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Alkaline Protease from Marine Streptomyces Albidoflavus and its Probable Applications

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ABSTRACT

A marine actinomycete isolate identified as Streptomyces albidoflavus; using chemotaxonomical, cultural and morphological techniques; was found to produce alkaline protease when cultivated on a culture broth medium containing a mixture of casein (5g/l) and peptone (5g/l) in the absence and presence of yeast extract (5g/l). It was also found that the presence of yeast extract slightly enhance the enzyme production. The effect of some metal ions on enzyme production was also studied and it was investigated that none of the tested metal ions were able to improve the productivity over the control which contains MgSO4.7H2O (1g/l). The crude alkaline protease from S. albidoflavus exhibited different applications, i.e., removing blood stain from cloth, degrade human hair, dissolve blood clot, degrade coagulated egg white, decomposition of protein layers on X-ray films as well as dehairing of cow skin.

Key words: Marine, identification, Streptomyces albidoflavus, alkaline protease and applications.

Introduction

Actinomycetes are considered as good decomposers in soil through the secretion of many extracellular hydrolyzing enzymes deserving studies for their potential applications as industrial enzymes (McCarthy, 1987; Edwards, 1993; Yeoman and Edwards, 1994). Actinomycetes, in addition to antibiotics, produce extracellular enzymes, e.g. proteases, chitinases, amylases etc. Compared to Bacillus spp., actinomycetes have been less explored for proteases. Several attempts are being done in this point of view (Ningthoujam et al., 2009). Streptomyces spp. are the most industrially important actinomycetes due to their ability to produce numerous secondary metabolites particularly antibiotics. The capability of this group of bacteria to produce large amount of enzymes offers another potentially intrsesting use (Pecznska-Czoch and Mordarski, 1988).

A protease catalyses the hydrolysis of proteins into peptides and amino acids, and consists of one of the most useful enzyme (Jayasree et al., 2009). Proteases are the most important group of industrial enzymes, which accounted for about 60% of total enzymes sales. Bacterial proteases are most significant compared with animal and fungal proteases and have wide range of industrial application. Among different proteases, alkaline proteases produced by microorganisms are of major importance from a biotechnological viewpoint, and are investigated not only in scientific fields of protein chemistry and protein engineering but also in applied fields such as detergents, foods, tannery, pharmaceutical, and leather industries. Proteases perform a great diversity of functions and have significant biotechnological applications. Proteases represent one of the three largest groups of industrial enzymes and find application in detergents, leather industry, food industry, pharmaceutical industry and bioremediation processes (Anwar and Saleemuddin, 1998; Gupta et al. 2002). Probably the largest application of proteases is in laundry detergents, where they help removing protein based stains from clothing (Banerjee et al. 1999). For an enzyme to be used as an detergent additive it should be stable and active in the presence of typical detergent ingredients, such as surfactants, builders, bleaching agents, bleach activators, fillers, fabric softeners and various other formulation aids. In textile industry, proteases may also be used to remove the stiff and dull gum layer of sericine from the raw silk fiber to achieve improved luster and softness.
Protease treatments can modify the surface of wool and silk fibers to provide new and unique finishes. Proteases have been used in the hide dehairing process, where dehairing is carried out at pH values between 8 and 10. An interesting application of alkaline protease was developed by Fujiwara and coworkers (Ishikawa et al., 1993). They reported the use of an alkaline protease to decompose the gelatinous coating of X-ray films, from which silver was recovered. Proteases are also useful and important components in biopharmaceutical products such as contact-lens enzyme cleaners and enzymic debriders (Anwar and Saleemuddin, 2000). The proteolytic enzymes also offer a gentle and selective debridement, supporting the natural healing process in the successful local management of skin ulcerations by the efficient removal of the necrotic material (Sjodahl et al., 2002).

Alkaline proteases with industrial applications were produced and studied from different microbial sources, mainly Bacillus and Streptomyces (Halpern, 1981; Hagiwara et al., 1958), but proteases from other actinomycetes were not common but little is known about proteases from Nocardiopsis (Kim et al., 1993).

Materials and methods

Microorganism and Culture Conditions:

Marine isolate of streptomycetes identified as Streptomyces albidoflavus was isolated using the following medium (g / l): glucose, 10.0; peptone, 5.0; yeast extract, 5.0; KH2PO4, 5.0; MgSO4, 7H2O, 10.0; Na2CO3, 10.0; Agar, 20. Na2CO3 was sterilized separately and added to the medium just before solidification giving a final pH of about 10.0 (Aftab et al., 2006). This strain was maintained on alkaline starch nitrate medium (g/l): starch, 20.0g; KNO3, 2.0g; K2HPO4, 1.0g; Mg SO4.7H2O, 0.5g; NaCl, 0.5g; FeSO4.7H2O, 0.01g; CaCO3, 3.0g; agar, 20.0g and distilled water 1000ml. The pH was adjusted to 9.5. For alkaline protease production cells from Streptomyces albidoflavus with CFU of about 9.6×10^8 (cells/ml) were cultivated on a production medium (g/l) as described by Rao and Narasu (2007) was: NaOH-treated depithed bagasse, 10.0; Casein, 5.0, Yeast extract, 5.0; KH2PO4, 2.0; K2HPO4, 2.0, MgSO4.7H2O, 1.0. The pH of the medium was adjusted to 9.0-9.5 and the culture was grown for 72h in a shaking incubator (170 rpm) at 37℃ after inoculation with 10% of cells/ml. At the end of fermentation period, the broth was centrifuged at 8,000 rpm, 4℃ for 20 min and the clear supernatant was used as the crude enzyme.

Enzymes Assay and Protein Measurement:

Alkaline protease activity in the cell-free culture filtrate was measured by modified method of Takami et al. (1989). Enzyme activity was determined by using casein as a substrate at a concentration of 1% (w/v) in 50mM Glycine-NaOH buffer (pH 10). The assay was carried out routinely in a mixture containing 0.5ml of a suitably diluted enzyme solution and 2.5 ml casein solution. After incubation for 1 hour at 30℃, the reaction was terminated by the addition of 2.5ml of 0.44M TCA (trichloroacetic acid) solution. After 10min the mixture was centrifuged at 8000 rpm for 10min. An aliquot of 0.5ml of supernatant was mixed with 2.5ml of 0.5M Na2CO3 and 0.5ml of Folin-Ciocalteu's phenol solution and kept for 30min at room temperature. The optical densities of the solutions were determined with respect to sample blanks at 660nm. One alkaline protease unit was identified as the amount of enzyme capable of producing 1µg of amino acid (measured as tyrosine) Protein was tested according to Lowry et al. (1951).

Effect of Different Nitrogenous Residues:

Different natural nitrogen sources (used as sole nitrogen sources) were used as sole nitrogen source for alkaline protease production from Streptomyces albidoflavus. These sources include: broad bean, soybean, bean, kidney bean, yellow lentils, cotton seed meal, baker's and fodder yeast, OFFAL and feather meal.

Taxonomic Studies of Streptomycete Isolate:

Morphological characteristics of the selected streptomycete isolate was studied by cultivating it on starch nitrate agar medium at 30 ℃ for 8 days and examined under light and scanning electron microscopy (Zeiss, West Germany, Model EM10). Physiological and biochemical characteristics: Lecithinase was conducted on egg-yolk medium according to the method of (Niith and Kutzner, 1969); Lipase (Elwan et al., 1977); Protease (Chapman, 1952); Pectinase (Hankin et al., 1971) amylase (Cowan, 1974); Catalase test (Jones, 1949); melanin pigment (Pridham, et al., 1957); degradation of Esculin and xanthine (Gordon et al., 1974); nitrate reduction (Gordon, 1966); hydrogen sulphide production and oxidase test (Cowan, 1974); the utilization of different
carbon and nitrogen sources (Pridham and Gottlieb, 1948) were also studied. Cell wall was performed by the method of (Becker et al., 1964; Lechevalier and Lechevaier, 1968). The cultural characteristics were studied in accordance with the guidelines established by the International Streptomycetes Project (Shirling and Gottlieb, 1966). Colors characteristics were assessed on the scale developed by (Kenneth and Deane, 1955). This isolate was then identified according to Szabo et al. (1975) and Bergey's manual of systematic bacteriology (1989).

Different Enzyme Applications:
Several Experiments Were Done with this Respect Including:
1. Blood Stain Removal:

   A clean piece of white cloth was soaked in blood and allowed to dry for 24 h at 60°C, then the cloth was soaked in 2% formaldehyde for 30 min. (as colour fixing agent) and washed with water to remove excess formaldehyde and then left to dry again at 60°C for 24 h. The cloth was cut into equal sizes (6.25 cm²) and they were incubated with buffered crude alkaline protease (glycine/NaOH, pH 9) of Streptomyces albidosflavus (Najafi et al., 2005). Different enzyme concentrations were used (1-5 ml). This test was carried out for 8 h. at 50°C. The same procedure has been done for control (without enzyme).

2. Degradation of Human Hair:

   Untreated Egyptian hair supplied from a barber shop was used in this experiment (Takami et al., 1992). Human hair (1 g) was suspended in 10 ml of 50 mM glycine/NaOH buffer (pH 9). Different concentrations from crude alkaline protease from S. albidosflavus (from 1-5 ml) were used provided that the total volume still constant (10 ml). The reaction mixture takes place at 50°C for 8 h.

3. Decomposition of Protein Layers on X-ray Films:

   Different crude alkaline protease concentrations from S. albidosflavus ranging from 1 to 5 ml were incubated with sheets (1x2 cm) of X-ray film in glycine/NaOH buffer (pH 9) for 2 h. at 50°C. A control has been constructed by incubating the sheet with the buffer and the experiment has been done as the sample.

4. Dissolution of Blood Clot:

   This test has been done by adding 1 ml alkaline protease from S. albidosflavus to 1 ml glycine/NaOH buffer (pH 9) containing blood clot and incubated at 50°C for 4 h. A control has been done at the same time.

5. Degradation of Coagulated Egg White:

   About 2 g of coagulated egg white were incubated with 5 ml of alkaline protease giving a total volume of 10 ml by using glycine/NaOH buffer (pH 9). The reaction mixture was incubated at 50°C for 8 h. A control was done at the same time using all the constituents except the enzyme.

6. Dehairing of Cow Skin:

   Different concentrations of crude alkaline protease from S. albidosflavus (1-5 ml) were added to cow skin (1 cm²) in glycine/NaOH buffer (pH 9) giving final volume of 10 ml and incubated at 50°C for 8 h. A control has been constructed at the same time having all the constituents except the enzyme.

Results:

Through studying the morphological properties of the selected isolate, it was found that the aerial mycelia of isolate was morphologically related to flexuous/spiral category as examined by light microscope (Fig. 1). While the mature spore mass was belonging to white series and had smooth surface as detected using TEM microscopy (Fig. 2). Moreover, the cultural properties of the chosen isolate were also studied and it was found that the isolate grew well on most of the tested organic and synthetic media. The color of the aerial and substrate mycelia varied depending on the type of the medium used. It has white color series on oat meal, yeast-malt extract and starch nitrate agar media.

The color of substrate mycelium was recorded. Diffused pigments were not observed. In addition, the physiological characteristics studies revealed that the isolate did not produce melanin pigments on peptone-yeast
extract-ion agar or tyrosine agar. It hydrolyzed starch, liquefied gelatin, reduced nitrate and relatively produced H2S. It utilized citrate and tolerated 10%NaCl. Fine growth was recorded at a temperature range of 15 to 37°C and at pH range of 5 to 9. The utilization of various carbohydrates by the selected isolate suggests a good pattern of carbon assimilation. Raffinose, rhamnose and inositol were poorly utilized while glucose, L-arabinose, D-xylose, D-galactose, mannose, D-mannitol, fructose, salicin, trehalose and sucrose sugars were well utilized. Results represented Table 1 showed the effect of different natural nitrogen sources on alkaline protease production from *Streptomyces albidoflavus* in comparison to two controls one containing yeast extract, casein and glucose and the other contains NaOH (1.5%)-treated bagasse pith instead of glucose and the other constitutes were not changed. It was found that all the used sources showed very low enzyme activity in comparison to the two controls Lima bean was the highest producer of the all tested sources (48.75U/ml and 15.38U/mg protein) followed by yellow lentils (25.83U/ml and 9.39U/mg protein) and kidney beans (18.33U/ml and 7.57U/mg protein). So, these all tested nitrogen sources were restudied in the presence of yeast extract (Table 2). It was found that the enzyme activities for all sources showed remarkable increase. In this case, Beans still had the highest enzyme activity of all tested sources (90.00U/ml and 11.18U/mg protein), followed by yellow lentils (79.58U/ml and 8.96U/mg protein) as the previous test. But cotton seed meal exhibited and increase in activity surpassing kidney bean (70.83U/ml and 7.26U/mg protein). The data in Table 3 explained alkaline protease production from the best natural nitrogen sources in the presence of NaOH-treated bagasse pith as sole carbon source. It was deduced that a slight increase in activity and productivity were achieved. On the other hand, the effect of different metal ion, in their chloride salts except silver at a concentration of 2mM, on alkaline protease production by *Streptomyces albidoflavus* was investigated (Table 4). It was distinguished that the enzyme activity was totally inhibited in the presence of Zn2+, Cu2+ and Ni2+. Fe3+, Hg2+, Ag+ and Co2+ showed a negligible enzyme activity as compared to controls (3.33, 3.33, 4.58 and 6.25U/ml, respectively). Moderate activity has been produced in case of Mn2+ and Ca2+ (57.92 and 71.67U/ml, respectively). Moreover, a little activity was found with Al3+ (14.58U/ml).

The effect of alkaline protease on different natural nitrogenous substrate was investigated using different enzyme doses and for different incubation periods. Blood-stained cloth was treated by different concentrations of alkaline protease from *Streptomyces albidoflavus* for eight hours. Results in Fig. 3 showed that the degree of blood stain removed was increased with increasing the enzyme concentration without any addition of any detergents. It was found that an enzyme concentration of 5ml (110.88U/ml) was approximately removed all the blood stain. In addition, human hair was incubated with different alkaline protease concentrations (1-5ml) from *Streptomyces albidoflavus* for 8h. at 50oC. Results in Fig. 4 investigated that with increasing the enzyme concentration the degree of hair degradation increased. An alkaline protease at concentration of 5ml showed a complete degradation of human hair, but enzyme concentration of 4ml showed the hair fibers clear softness. Moreover, the degradation of human hair was also observed using the scanning electron microscope (SEM) for the 4ml enzyme-treated sample. Human hair treated sample was first twice washed with distilled water and then subjected to SEM technique. Results in Fig. 5a revealed that with no enzyme treatment (control), the hair cuticle exhibited a mosaic structure on the hair surface. On the other hand, the 8h.-treated hair sample with 4ml alkaline protease enzyme showed that the hair cuticle was bristled up and partially peeled (Fig. 5b).

Several alkaline protease concentrations (1-5ml) from *Streptomyces albidoflavus* were incubated with X-ray film for 2h. It was found that all the enzyme concentrations were able to dissolve the gelatin layer on the X-ray film (Fig. 6). The ability of alkaline protease from the potent alkaline protease-producing isolate (*Streptomyces abidoflavus*) to degrade blood clot was tested (Fig. 7). One-milliliter enzyme was incubated with a blood clot in 1.5ml glycine/NaOH buffer (pH 9) at 50°C for 3h. and it was found that the enzyme was able to completely dissolve the blood clot. This explained the ability of this enzyme to digest some natural proteins.

Results in Fig. 8 investigated that crude alkaline protease from *Streptomyces albidoflavus* showed that it could degrade the coagulated egg white. The test has been constructed by incubating 5 ml enzyme with 2 g coagulated egg white in 5ml glycine /NaOH buffer (pH9) at 50°C for 8h. On the other hand, incubation of alkaline protease from *Streptomyces albidoflavus* at different enzyme concentrations (1-5ml, each ml has 110U) for 8h. at 50°C with cow skin, hair was removed very easily as compared to the control and the degree of dehairing increased with the increasing of enzyme concentration. Since this alkaline protease can digest collagen, the process of dehairing must be controlled to avoid reducing the quality of the leather. As against traditional chemical methods, enzymatic process yield products of improved quality and reduce the use of hazardous and polluting chemicals (Fig. 9).
**Fig. 1:** Photograph showing spore chain morphology of streptomycete isolate (1000X).

**Fig. 2:** Transmittance electron micrograph (TEM) of spores of streptomycete isolate (X 6,300).

**Fig. 3:** Blood stain removal from cloth. Alkaline protease (1&5ml) from Streptomyces albidoflavus was incubated with the buffered (glycine/NaOH) blooded cloth at 50°C for 8h.

**Fig. 4:** Human hair degradation using different alkaline protease concentrations.

**Fig. 5:** SEM of the untreated (a) and crude alkaline protease-treated human hair (b).
Fig. 6: Decomposition of X-ray films by different concentrations of crude alkaline protease from *Streptomyces albidoflavus*.

Fig. 7: Effect of crude alkaline protease from *Streptomyces albidoflavus* on the dissolution of blood clot.

Fig. 8: Degradation of coagulated egg white by crude alkaline protease from *Streptomyces albidoflavus*.

Fig. 9: Digestion of natural proteins: Removal of cow skin hair using alkaline protease from *Streptomyces albidoflavus*.

Table 1: Effect of different nitrogenous sources as sole nitrogen sources in absence of yeast extract.

<table>
<thead>
<tr>
<th>Source</th>
<th>Final pH</th>
<th>Activity (U/ml)</th>
<th>Protein (mg/ml)</th>
<th>Specific activity (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 1</td>
<td>7.45</td>
<td>107.08</td>
<td>4.04</td>
<td>26.50</td>
</tr>
<tr>
<td>Control 2</td>
<td>8.63</td>
<td>103.75</td>
<td>5.23</td>
<td>19.84</td>
</tr>
<tr>
<td>Broad bean</td>
<td>8.45</td>
<td>13.71</td>
<td>3.01</td>
<td>4.55</td>
</tr>
<tr>
<td>Soybean</td>
<td>8.28</td>
<td>7.25</td>
<td>3.59</td>
<td>2.02</td>
</tr>
<tr>
<td>Bean</td>
<td>8.58</td>
<td>48.75</td>
<td>3.17</td>
<td>15.38</td>
</tr>
<tr>
<td>Kidney bean</td>
<td>8.45</td>
<td>18.33</td>
<td>2.42</td>
<td>7.57</td>
</tr>
<tr>
<td>Yellow lentils</td>
<td>8.77</td>
<td>25.83</td>
<td>2.76</td>
<td>9.36</td>
</tr>
<tr>
<td>Cotton seed</td>
<td>8.51</td>
<td>6.67</td>
<td>3.98</td>
<td>1.68</td>
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<tr>
<td>Baker’s yeast</td>
<td>8.68</td>
<td>10.42</td>
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<td>2.97</td>
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<tr>
<td>Fodder yeast</td>
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<td>3.17</td>
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<tr>
<td>Offal</td>
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<td>9.58</td>
<td>4.32</td>
<td>2.22</td>
</tr>
<tr>
<td>Feather meal</td>
<td>8.43</td>
<td>8.75</td>
<td>5.02</td>
<td>1.74</td>
</tr>
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</table>

Control 1 (glucose+casein+yeast extract), Control 2 (treated bagasse pith+casein + yeast extract)
Table 2: Effect of different nitrogenous products as nitrogen sources in the presence of yeast extract.

<table>
<thead>
<tr>
<th>Source</th>
<th>Final pH</th>
<th>Activity (U/ml)</th>
<th>Protein (mg/ml)</th>
<th>Specific activity (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 1</td>
<td>7.45</td>
<td>107.08</td>
<td>4.04</td>
<td>26.50</td>
</tr>
<tr>
<td>Control 2</td>
<td>8.63</td>
<td>103.75</td>
<td>5.23</td>
<td>19.84</td>
</tr>
<tr>
<td>Broad bean</td>
<td>8.45</td>
<td>18.75</td>
<td>8.32</td>
<td>2.25</td>
</tr>
<tr>
<td>Soybean</td>
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<td>90.00</td>
<td>8.05</td>
<td>11.18</td>
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<td>22.50</td>
<td>8.39</td>
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<tr>
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<td>70.83</td>
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<tr>
<td>Baker's yeast</td>
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<tr>
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<td>39.58</td>
<td>9.49</td>
<td>4.17</td>
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Control1 (glucose+casein+yeast extract), Control2 (treated bagasse pith+casein + yeast extract).

Table 3: Effect of the best nitrogenous product in the presence of treated bagasse pith as a carbon source and yeast extract.

<table>
<thead>
<tr>
<th>Source</th>
<th>Final pH</th>
<th>Activity (U/ml)</th>
<th>Protein (mg/ml)</th>
<th>Specific activity (U/mg protein)</th>
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<tbody>
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<td>111.67</td>
<td>4.95</td>
<td>22.56</td>
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<td>Control 2</td>
<td>8.63</td>
<td>97.92</td>
<td>6.26</td>
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<tr>
<td>Lima bean</td>
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<td>94.17</td>
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<td>14.81</td>
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<td>Yellow lentils</td>
<td>8.71</td>
<td>80.83</td>
<td>6.37</td>
<td>12.69</td>
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<tr>
<td>Cotton seed</td>
<td>8.98</td>
<td>92.92</td>
<td>8.31</td>
<td>11.18</td>
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</tbody>
</table>

Control1 (glucose+casein+yeast extract), Control2 (treated bagasse pith+casein + yeast extract).

Table 4: Effect of different metal ions (2mM) on alkaline protease production from Streptomyces albidoflavus.

<table>
<thead>
<tr>
<th>Metal salt (2mM)</th>
<th>Final pH</th>
<th>Activity (U/ml)</th>
<th>Protein (mg/ml)</th>
<th>Specific activity (U/mg protein)</th>
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<tr>
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<td>105.42</td>
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<tr>
<td>FeCl3</td>
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<td>3.33</td>
<td>4.77</td>
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<tr>
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<td>7.26</td>
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<tr>
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<td>3.33</td>
<td>4.60</td>
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<tr>
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<td>0.00</td>
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<td>4.58</td>
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<td>8.15</td>
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Discussion:

Alkaline protease from microbial origin found a broad range of industrial applications. By studying the effect of different carbon and nitrogen sources, it was found that the optimum enzyme yield had been established in case of glucose, casein and yeast extract. Kathiresan and Mannivanan (2007) used the same constituents in addition to peptone for alkaline protease production from the coastal mangrove isolate, *Streptomyces* sp. On the other hand, Narayana and Vijayalakashi (2008) used the same constituent except casein for protease production from *Streptomyces albidoflavus*. Alkaline protease production using agroindustrial wastes has been also studied. Khosravi-Darani et al. (2008) studied the production of alkaline protease from *Bacillus* sp.2-5 using date palm as sole carbon source. Moreover, Agroindustrial wastes were also used for alkaline protease from *B. licheniformis* N-2 (Nadeem et al., 2008) and *B. circulans* (Jaswal et al., 2008). In addition, several attempts have been done for applying crude alkaline protease from the potent isolate *Streptomycyces albidoflavus*. It has been investigated that this enzyme was able to degrade several natural proteins including blood stain, cow skin hair, human hair, coagulated egg white protein, blood clots, etc. Crude alkaline protease from this isolate was able to degrade the proteinous (gelatin) layer of X-ray films. This interesting application of alkaline protease was previously studied (Ishikawa et al., 1993; Masui et al., 1999; Karadzic et al., 2004; Shankar et al., 2010). They reported the use of an alkaline protease to decompose the gelatinous layer of X-ray films from which silver and polyethylene tetrathalate film base were recovered.

Alkaline protease produced in this study was obtained using less expensive carbon source. It was produced using an agricultural waste (bagasse pith) so it is valuable to use it as a commercial source in different applications. This enzyme was used in the removal of blood stains from cloth. The enzyme showed promising results in the removal of blood stains from cloth and can also be used to remove blood from surgical instruments and this means that haem is considered as a substrate for the enzyme. These properties of the enzyme indicated the possibilities of its use in the manufacture of surgical cleaning detergent industry (Anwar and Saleemuddin 1997; Vishalakshi et al., 2009).
Crude alkaline protease from *Streptomyces albidoflavus* was found to be able to dehair buffalo skin. The enzyme approximately removed intact hairs completely from the hide after 24h incubation at 50°C. Since protease can digest collagen, the process of dehairing must be controlled to avoid reducing the quality of the leather. As against traditional chemical methods, enzymatic processes yield products of improved quality and reduce the use of hazardous and polluting chemicals (Najafi *et al*., 2005; Zambare *et al*., 2007; Sivasubramanian *et al*., 2008). Moreover, keratin has a lot of disulfide (S-S) bonds and form a mammalian outer tissues such as hair, nails, feather and horn. Keratins of various origins have different numbers of S-S bonds. Science the S-S bonds contributes to the rigidity of keratin, keratinous proteins which are bridged by a lot of S-S bonds are not easily hydrolyzed by proteolytic enzymes. Human hair generally has three types of tissues, i.e. cuticle, fibrous cortex and medulla (Baden, 1981). Each tissue is composed of keratinous protein with a half cystine content of 17-19% (Gillespie and Marshall, 1981). Human hair often causes clogging in the bathtub drains with bad odor at home and hotel. However, an effective method to remove these clogs has not been developed. So, we tried to enzymatic treatment of an alkaline protease from *Streptomyces albidoflavus* for safe degradation of human hair. Untreated Egyptian black hair supplied from barbershop was used in this experiment and it has been found that this enzyme was able to completely degrade the human hair after 14-24hr. Takami *et al*. (1992) reported that alkaline protease from alkaliphilic *Bacillus sp* no. AH-101 was able to completely degrade Japanese human hair. Finally, the ability of crude alkaline protease from the alkaliphilic isolate, *Streptomyces albioflavus*, to digest some other natural proteins was also studied. The enzyme was found to degrade the insoluble form of human blood clots as well as coagulated egg white to their soluble form. These results suggest the ability to use this enzyme in collagen extraction, waste treatment and other related applications. Ahmed *et al*. (2008) found that, after incubation of alkaline protease from *Pseudomonas aeruginosa* with blood clot and coagulated egg white, the solid form of these natural proteins were converted to their soluble forms.

References


