Molecular recognition of esterase plays a major role on the removal of fatty soils during detergency

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ABSTRACT

In this work it is describe for the first time, the use of an esterase with null activity (Tfu.0883 bacterial cutinase from *Thermobifida fusca*) on the removal of fat from the surface of a cotton substrate. Similar levels of fat removal were found for both null and wild-type proteins, despite that only wild type protein yielded fatty acids. Our results show that molecular recognition of esterase plays a major role on the removal of fatty soils, allowing important guidelines for the design of detergent enzymes. Furthermore, the advantage of using null esterase enzymes lies in the avoidance of the rancid smell of short chained fatty acids, typical after esterase treatment.

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1. Introduction

Enzymes have been used to improve the cleaning efficiency of detergents for more than 35 years, and are now well accepted as ingredients in powder and liquid detergents, stain removers/laundry pre-spotters, automatic dishwashing detergents and industrial/institutional cleaning products. As catalysts, enzymes speed up specific chemical reactions, in mild conditions of temperature and pH, without being altered or consumed in the process. Consequently, small quantities of enzyme can repeatedly catalyze the breakdown of millions of molecules in minutes. Enzymes operate optimally in detergents at temperatures of 20–60 °C and within a pH range of pH 7.5–10.5. Detergency is the most successful application area of industrial enzymes like proteases, lipases, amylases and cellulases, representing more than 30% of the worldwide market (Olsen and Falholt, 1998). Today, the most widely used industrial enzymes are hydrolases, which hydrolyze and solubilize the substrate soils, based on proteins, lipids and polysaccharides, facilitating their further removal from the matrix where they “sit on” (Hasan et al., 2006, 2010; Jaeger et al., 1994; Maurer, 2004; Olsen and Falholt, 1998). Cellulases are claimed to break cellulosic microfibrillar material, releasing entrapped soils from textile surface (Ahuja et al., 2004; Kirk et al.,

2002; Olsen and Falholt, 1998; Sukumaran et al., 2005). Moreover, they can be used as softening agents, to remove soil particles and to revive colors.

The washing performance of an enzyme detergent depends on many factors namely detergent composition and dosage, pH and buffer capacity, water hardness, washing time and temperature, mechanical handling, soiling agents and textile type. Furthermore, its specificity should be as higher and wide as possible. A specific enzyme would be able to hydrolyze fats and soils with the consequent production of protein and peptide fragments, which can be easily removed during the washing process.

Being generally accepted that enzymes play a major roll in detergency since they speed up the soil removal process (Ahuja et al., 2004; Hasan et al., 2006; Olsen and Falholt, 1998), it is also expected that surfactants would help on the enzyme accessibility towards the soils, but any chemical breakage would make the presence of the enzymes at the detergent formulation redundant, since the major objective of a detergency process is the removal and dispersion of soils itself and not their molecular hydrolysis. Studies have demonstrated that the use of lipases together with a nonionic surfactant led to detergency values similar to those reached at higher surfactant concentrations, revealing a synergistic effect between them (Jurado et al., 2006). It has also been consistently verified that very low amounts of enzyme are required to greatly enhance soil release, leading to the assumption that other phenomena could be involved. The hydrolysis at a molecular level by an enzyme involves a recognition process of the substrate and docking at the active

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site, before the catalytic action itself. If the substrate is at solid or in a different aggregation state, some binding domains/modules have been designed by nature to perform this recognition process (Laurent et al., 2008). Amylases and cellulases are known to have binding modules and some lipases require interfacial activation, i.e., they have a boost of their activity above critical micellar concentration (Olsen and Falholt, 1998; Reetz, 2002).

There are also some evidences that molecular recognition described as a process by which biological entities specifically interact with each other or with small molecules to form complexes, might include the physical breaking of aggregate substrates making it accessible towards the enzymes (like cellulases) (Jurado et al., 2006; Maurer et al., 2009; Mohan et al., 2006). Those indications are of prime importance for enhancement of detergency phenomena, since the major goal it is the removal of soils, either in dispersed or soluble form. To our knowledge, the molecular recognition effects of proteins have never been specifically tested for detergency purposes until now and very little work dealing with odor formation/removal in laundry during washing has been published.

In this work, we have tested a non-industrial fitted esterase, a bacterial cutinase from *Thermobifida fusca*, for the removal of butter fat from a cotton fabric substrate. This enzyme has been suggested in literature for PET (polyethyleneterephthalate) hydrolysis, recycling and for antipilling treatment in detergents (Kellis et al., 2002; Maurer et al., 2009). Due to their strong hydrophobicity, fats and oils (triglycerides) are difficult to remove from laundry at low temperatures. Thus, cutinases hydrolyze triglycerides to more hydrophilic and soluble mono- and diglycerides, free fatty acids and glycerol, which can give the traditional rancid smell after washing (Ahuja et al., 2004; Dutta et al., 2009; Hasan et al., 2010; Munk et al., 2000; Obendorf et al., 2001). For this purpose, the genetic engineering of wild-type Tfu.0883 was assessed nullifying its active site avoiding at the same time its inactivation during storage due to proteolytic and autolytic degradation. Our main goal was to exploit the molecular recognition ability of cutinase to remove fat soils, avoiding their chain breakage into small fragments that would be hardly removed during washing procedure. Besides, the characteristic rancid smell of the low chain fatty acids, normally obtained when an active catalyst is used, will be reduced as well as the sebum, that is normally difficult to remove from the interior of the fibres by normal detergency.

2. Materials and methods

2.1. Materials

The *T. fusca* wild-type cutinase and null mutant cutinase, used in this work, were partially purified from engineered *Escherichia coli* BL21(DE3) and produced at the State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi, China (Chen et al., 2008, 2010).

The substrate used for fatty soils removal was gently supplied by Procter & Gamble, NewCastle, United Kingdom. The substrate consisted on butter fat deposited on cotton fabric.

2.2. Methods

2.2.1. Preparation of cutinase mutant

Cutinase S130C mutation was generated by the standard QuikChange mutagenesis methodology using complementary primers. The forward primer was 5'-CTggCggTCATgggCCACTgTATgggCggCggCggCAC-3', and the reverse primer was 5'-gTgCCgCCgCCgCCCATACAgTggCCCATgACCgCCAg-3'. The gene sequence was confirmed by DNA sequencing. The

plasmid (pET20b-Tfu.0883) with the correct sequence was used to transform chemically competent *E. coli* BL21 (DE3) cells (Chen et al., 2008).

The recombinant cutinase was partially purified through $(\text{NH}_4)_2\text{SO}_4$ precipitation. The *E. coli* BL21(DE3) cells harboring pET20b-Tfu.0883 S130C were grown in TB medium containing ampicillin (100 mg/l) at 37 °C with shaking (200 rpm). Isopropyl- β -D-thiogalactoside was added to a final concentration of 0.4 mM when the culture reached an OD₆₀₀ of 1.5. The culture supernatant was collected by centrifugation (40,000 × g, 20 min, 4 °C) 50 h after induction. Solid $(\text{NH}_4)_2\text{SO}_4$ was slowly added to the supernatant to final 70% saturation, on ice. The precipitated protein was collected by centrifugation (40,000 × g, 30 min, 4 °C) and immediately dissolved in buffer (20 mM Tris-HCl, pH 8.0). The sample was dialyzed against 2 L of buffer (20 mM Tris-HCl, pH 8.0) at 4 °C overnight and then lyophilized (Chen et al., 2010).

As control of the experiments an endogenous crude protein extract was obtained from *E. coli* BL21(DE3) cells transformed with an empty vector; the culture growth and protein purification were performed in the same way as described for the bacteria cultures harboring the *T. fusca* wild-type and null cutinase genes.

2.2.2. Detergent efficiency of cutinase on fatty soils removal

A detergency test which uses a natural soil (butter fat) and closely simulates laundry practice has been performed. For this purpose, samples of cotton butter fat substrate (≈0.55 g each) were incubated with cutinase from *T. fusca* (0.055 mg/g_{substrate}) in Tris-HCl (0.1 M) buffer (pH 8). The incubation was performed using wild-type and null cutinase. As control of the experiments an endogenous crude protein extract, obtained from *E. coli* BL21(DE3) cells transformed with an empty vector was used to eliminate the effect of other proteins on fat removal.

The experiments were carried out at 37 °C for 2 h under continuous shaking, 40 rpm (using an AHIBA Spectradye, from Datascolor, with vertical agitation), simulating the domestic washing machines. After treatment the samples were rinsed twice with cold water. The assay was performed in duplicate. The cleaning degree was defined as the color difference in terms of *K/S* quantification before and after washing procedure. The color measurements were carried out using a spectrophotometer (illuminant D65) (Spectraflash 600 Plus, from Datacolor International). The color strength was evaluated as *K/S* at maximum absorption wavelength (360 nm). The ratio between absorption coefficient (*K*) and scattering coefficient (*S*) is related to reflectance data by applying Kubelka–Munk's law (Eq. (1)) at each wavelength, and it is proportional to dye (color) concentration.

$$\frac{K}{S} = \frac{1 - R^2}{2R} \quad (1)$$

Eq. (1) – determination of *K/S* using the simplified Kubelka–Munk's law (*R* – light reflection; *K* – absorption coefficient; *S* – scattering coefficient). The relative *K/S* was calculated for each sample, comparing the *K/S* value of samples after and before incubation.

All measurements were recorded using duplicate samples and the results were expressed as mean values. The final incubation solutions were kept for further hydrolysis products determination (fatty acids).

2.2.3. Fatty acids determination

Fatty acids determination was assessed by using titration method with 0.02 N NaOH. Aliquots of resulting incubation solutions (3 mL) were titrated with 0.02 N of NaOH using 1% phenolphthalein in 95% ethanol.

2.2.4. Study of the interaction between cutinase mutant and a fat model

There are no X-ray structures available for *T. fusca* cutinase. In order to perform simulation studies with *T. fusca* cutinase is necessary to derive its 3D structures by homology modeling. To perform these homology modeling studies the SWISS-MODEL tool was used (Arnold et al., 2006; Kiefer et al., 2009; Peitsch, 1995). The results from the SWISS-MODEL show that the alignment was performed with *Streptomyces Exfoliatus* lipase (PDB code 1jfr (Wei et al., 1998)), with 64% of sequence identity. From this, it was built the structure of the mutation S130C by replacing the serine by a cysteine residue.

To obtain a more realistic representation of *T. fusca* cutinase some molecular dynamic simulations of this structure in water were performed. The simulations were carried out with GRO-MACS 4.0.7 (Hess et al., 2008) package using the GROMOS 53A6 force field (Oostenbrink et al., 2004). The system was solvated using a pre-equilibrated box of water. The system size was chosen according to the minimum image convention taking into account a cut-off of 1.4 nm. The bond lengths of the proteins were constrained with LINCS (Hess et al., 1997) and those for water with SETTLE (Miyamoto and Kollman, 1992). Non-bonded interactions were calculated using a twin-range method, with short and long range cut-offs of 0.8 and 1.4 nm, respectively. Neighbor searching was carried out up to 1.4 nm and updated every five steps. A time step of integration of 2 fs was used. A reaction field correction for the electrostatic interactions was applied using a dielectric constant of 54 (Smith and van Gunsteren, 1994). The single point charge (SPC) model (Hermans et al., 1984) was used for water molecules. The initial systems were energy minimized for 2000 steps using the steepest descent method, with all heavy atoms harmonically restrained using a force constant of 10^3 kJ/mol nm². The systems were initialized in the canonical ensemble (NVT – number of molecules, volume and temperature constant) for 50 ns, with all heavy atoms harmonically restrained using a force constant of 10^3 kJ/mol nm². The simulation was then continued for 50 ns in the isothermal–isobaric ensemble (NPT – number of molecules, pressure and temperature constant), with the heavy atoms harmonically restrained with the same force constant. Finally, for allowing the equilibration of the system properties, the simulations were further extended in the NPT ensemble with positional restraints applied to the C α atoms. Pressure control was implemented using the Berendsen barostat (Berendsen et al., 1984), with a reference pressure of 1 bar, 0.5 ps of relaxation time and isothermal compressibility of 4.5×10^{-5} bar⁻¹. Temperature control was set using the V-rescale (Berendsen et al., 1984; Bussi et al., 2007) thermostat at 300 K. The protein and water were grouped in different heat baths, with temperatures coupling constants of 0.025 ps in the first two initialization steps and with 0.1 ps for the rest of the simulations. Five replica simulations of 50 ns in length were carried out for each enzyme, using different initial velocities taken from a Maxwell–Boltzman distribution at 300 K.

After obtaining the structures for *T. fusca* (native state and S130C mutation) the AutoDockVina (Trott and Olson, 2010) was used to study the possible interactions between this enzymes and a butter fat molecule. For the docking, the enzyme was assumed as the receptor, fully rigid and the fat molecule as the ligand, with no rigid bonds. A box of size $70 \times 70 \times 70$ Å, centered at the geometric center of *T. fusca* structure was considered allowing the fat model to explore all the positional space surrounding the enzyme. The standard configurations of AutodockVina were used, except for the exhaustiveness value (we used 800), allowing the program to generate 20 binding.

2.2.5. Cutinase formulations

The nanoemulsions, containing wild-type and null cutinase, were produced using an high pressure homogenizer. Briefly, the

organic solvent (vegetable oil) was added to the aqueous solution of protein (0.5 g/L cutinase) in order to achieve a ratio of 99.5:0.5 (aqueous/organic phase). The mixture was added to 1% of Tween 80 (anionic surfactant) in order to decrease the particle size. The homogenization was carried out using a high pressure homogenizer (1000 bar) for 13 min (39 homogenization cycles).

The detergency tests on cotton butter fat substrate were carried out by adding 0.55 mg/g_{substrate} of protein formulations (null and wild-type) in Tris–HCl buffer (pH 8) at different temperatures (40, 60 and 90 °C) simulating laundry washing practice. Incubations with free cutinase (null and wild-type) were performed to compare with protein formulations behavior. The cleaning degree was defined as the color difference in terms of K/S quantification before and after washing procedure.

2.2.6. Cutinase formulations characterization

The zeta-potential and size distribution of nanoemulsions were determined at 25 ± 0.1 °C using a Malvern zetasizer NS (Malvern Instruments) by electrophoretic laser Doppler anemometry and photon correlation spectroscopy (PCS), respectively.

2.2.7. Transmission electron microscopy (STEM) characterization

The microscopic aspect of nanoemulsions was determined by STEM analysis. The suspensions were placed in Copper grids with carbon film 400 meshes, 3 mm diameter. The shape and morphology of nanoemulsions were observed using a NOVA Nano SEM 200 FEI.

3. Results

The concept of molecular recognition involved in cutinase action towards fat was firstly exploited. For this a wild-type cutinase (Tfu_0883) and a mutant cutinase (S130C), genetically modified by site directed mutagenesis to eliminate catalytic activity, were applied onto cotton fabric substrates, totally covered with yellow butter fat. The washing conditions followed, simulated the laundry procedures and the level of washing efficiency was evaluated in terms of K/S quantification (% color removal) and weight loss. In addition, the concentration of fatty acids produced was calculated by titration. The results presented in Table 1 show that cutinase with null activity (S130C) was able to remove almost the same amount (80.42%) of fat as the wild-type (82.46%). However, it can be depicted that after washing with null enzyme, the quantity of fatty acids produced was much lower (1.67 mM) than that produced with wild-type (13.33 mM). It seems evident that molecular recognition overlapped the substrate specificity of cutinase. Thus, null cutinase was able to recognize fat at fabric's surface which was further removed by mechanical agitation. Low quantity of fatty acids was produced since no hydrolysis occurred, avoiding therefore the small chain fatty acids rancid smell. The results obtained for the extract control corroborate this assumption since this medium was not able to remove the same amount of fat as null cutinase did. The removal of fat by cutinase is not a product of an un-specific binding, instead a molecular recognition phenomena is involved.

To emphasize the importance of molecular recognition in this process we performed some docking analyses on both wild type and mutated cutinase, and the results are depicted in Fig. 1. The structure of *T. fusca* cutinase was obtained by homology modeling and the alignment was made with *S. Exfoliatus* lipase. As both enzymes belong to the same family, it was expected that the 3D structures would be very similar, but in order to obtain a more realistic representation of Tfu_0883, the structure was allowed to relax in water for 50 ns, doing some molecular dynamics simulations with Gromacs. Five different simulation replicas were used as the starting point for the docking calculations. From all the docking results, the replicas presenting the most favorable affinity values were chosen

Table 1

Concentration of fatty acids (mM) in solutions after detergency tests during 2 h at 37°C in a shaking water bath; % of color removal (K/S) after samples incubation and respective bath pH.

	Conc. fatty acids (mM)	pH ^a	% color removal (K/S)
Cotton fat substrate + cutinase Tf.0883 null activity + Tris–HCl buffer	1.67	6.18	80.42
Cotton fat substrate + cutinase Tf.0883 wild-type + Tris–HCl buffer	13.33	4.91	82.46
Cotton fat substrate + Tris–HCl buffer	2.00	7.17	58.76
Cotton fat substrate + endogenous crude protein extract + Tris–HCl buffer	2.6	7.21	39.26

^a After 2 h of incubation.

(Fig. 1). From Fig. 1 it is possible to observe the best docking results for the butter fat molecule interacting with the catalytic moiety of the enzyme, represented by the position 130 at light green. Apparently the mutation S130C did not change significantly the affinity of the catalytic triad for the ester groups. The butter fat molecule binds at the same places for both enzymes, confirming their molecular recognition role. From the bottom pictures in Fig. 1 it can be also concluded that the butter fat interaction with the enzyme occurs in the positively charged areas, in accordance with the negative character of ester groups.

Afterwards, formulations containing wild-type and null cutinase were produced by high pressure homogenization, in order to increase their stability and diffusion rate onto the fabric substrates. The high-pressure homogenization, extensively used in food, pharmaceutical and general chemical engineering applications, was applied to create emulsions of wild-type and null cutinase (i.e. liquid-in-liquid dispersions) with small and narrow drop size distribution. The emulsification in the homogenizer can be described as a combination of fragmentation, recoalescence and adsorption of emulsifiers (Håkansson et al., 2011). There are also some studies showing that cavitation takes place in the homogenizer and could also participate in fragmentation (Floury et al., 2004; Håkansson et al., 2011). This process involves the formation of $\cdot\text{OH}$ and $\cdot\text{H}$ radicals, that will form H_2 and H_2O_2 , and, in the presence of O_2 will form $\cdot\text{HO}_2$ hydroxyl, superoxide and peroxide radicals that are all potential protein cross-linking agents. The cysteine residues, a key requirement for stable microemulsions formation, present

in cutinase are held together by protein cross-linking through disulfide linkages from cysteine oxidation (Avivi and Gedanken, 2005; Gedanken, 2008). Together with the cysteine residues of the protein, the amount of hydrophobic aminoacids played also a significant role on the microemulsion production (Bidyut and Moulik, 2001).

Oil-in-water microemulsions with narrow and small sizes (150 nm and 230 nm for null and WT, respectively) were obtained, as shown in Table 2. The morphology of the microemulsions produced, determined by transmission microscopy (STEM) is presented in Fig. 2, demonstrating that the proteinaceous microemulsions are spherical with a regular surface. This morphology would allow a higher protein stabilization, since a minimum contact with water is provided, requiring at the same time a small amount of surface-active agent for stabilization (Dickinson, 1992). STEM technique allowed to show the average particle size of around 150–250 nm, corresponding to the values obtained by DLS measurements. Herein, after 16 weeks of storing the new protein formulations produced remained stable (Table 2). In addition, the particles size and zeta-potential did not suffer significant changes that would affect their detergency performance.

The detergency ability of the produced nanoemulsions was tested through cotton fabrics totally covered with yellow fat. Afterwards, the concentration of fatty acids was determined by titration and the results are presented in Fig. 3A. The results obtained indicate that the production of fatty acids at detergency process using nanoemulsions of null cutinase was much lower than that obtained

Fig. 1. Docking results with wild-type cutinase (on the left) and a mutant cutinase (on the right). On the top pictures it is possible to see, at light green, the position 130 of the enzyme. The bottom pictures represent the enzyme surface potential, constructed using Pymol (Schrodinger, 2010).

Table 2
Particle size, zeta-potential and polydispersity (PDI) of nanoemulsions produced by homogenization method.

	Size (nm)	Size (nm) 16 weeks	Zeta-potential (mV)	Zeta-potential (mV) 16 weeks
Null cutinase	150 (PDI = 0.16)	173.1 (PDI = 0.25)	−8.02	−16.8
WT cutinase	230 (PDI = 0.24)	221.5 (PDI = 0.38)	−26.2	−32.5

Fig. 2. STEM of nanoemulsions of null (A) and wild-type cutinase produced by high pressure homogenization using vegetable oil as organic solvent ($\times 50,000$ magnification).

for wild-type. Nevertheless, null enzyme was able to remove higher amount of fat from the cotton fat surface (Fig. 4). These findings suggest that catalytic activity is not an essential issue on detergency phenomena, molecular recognition played the higher role on this process. The behavior, in terms of total weight loss, of both null and wild-type cutinases in the free or formulated state is similar.

Fig. 3. Fatty acids (mM) production (A) and weight loss (%) (B) after fat removal with of free and formulated cutinase protein at 40, 60 and 90 °C; the Tris–HCl buffer was used as control on fat removal.

All of them were able to remove similar amount of fat from cotton surface (Fig. 3B), however the production of fatty acids was considerably higher when wild-type was applied. Thus, the ability of wild-type to hydrolyze fat producing small and chain fatty acids is demonstrated. These fatty acids are partially removed and others remain at cotton surface forming a sebum layer and an undesirable rancid smell. In the case of null cutinase, the enzyme does not hydrolyze fat, removing it by molecular recognition. Despite the similar weight loss obtained, null cutinase was able to remove higher fat chains by molecular recognition, which can be translated in a higher color (fat) removal from the cotton surface (Fig. 4).

Thus, the detergency ability of both null and wild-type cutinase was quantified in terms of fat removal, measured by *K/S* determination. The lower is the amount of fat at cotton surface, the lower is the *K/S* value and thus the higher is the fat removal. Null cutinase, as mentioned before, was responsible for the highest amount of fat removal, decreasing therefore the *K/S* values of cotton samples after detergency.

Fig. 4. Detergency efficiency measured in terms of *K/S* of fabrics after washing with different cutinase formulations.

4. Discussion

Due to their strong hydrophobicity, fats are difficult to remove from laundry at low temperatures. Furthermore, a sebum production and rancid smell are often related to the fat hydrolysis by esterases (Olsen and Falholt, 1998). The results obtained in this work demonstrated that the lipolytic cutinase showed to be potentially suitable for fat removal. A different behavior was observed for both null and wild-type cutinases. By molecular recognition, null cutinase efficiently removed fat from cotton surface with low production of fatty acids, reducing or/and avoiding the inherent rancid smell and the sebum formation. This behavior can probably be explained by analyzing and comparing the structure of the native enzyme with the published cutinase from *Fusarium solani pisi* structure that reveals the active site serine accessible to the solvent. This serine is part of the Ser-Asp-His catalytic triad and is located in a predominantly hydrophobic cleft. At both sides of the cleft two hydrophobic areas are located (loops 80–90 and 180–190, respectively) that are involved in the molecular binding of the substrate (Longhi and Cambillau, 1999). This hydrophobic lipid binding region close to the active site is surrounded by a positively charged collar mainly formed by hyper-exposed arginines. This surface with hydrophobic and positively charged residues is probably involved in the binding to the mostly negatively charged interface (Flipsen et al., 1999). This assumption is corroborated by the docking studies performed as shown in the bottom pictures of Fig. 1.

In this study, stable cutinase emulsions produced by high pressure homogenization were able to efficiently remove the butter fat from cotton substrates. It would be expected that these formulations removed higher amount of fat, due to the higher penetration ability onto the inner parts of cotton fibres. However, this behavior was not observed. Enzymes acted only at cotton surface and their size was not a crucial parameter for detergency efficiency. These results suggest that molecular recognition of the substrate overlap enzyme activity. The content of fatty acids produced was much lower and therefore the rancid smell, characteristic of esterase action, was avoided. As published by others, the removal of fat is not a simple hydrolysis but involves molecular recognition phenomenon where (R)-ester is readily recognized and efficiently bond to the active site (Bhalerao et al., 1991).

Another aspect to consider is that the color removal was higher for lower temperatures, suggesting that using enzymes, specifically esterases, it is possible to decrease the washing temperature with considerable saving costs.

Until now, only few works about experimental structural data were published about the interaction of lipases and cutinases with a triglyceride. Mannesse and co-workers studied the substrate selectivity and stereopreference of a *Fusarium solani* cutinase and their mutants to some triglyceride analogues (Mannesse et al., 1995). Flipsen et al. investigated which factors determine the value of the interfacial tension caused by cutinase binding (Flipsen et al., 1999). With this work it was proved that the use of an esterase, namely cutinase, showed to be highly space-efficient and of particular advantage in concentrated detergent formulations. Moreover, genetic engineering has contributed to improvements in the specificity, economy and potentiality for use on a detergent composition. The use of an enzyme without catalytic activity allows the development of a wide range of detergent formulations with no concerns about enzyme stability during storage.

5. Conclusions

Overall, our results suggest important guidelines for the design of detergent formulations through modern biotechnology. The results obtained offer the advantage of using null esterase enzymes for fat removal avoiding the rancid smell of short chained fatty acids

after enzymatic treatment. Preliminary molecular modeling studies allowed a new insight into the origin of enantioselectivity in cutinase-catalyzed reactions and provide guidelines for improving selectivity by protein engineering.

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