materials.

Enzymatic processes have been suggested as an alternative pre-treatment meeting these requirements (Zahn and Hoffmann 1996). Wool properties like handle, lustre and whiteness have been reported to be enhanced by enzyme-catalyzed

reactions (Heine and Höcker 1995; El-Sayed et al. 2001) described the use of the protease papain on pre-treated wool (lipase/sodium monoperoxyphthalate/sodium sulphite) resulting in a shrink-proofed effect. A commercial enzyme preparation combined with an alkaline peroxide treatment followed by application of chitosan biopolymer showed good effects concerning shrink resistance of wool, moreover the wettability and whiteness were enhanced (Jovancic et al. 2001, 2003) showed that during enzymatic treatments the F-layer of wool was completely removed and the content of cysteic acid was significantly decreased whereas during plasma treatment a partial oxidation of hydrocarbon chains of the F-layer and the formation of cysteic acid residues at the wool surface was detected.

Despite the promising results of protease-based pre-treatment processes of wool, partial damage of the fibres by the proteases still remains a major problem. While the use other enzymes such as protein-cross linking enzymes (transglutaminases) has been recently suggested to avoid this risk associated with proteases, other promising approaches involve chemical modification of proteases (Schroeder et al. 2004). Finally, the screening for new proteases with high specificity to cuticles could be an interesting alternative to existing enzymes.

In this study, a new protease with higher specificity to the outer part of wool fibres than bacterial proteases was isolated from *Beauveria brongniartii*. This enthomopathogenic fungus is mainly used as a biocontrol agent against the May beetle (Koller et al. 2005) and longicorn beetles (Higuchi et al. 1997).

Materials and methods

Screening and cultivation of microorganisms

A total of nine fungal strains deposited at the culture collection of the Department of Environmental Biotechnology, Graz University of Technology, Austria were screened for their ability to degrade wool cuticle scales: *Aspergillus tereus* A9, *Bjerkandera adusta* BAG1, *Penicillium simplicissimum*, *Trichoderma viride* BJG 102, *Aspergillus niger*, *Curvularia lunata* IT 0005, *Beauveria brongniartii*, *Fusarium solanii* VAI 1 and *Cylindrocarpon radicicola* DSM 837. The fungi were usually grown on pepton glucose Agar (Fluka, Buchs, Switzerland) plates at 30°C for 3–7 days and stored at 4°C thereafter. The medium for production of proteases consisted of 10 g wool or wheat bran flakes L^{-1}, 4 g $MgSO_4 \cdot 7H_2O$ L^{-1}, 1.0 g $(NH_4)_2SO_4$ L^{-1}, 1.5 g yeast extract L^{-1}, 5 g KH_2PO_4 L^{-1} and 1 g glucose L^{-1}. The pH was adjusted to 4.0. All chemicals were obtained from Sigma. For cultivation of the fungi 250 ml of this medium in Erlenmeyer flasks (500 ml) were inoculated with 1 cm^2 of the actively growing fungus from PDA-plate and incubated 30°C on a rotary shaker (160 rpm). Growth was monitored by daily measurement of the protease activity using azocasein as substrate. Every few days the pH was controlled and the broth was observed for possible infections under the microscope. Cultures were harvested by centrifugation (10 min at 9,500 *g*).

Before addition to the cultivation medium 20 g of raw wool (Drummond Parkland of England, Huddersfield, UK) were washed in 800 ml H_2O (pH 9.0) containing 0.80 g Lutensol® A 7 N (BASF), 1.34 g $NaHCO_3$ and 15.12 g Na_2SO_3. The reaction mixture was shaken at 60°C in a waterbath for 30 min.

Protease activity with azocasein

Two hundred and fifty microlitre of 2% (w/v) azocasein solution in 50 mM Tris HCl buffer (pH 8.0) were equilibrated in a thermomixer comfort (Eppendorf, Hamburg, Germany) at 37°C. The reaction was initiated by pipetting 150 µl sample to the substrate. The mixture was incubated at 37°C for 30 min and the reaction was stopped by addition of 1.2 ml of 10% (w/v) trichloroacetic acid (TCA). The samples were shaken for a short time and allowed to stand at room temperature for 15 min to ensure complete precipitation of the remaining azoprotein and azoprotein fragments. Thereafter, the samples were centrifuged at 9,500 *g* for 3 min and 600 µl of the clear supernatant were transferred to a cuvette containing 700 µl of 1.0 M NaOH. The absorbance was read at 440 nm with a spectrophotometer (Kontron,

Uvikon 940, München, Germany). One unit of protease activity is defined to be the amount of enzyme required to produce an absorbance change of 1.0 in a 1 cm cuvette under the conditions of the assay.

Enzyme purification

The fermentation broth containing the wool substrate was filtered with a sieve to separate the wool from the medium. The resulting solution was centrifuged at 13,700 *g* with a Sorvall® RC-5B Refridgerated Superspeed Centrifuge (DuPont Instruments, Wilmington, USA) at a temperature of 10°C for 20 min. The supernatant was collected, filtered and aliquots were stored at −20°C until further processing.

Defrosted samples were filtered through a 0.2 μm Whatman filter via pressure filtration to eliminate the precipitated polysaccharides and to obtain a clear solution. After centrifugation, the culture supernatant was desalted (3x washing with buffer containing 20 mM $CaCl_2$) and concentrated by ultrafiltration with VivaSpin 20 (Vivascience, Hannover, Germany), equipped with polyethersulphone membranes with a 5 kDa cut off at 3,000 g. All chromatographic purification steps were carried out using an Aekta Purifier FPLC system from Amersham Pharmacia Biotech. As a first step a cation exchange column HiTrap^{TM} SP HP (Amersham Biosciences, Uppsala, Sweden), pH 7.5 (50 mM HEPES; for elution 1 M NaCl was added) was chosen. 100 μl of sample were applied to the column (1 ml column volume) at a flow rate of 1 ml min^{-1}. Fractions with a volume of 1 ml were collected.

For size exclusion chromatography, the concentrated pooled fractions from cation exchange chromatography (100 μl) were subjected to a Superdex 75 HR 10/30 with a fractionation range from 3 to 70 kDa (Amersham Pharmacia Biotech) which was equilibrated with phosphate buffer (0.1 M) pH 8.0 containing 0.1 M NaCl. The column was run with the same buffer with a flow rate of 0.5 ml min^{-1}. All desalting and concentration steps were done using ultrafiltration (VivaSpin 20) with 5 kDa cut off at 3,000 g as described above.

Characterization

In order to study the pH stability of the *Beauveria brongniartii* protease, enzyme samples (47 ng enzyme protein mL^{-1}) were incubated at various pH values between pH 4 and pH 10 (Britton Robinson buffer) at room temperature. Residual enzyme activity was measured using the standard azocasein assay procedure. Temperature stabilities (20–60°C) were measured similarly using 50 mM Tris-HCl buffer, pH 8.0. The reaction was started by incubating 1.47 ml of buffer with 30 μl enzyme solution. Common protease inhibitors were tested for their effect on the activity of the *B. brongniartii* protease. 20 μl of the protease were pre-incubated with varying amounts of inhibitor-solution and 50 mM Tris-HCl buffer, pH 8 for different time intervals in a final volume of 1 ml at room temperature. The final concentration of inhibitor was 1.46 μM for Pepstatin A, 10 μM for E-64, 0.3 μM for Aprotinin and 1 mM for EDTA. Additionally, the effect of the double charged ions Ca^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+}, Cu^{2+} was assessed at a concentration of 5 mM in 50 mM Tris-HCl buffer pH 8.0 incubated for different time intervals at room temperature. The effect of $CaCl_2$ was assessed at 5, 10, 20, 30, 50 and 100 mM concentration. Different organic solvents (DMSO, DMF, MeOH, EtOH, ACN and isopropanol) were tested on their effect to the isolated enzyme while incubating at 37°C for certain times. Deglycosilation was performed with the endoglycosidase Endo H P0702 L (New England Biolabs, Ipswich, USA). Its activity was 500,000 U ml^{-1} and the molecular weight was 29 kDa. 27 μl of the purified enzyme were mixed with 3 μl denaturation buffer (5% SDS, 10% β-mercaptoethanol) and heated at 100°C for 5 min.

To 8.25 μl of the denaturated sample 1 μl 1/10 G5 buffer (sodium citrate, pH 5.5) was added. The reaction was started by adding 0.75 μl Endo H and was performed at 37°C over night. After the incubation time samples were run on a SDS gel and further on silver stained.

The 1D SDS gels were performed according to Laemmli and the 2D gel was operated according to the Amersham method. Isoelectric focusing (IEF) was performed on 5% polyacrylamide gels with a thickness of 1 mm and a pH gradient from

3 to 10. IEF gels were stained with Coomassie brilliant blue R-250.

Substrate specificities of the B. brongniartii protease

Hydrolytic activity of the enzyme with *p*-nitroanilide (*p*NA) and ester substrates was tested at 30°C in 50 mM Tris-HCl buffer, pH 8.0. The substrates used were N-Suc-Ala-Ala-Pro-Phe-*p*NA, N-Suc-Ala-Ala-Pro-Leu-*p*NA, L-Leucine-*p*NA and Gly-Phe-*p*NA. All tested substrates were purchased from Sigma (St. Louis, USA). The release of *p*NA was measured at 410 nm (ϵ = 8,480 M^{-1} cm^{-1} (Bakhtiar et al. 2005)). Initial reaction rates were determined at different substrate concentrations and kinetic parameters were calculate by non-linear regression using the program Origin 5.0. Stock solutions were prepared in DMSO (4 mM) and further dilutions were done with buffer.

Digest and MS-analysis

Bands of interest were cut out of a SDS gel and the tryptic in-gel-digest was optimized following the procedure after Shevchenko et al. (1996). MS analysis was performed using a nanoHPLC-ESI-IonTrap instrument from Agilent. For HPLC separation a multistep-gradient was run from 100% A (water with 0.1% formic acid) to 95% B (ACN with 0.1% formic acid) within 80 min. The flow was maintained at 300 nl min^{-1}. The enrichment column was a ZORBAX SB-C18, 5 × 3 mm (Agilent) and as separation column a ZORBAX 300SB-C18, 3.5 μm, 50 × 0.075 mm (Agilent) was used. The injection volume was 10 μL.

Spectra were obtained with an ion trap mass spectrometer (Agilent 1100 series LC/MSD Trap, Agilent, Waldbronn, Germany). The spray voltage was set to 1.1 kDa and the capillary temperature was set to 100°C. Dry gas was set to 6.00 l min^{-1}, the accumulation time was 50 ms and the spectra were obtained by scanning a range from 200 to 2200 m/z. The spectrum was acquired with the Auto MS (2) mode. The compound mass spectra were analyzed using the database program MASCOT (MatricScience LTD, Boston, USA) choosing the NCBInr database (NCBI Resources, Bethesda, USA).

Wool treatment

Raw wool was obtained from Drummond Parkland of England, (Huddersfield, UK). To obtain wool fibres with partially removed cuticles, the method of Ley (1988) was adapted. To 5 g of washed wool (cut with scissors into small pieces) 250 ml 0.1% SDS (w/v) solution was added and removal of cuticles was monitored microscopically (Laborlux S, Leitz, Oberkochen, Germany). The mixture was shaken at 27°C. After 42 h of incubation, the reaction was stopped by washing the wool with plenty of demineralised water. The effect of detergent on wool was observed with a brightfield light microscope.

Alternatively, 5 g of washed wool snippets were transferred to 200 ml of formic acid (100%) and heated at 100°C for 1 h (Bradbury 1970). Then the wool was washed with plenty of water and the pH of water and wool was monitored. The effect of formic acid on wool was observed by light microscopy. Hydrolysis of these wool substrates was studied both with the isolated *B. brongniartii* protease and a bacterial protease from *Bacillus sp.* (Sousa et al. 2005).

Enzyme treatment of native and pre-treated wool was started by adding a defined activity of protease (10 mU min^{-1}) to 0.5 g washed wool in 50 ml of 50 mM borate buffer with 20 mM $CaCl_2$ (only for *B. brongniartii* protease) in 100 ml Erlenmeyer flasks. Incubation was carried out at 37°C with shaking at 150 rpm. As blanks the enzyme alone and wool without enzyme were incubated. 5 ml samples were taken in certain time intervals protease activity was immediately determined to follow the stability of the enzyme during the reaction. Thereafter, samples was denaturated in a water bath at 100°C for 5 min and stored at room temperature until the measurement of C and N-Content (dissolved organic carbon (DOC), TN; TOC-VCPH Total carbon analyzer, equipped with an ASI-V autosampler and a TNM−1 Total measuring unit from Shimadzu (Kyoto, Japan) and protein absorption (Biophotometer, Eppendorf 280 nm).

Results and discussion

Screening

To isolate proteases with high specificity to wool cuticles nine different fungi (see Materials and methods) were tested for their ability to produce such enzymes with wool as carbon source. Out of these fungi *Beauveria brongniartii* showed best results in producing proteases when grown on wool. In Fig. 1 protease activity produced by *B. brongniartii* when grown on wool is shown.

Enzyme purification and characterisation

Concentration of *B. brongniartii* wool protease was performed by ultrafiltration (5 kDa) including three washes of in the presence of 20 mM $CaCl_2$. The loss of activity was only 6% with the optimized method which was quite reasonable as first concentration step. Cation exchange chromatography turned out to be superior to both other ion exchangers and hydrophobic interaction chromatography as first chromatographic separation step although further improvement of yields will be required (Table 1). In subsequent gel filtration chromatography only one dominant peak with protease activity was observed. In this peak, a single band was detected on SDS gels corresponding to a molecular weight of 27 kDa. This *B. brongniartii* wool protease had a pI value of 8.0 and was most active at pH 8.0 and 20°C. The half life times of the *B. brongniartii* protease at different conditions are shown in Table 2.

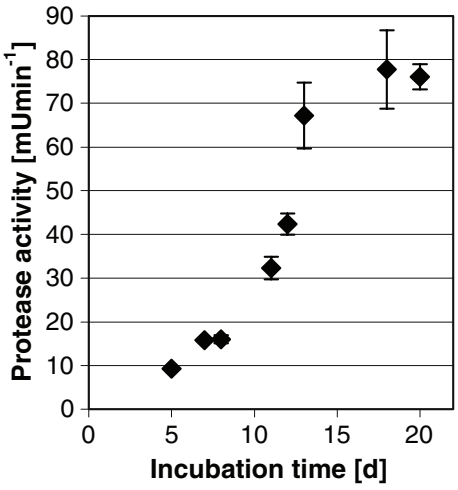

Fig. 1 Protease activity produced by *B. brongniartii* when grown on wool

Although the enzyme showed little effects against various protease inhibitors, E-64, a cysteine inhibitor resulted in an IC_{50} value of 200 μM at an incubation time of 2 h. However, this rather small effect could also be due interaction with cysteine residues not involved in catalysis but e.g. in enzyme/substrate binding. Metal ions enhanced the stability of the protease, especially Ca^{2+} and Mg^{2+} increased the stability by 34% and 33%, respectively at 5 mM concentration. The optimum effect with Ca^{2+} was seen at 20 mM. Organic solvents also increased the stability of the *B. brongniartii* protease, shown for various alcohols (isopropanol, ethanol, methanol), DMF and DMSO. The enzyme hydrolysed the derivatized peptide substrates N-Suc-Ala-Ala-Pro-Phe-*p*NA and N-Suc-Ala-Ala-Pro-Leu-*p*NA and based on the catalytic efficiency obviously had a preference for the aromatic amino acid in the P1 position (Table 3). N-Suc-Ala-Ala-Pro-Phe-*p*NA is described as a specific substrate for chymotrypsin and subtilisin and N-Suc-Ala-Ala-Pro-Leu-*p*NA can be cleaved by both elastase and chymotrypsin (Larcher et al. 1996). Both of these substrates are specific for serine proteases which is in contrast to the fact that the enzyme was slightly inhibited by E-64, known as cysteine protease inhibitor

Table 1 Purification of the *B. brongniartii* protease

	Volume (ml)	Total activity (U)	Yield activity (%)	Total protein (mg)	Yield protein (%)	Specific activity (U mg^{-1})	Fold of purification
Fermentation broth	120	6.60	100	69.92	100	0.09	1.0
Concentration-VivaSpin	10.25	6.34	96	38.03	54	0.17	1.8
Cation exchange chromatography	2.36	0.64	10	5.53	8	0.59	6.3
Gel filtration chromatography	9.20	0.35	5	2.12	3	0.76	8.0

Table 2 Stability of the *B. brongniartii* protease at different conditions. Effect of different compounds and solvents were tested at pH 8 and 20°C

Conditions	Half life time (h)
pH 8* 50°C	2
pH 8* 30°C	25
pH 8* 20°C	33
pH 9* 20°C	20
pH 10* 20°C	13
pH 7* 20°C	24
ACN	3
MeOH	6
DMF	10
DMSO	19
EtOH	24
Isopropanol	31
Ca^{++} (5 mM)	18
Mg^{++} (5 mM)	18
Zn^{++} (5 mM)	11
EDTA (5 mM)	3

*in the presence of 20 mM $CaCl_2$

Table 3 Kinetic parameters of the *B. brongniartii* protease on selected substrates. No activity was detected on Gly-Phe-*p*NA and L-Leu-*p*NA

Substrate	K_m (mM)	K_{cat} (s^{-1})	K_{cat}/K_m
N-Suc-Ala-Ala-Pro-Phe-*p*NA	0.228	0.354	1.553
N-Suc-Ala-Ala-Pro-Leu-*p*NA	0.202	0.056	0.277

(Sigma). In contrast to other alkaline proteases, the *B. brongniartii* protease did not hydrolyse substrates with smaller chain length (Bakhtiar et al. 2005). No homology was found to existing enzymes with peptide mass mapping based on tryptic digestion and analysis of the peptide fragments with nano-HPLC-ESI-MS.

Wool treatment

Wool cuticle scales can be efficiently removed with both SDS and formic acid (Fig. 2). In contrast to formic acid, the SDS treatment allows better control with less damage to the whole fibres. Interestingly, the C/N ratio of material released from wool during hydrolysis with the *B. brongniartii* protease increased with longer incubation times (Fig. 3). This might be due to the fact that the enzyme initially hydrolysed cuticles of the wool fibre. The dominant amino acid of cuticles is cysteine (C/N = 3) with a small C-content compared to amino acids constituting proteins located more inside the fibre with a higher C/N ratio $C/N_{average} = 4.3$ which were obviously subsequently hydrolysed. The A-layer has the highest half-cysteine content with 37% and a high isopeptide amount (Zahn et al. 1997). Therefore, the C/N ratio is small at the beginning and when the *B. brongniartii* protease has removed the cuticle material it starts to hydrolyze proteins in the cortex cells and cell membrane complex proteins until a plateau is reached after 32 h.

The *Bacillus* protease shows a different behaviour. This enzyme seems to penetrate into the fibres from the beginning on. This results in a higher C/N ratio of released material at the

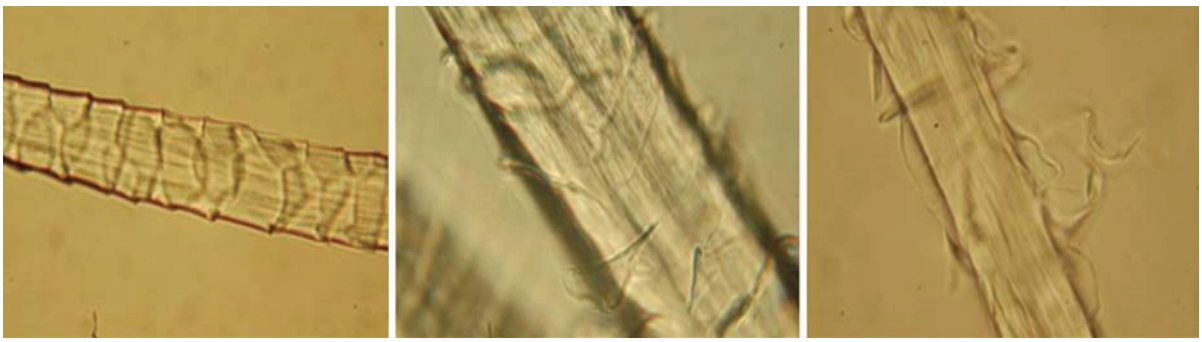

Fig. 2 Washed wool (left picture) compared to pre-treated wool (middle: wool pre-treated with SDS, right: wool pre-treated with formic acid)

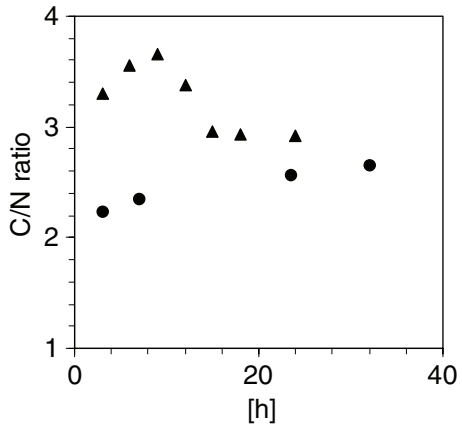

Fig. 3 C/N ratio of material released from wool by the *B. brongniartii* (●) and *Bacillus* sp. protease (▲). All data result of four repetitions with a standard deviation of below 18%

beginning compared to the *B. brongniartii* protease. When the reaction proceeds, cuticles (with high cysteine content) are also hydrolysed resulting in a decreasing C/N ratio.

These results concerning the C/N ratio of material released by the two different proteases correlate very well with the SEM pictures (Fig. 4). In contrast to the *B. brongniartii* protease, cracks (see arrows in Fig. 4) in the cuticle can clearly be seen after treatment with the *Bacillus* protease. This indicates that the enzyme was penetrating through the cuticle into the wool fibre and thereby destroying the fibre. Furthermore, treatments with the *B. brongniartii* protease lead to a smoother surface.

The substrate specificities of the two proteases were compared on wool and wool after partial removal of cuticles. Interestingly, the activity of the *B. brongniartii* protease decreases significantly with the removal of cuticles from wool while the activity of the *Bacillus* proteases remains the same (Fig. 5). These findings correlate both with the results of the C/N measurements and microscopic observations indicating that the *B. brongniartii* protease preferentially hydrolyzes proteins of the cuticle scales.

Conclusion

The C/N ratio of material released from wool after enzyme treatment can be used as an indication for the mode of action proteases on this substrate (on the surface or more inside the fibres). Together with other analytical methods (SEM, specificity on wool with removed cuticles) it was proven that the newly isolated protease from *B. brongniartii* preferentially hydrolysed wool cuticles when compared to a *Bacillus* sp. protease. Thus, this enzyme has a potential for wool anti-shrinkage treatment.

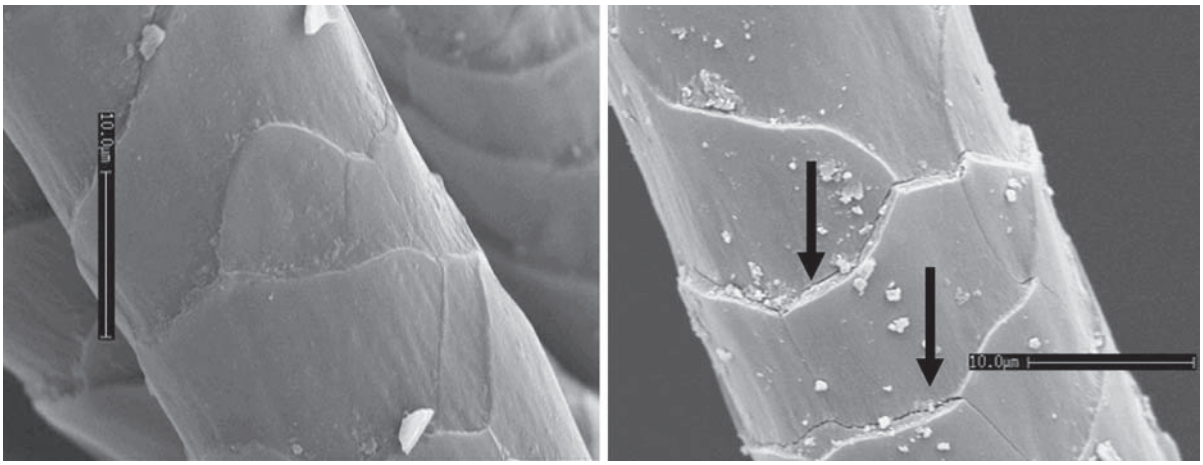

Fig. 4 SEM images of wool fibres treated (30 min) with the *B. brongniartii* protease (left picture) and the *Bacillus* sp. protease (right picture)

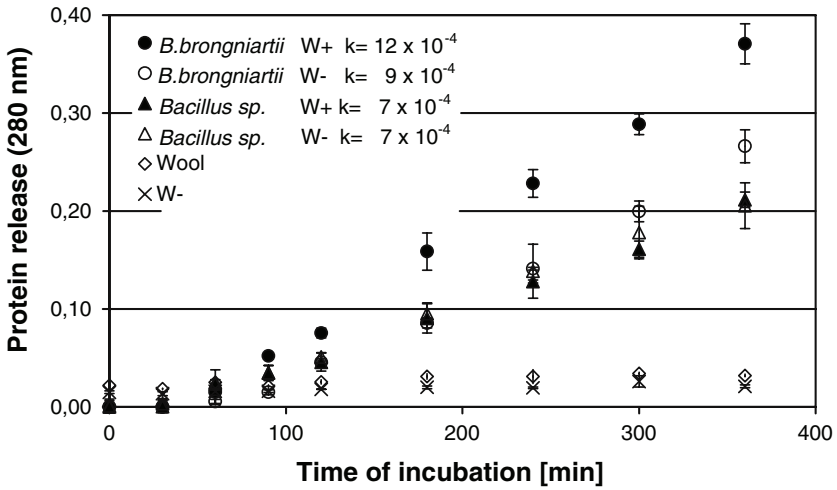

Fig. 5 Release of protein from wool and wool (W) with partially removed cuticles (W-) by the *B. brongniartii* protease and *Bacillus* sp. proteases. Cuticles were removed by SDS treatment. All data result of four repetitions

References

Bakhtiar S, Estiveira RJ, Hatti-Kaul R (2005) Substrate specificity of alkaline proteases from alkalophilic feather-degrading *Nesterenkonia* sp AL20. Enzyme Microb Technol 37:534–540

El-Sayed H, Kantouch A, Heine E, Höcker H (2001) Developing a zero-AOX shrink-resist process for wool, I: preliminary results. Color Technol 117:234–238

Heine E, Höcker H (1995) Enzyme treatments for wool and cotton. Rev Prog Col 25:57–63

Higuchi T, Saika T, Senda S, Mizobata T, Kawata Y, Nagai Y (1997) Development of biorational pest control formulation against longicorn beetles using a fungus, *Beauveria brongniartii* (Sacc.) Petch. J Ferment Bioeng 84(3):236–243

Jovancic P, Jocic D, Molina R, Julia MR, Erra P (2001) Shrinkage properties of peroxide-enzmye-biopolymer treated wool. Text Res J 71(11):948–953

Jovancic P, Jocic D, Molina R, Julia MR, Erra P (2003) The combined low-temperature, plasma/enzyme wool shrink/resist treatment. AATCC Rev 3(2):25–28

Koller R, Jung K, Scheu S, Zimmermann G, Ruther J (2005) Biocontrol of the forest cockchafer (*Melolontha hippocastani*): experiments on the applicability of the ''catch and infect''-technique using a combination of attractant traps with the entomopathogenic fungus *Beauveria brongniartii*, insect pathogens and insect parasitic nematodes: melolonatha. IOBC/WPRS Bull 28(2):37–44

Larcher G, Cimon B, Symoens, Tronchin G, Chabasse D, Bouchara JP (1996) A 33 kDa serine proteinase from *Scedosporium apiospermum*. Biochem J 315:119–126

Schroeder M, Schweitzer M, Lenting HBM, Guebitz GM (2004) Chemical modification of proteases for wool cuticle scale removal. Biocat Biotrans 22(5/6):299–305

Sousa F, Cavaco-Paulo A, Erlacher A, Schroeder M, Guebitz GM (2005) Novel bacterial and modified proteases for wool treatment COST847 Congr Text Biotechnol Maspalomas, Spain Feb. 20–23

Shevchenko A, Wilm M, Vorm O, Mann M (1996) Mass spectrometric sequencing of protein–silver-stained polyacrylamide gels. Anal Chem 68(5):850–858

Simpson WS, Crawshaw GH (2002) Wool: science and technology. CRC Press, Boca Raton ISBN 0 8493 2820 9

Zahn H, Hoffmann R (1996) ''Wool'' in Ullmann's Encyclopedia of Industrial Chemistry, Vol. A 28, VCH Verlagsgesellschaft. Weinheim, Germany, ISBN 3 527 20128 9

Zahn H, Wortmann FJ, Höcker H (1997) Chemie und Aufbau der Wolle, Chemie in unserer Zeit, Wiley-VCH, Nr. 6