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ORIGINAL PAPER

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# Compatibility of alkaline xylanases from an alkaliphilic *Bacillus* NCL (87-6-10) with commercial detergents and proteases

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**Abstract** Alkaline xylanases from alkaliphilic *Bacillus* strains NCL (87-6-10) and Sam III were compared with the commercial xylanases Pulpzyme HC and Biopulp for their compatibility with detergents and proteases for laundry applications. Among the four xylanases evaluated, the enzyme from the alkaliphilic *Bacillus* strain NCL (87-6-10) was the most compatible. The enzyme retained its full activity (40 °C for 1 h) in the presence of detergents, whereas Pulpzyme HC and Sam III showed only 30% and 50% of their initial activity, respectively. Biopulp, though stable to detergents, had only marginal activity (5%)at pH 10. However, all four enzymes retained significant activity (80%) for 60 min in the presence of the proteases Alcalase and *Conidiobolus* protease. Supplementation of the enzyme enhanced the cleaning ability of the detergents.

**Keywords** Alkaliphilic *Bacillus* · Alkaline xylanase · Detergent compatibility · Protease compatibility

## Introduction

Cellulase-free xylanase finds extensive application in the pulp and paper industry as an effective adjuvant in the bio-bleaching of wood pulps. This pre-treatment lowers the consumption of bleach chemicals, such as molecular chlorine, chlorine dioxide and hydrogen peroxide, with a concomitant increase in brightness [21]. Xylanases are also used in the baking industry [11] and as animal feeds [3, 20]. However, there are no published reports on the use of xylanases in detergents, except for patents by Herbots et al. [6, 7].

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For an enzyme to be used as a detergent additive, it should be stable to such detergent components as surfactants, builders, bleaching agents, bleach activators and other formulation chemicals. In general, alkaline proteases are the standard choices for detergent enzymes with formulations ranging from household laundering to solutions for cleaning contact lenses and dentures [14]. Site-directed mutagenesis has made significant contributions in improving catalytic efficiency and stability towards pH, temperature and oxidising agents. Newer enzymes with improved properties, such as Durazym and Maxapem, have been reported [4, 5]. Of late, hydrolytic enzymes, namely alkaline amylase, cellulase and lipase, have been incorporated in detergent formulations to increase the efficiency of the washes [9, 15, 19]. Vegetable- or fruit-derived stains, such as those from mango and grasses, are difficult to remove; and coffee, tea and tobacco stains are even more challenging. Hence, it would be advantageous to incorporate alkaline xylanases along with proteases in detergent formulations to solubilise stubborn stains of plant origin. The present communication details the compatibility and potential application in detergents of the alkaline xylanases from alkaliphilic *Bacillus* sp. NCL (87-6-10).

## Materials and methods

### Materials

Media chemicals (HiMedia Laboratories, Mumbai, India), Hammarsten's casein (Sisco Chemicals, Mumbai, India), xylan (oat spelts) and 3,5-dinitrosalicylic acid (DNS); (Sigma Chemical Co., USA) were used. Alcalase and Pulpzyme HC were from Novo Industries, Denmark. Biopulp is a product of Biocon India. All other chemicals used were of analytical grade.

Wheat bran and soybean meal were obtained locally. Commercial detergents, namely Surf Excel, Surf, 501 bar soap and Rin Shakthi (Hindustan Lever, Mumbai, India), Ariel (Procter & Gamble, Mumbai, India), Nirma soap (Nirma, Ahmedabad, India), Dettol liquid soap (Reckitt Piramal, Mumbai, India), Snow White (Mehta Soap Factory, Pune, India) and Denturite (Bharucha Brothers, Mumbai, India), were purchased from a local market.

### Cultures

The two alkaliphilic *Bacillus* strains, NCL (87-6-10) and Sam III (from the National Collection of Industrial Microorganisms, National Chemical Laboratory, Pune, India, with accession numbers 2128 and 5122 respectively), were maintained on NAX slants (0.2% peptone, 0.2% beef extract, 0.1% NaCl, 1% xylan, 2.5% agar) at 10 °C. *Conidiobolus* sp. NCL (97-7-11) for protease production was maintained on MGYP slants (0.3% malt extract, 0.5% yeast extract, 0.5% peptone, 1% glucose, 2% agar) at 14 °C.

### Xylanase assay

Xylanase activity was determined at pH 10 and 40 °C by measuring the release of reducing sugars from oat spelt xylan by the DNS method [1]. One unit of xylanase activity was defined as the amount of enzyme that produced 1 μmol xylose equivalent $min^{-1}$ under the assay conditions.

### Production of xylanases from alkaliphilic *Bacillus* strains

Xylanase production from the alkaliphilic *Bacillus* strains NCL (87-6-10) and Sam III was carried out as described earlier [1, 18]. The cell-free broth was concentrated by ultrafiltration using a YM-3 membrane and precipitated with ammonium sulphate (60% saturation). The precipitate was dissolved in a minimum volume of 20 mM potassium phosphate buffer, pH 7.0, dialysed against the same buffer and used as the source of enzyme for further studies. The specific activity of this enzyme was 250 units $mg^{-1}$ protein.

### Purification of xylanases

Xylanases A and C were purified according to Balakrishnan et al. (2). The specific activity of the purified enzymes were 370.8 units $mg^{-1}$ protein and 3045 units $mg^{-1}$ protein, respectively.

### Protease assay

The protease activity was determined at pH 10 and 37 °C by the method of Kunitz [10]. One unit of protease activity was defined as the amount of enzyme resulting in an increase of 1.0 absorbance unit $ml^{-1}$ reaction mixture $min^{-1}$ at 37 °C.

### Production of protease

Protease production was carried out in a medium containing defatted soybean and MGYP, according to Ingle et al [8]. The broth was concentrated by ultrafiltration using a YM-3 membrane, precipitated with ammonium sulphate (90% saturation), dissolved in 10 mM potassium phosphate buffer, pH 7, dialysed against the same buffer and used as the source of enzyme. The activity of the crude enzyme ranged from 250 Kunitz $ml^{-1}$ to 300 Kunitz $ml^{-1}$.

### Effect of detergents on enzyme activity

Various commercial detergents, including Surf Excel, Surf, Ariel, Nirma, 501 bar soap, Snow White, Rin Shakthi, Dettol liquid soap and Denturite (7 mg $ml^{-1}$) were incubated with 15 units of the alkaliphilic *Bacillus* NCL (87-6-10) xylanase at 40 °C for 5 h. Aliquots were withdrawn at intervals of 60 min and the residual activity was determined under standard assay conditions. Enzyme samples incubated in the absence of detergents served as controls.

### Stability of xylanases to Surf Excel

Stability studies were carried out by incubating 0.1 unit of different commercial xylanases, including Pulpzyme HC, Biopulp and the alkaliphilic *Bacillus* strains NCL (87-6-10) and Sam III, with Surf Excel (7 mg $ml^{-1}$) at 40 °C for 1 h, followed by determining the residual activity under standard assay conditions.

### Effect of proteases on xylanases

The effects of the alkaline proteases Alcalase and *Conidiobolus* protease on the various xylanase preparations were determined by incubating 0.1 unit of the enzyme with 3 Kunitz of Alcalase and *Conidiobolus* protease at pH 10.0 at 40 °C for 60 min, followed by determining the residual activity under standard assay conditions. The control consisted of samples incubated in the absence of proteases. *Conidiobolus*protease at 3 Kunitz corresponded to 0.01% active enzyme by weight of composition, which was calculated from the specific activity (45 kilounits $mg^{-1}$) of the purified *C. coronatus* protease [12].

### Effect on purified xylanases

Studies at the effects of Surf Exel and alkaline proteases on purified xylanases (A, C) were carried out in a similar manner.

### Washing test with xylanase preparations

The application of xylanase as a detergent additive was evaluated on pieces of white cloth (2×2 cm) stained with tea and grass extracts. The stained cloth pieces were dried overnight at 50 °C. The soiled cloth pieces were washed with 50 ml of water containing the detergent solution (0.7 mg $ml^{-1}$) and crude or purified xylanases (0.1 unit $ml^{-1}$) for 10 min at 40 °C. Controls consisted of soiled cloth pieces without enzyme treatment. The treated and untreated samples were compared visually, to evaluate the efficacy of the enzyme treatments.

## Results and discussion

### Activity as a function of pH

The alkaline xylanase from alkaliphilic *Bacillus* sp. NCL (87-6-10) was active over a broad pH range (pH 6.0–10.0). Since the xylanase was active at alkaline pH, studies on its compatibility and its applications in detergents were undertaken. Activities of the different xylanases at alkaline pH (pH 8.0, pH 10.0) and 40 °C are shown in Table 1. The alkaliphilic xylanase from *Bacillus* NCL (87-6-10) retained 50% of its initial activity at pH 10.0, while Biopulp exhibited only 10% of its activity.

**Table 1** Comparative activities of alkaline xylanases as a function of pH

| Xylanase | Relative activity (%) | |
|---|---|---|
| | pH 8 | pH 10 |
| NCL (87-6-10) | 100 | 52 |
| Sam III | 100 | 34 |
| Pulpzyme HC | 100 | 26 |
| Biopulp | 100 | 5 |

Pulpzyme HC and Sam III showed 30–40% of their initial activities, respectively. Optimal activity at pH 10.0 at 40 °C is ideal for enzyme formulations in detergents, as most washing operations are generally carried out at lower temperatures, due to high energy costs.

## Compatibility of alkaliphilic *Bacillus* xylanase NCL (87-6-10) with commercial detergents

The enzyme showed high stability in the presence of Denturite (a detergent used for cleaning dentures; Fig. 1). The stability of xylanase in the presence of other detergents ranged from 88% to 60% for 1 h. Dettol liquid soap was most inhibitory; and the enzyme lost more than 60% of its activity within 1 h.

Thus, the alkaline xylanase from alkaliphilic *Bacillus* was compatible with a wide range of commonly used detergents. Of these, Surf Excel gave the best results. Further experiments were carried out using Surf Excel.

## Effect of Surf Excel on xylanase preparations

In order to compare the properties of the xylanase from alkaliphilic *Bacillus* NCL (87-6-10) with other xylanases, two commercially available xylanases, namely Pulpzyme HC and Biopulp, were also evaluated for their compatibility with Surf Excel. The xylanase from an isolate of alkaliphilic *Bacillus* Sam III was also included. Enzymes are generally incorporated at low concentrations (from 0.0005% to 0.5% active pure protein by weight) in detergent formulations [6, 7]. The enzymes were evaluated at a concentration of 0.1 unit $ml^{-1}$ for 1 h. Alkaliphilic *Bacillus* NCL (87-6-10) xylanase retained approximately 75% of the initial activity after 60 min, whereas Pulpzyme HC exhibited only 10% of its activity. However, Biopulp and Sam III showed 60% and 40% of their initial activities respectively (Fig. 2). These studies indicate that the xylanase from the NCL strain retained a significant amount of its activity at concentrations as low as 0.1 unit. An enzyme concentration of 0.1 unit corresponded to 0.0005% of the composition by weight of active enzyme, based on a specific activity of 3045 units $mg^{-1}$ protein [2], which lies within the desired range of 0.0005% to 0.5% by weight of active enzyme in Surf Exel.

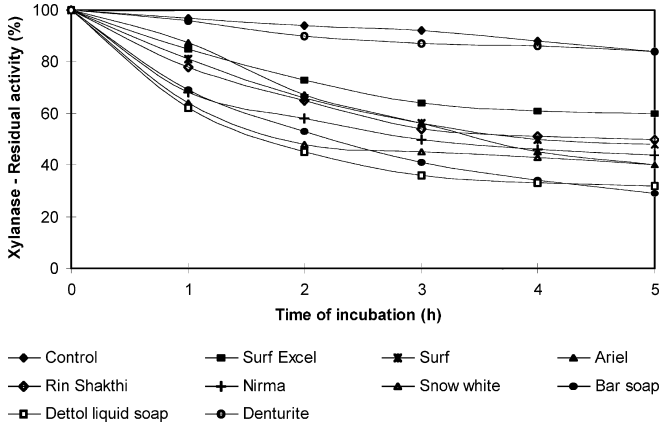


**Fig. 1** Effect of commercial detergents on alkaline xylanases from alkaliphilic *Bacillus* NCL (87-6-10). Xylanases (15 units) were incubated with detergents in water at a concentration of 7 mg $ml^{-1}$ at 40 °C for 5 h

## Xylanase activity in the presence of Surf Excel

The xylanase was assayed in the presence of xylan at two different concentrations of Surf Excel (7.0 mg $ml^{-1}$, 0.7 mg $ml^{-1}$). Suitable controls were included and the results are shown in Table 2. The enzyme is not only stable but is active in the presence of detergents, even at concentrations as low as 0.03 unit in 7 mg Surf Excel $ml^{-1}$.

## Compatibility of xylanases with proteases in detergents

To increase the efficiency of detergents, a combination of proteases, amylases, cellulases and lipases are incorporated in the detergents. Among these, proteases form an important component of most detergent formulations. It is therefore desirable to have an enzyme that is active in the presence of proteases. Hence, the action of Alcalase and *Conidiobolus* protease on the different xylanases

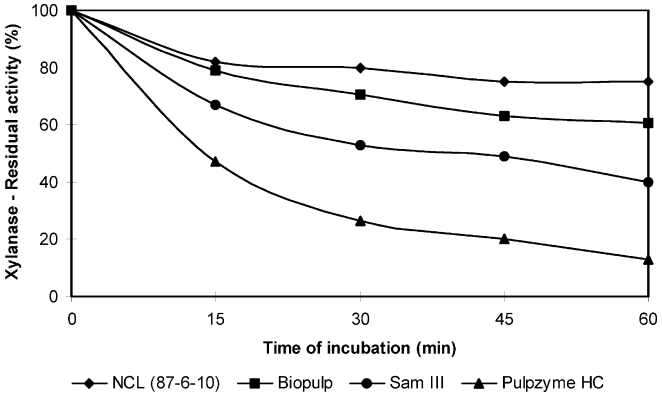


**Fig. 2** Effect of Surf Excel on xylanases. Each xylanase (0.1 unit) was incubated with Surf Excel in water at a concentration of 7 mg $ml^{-1}$ at 40 °C and assayed at times up to 1 h

**Table 2** Xylanase activity from alkaliphilic *Bacillus* NCL (87-6-10) in the presence of Surf Exel at 40 °C for 30 min

| Surf Excel detergent (mg $ml^{-1}$) | Residual activity (%) of xylanases (units $ml^{-1}$) | | |
|---|---|---|---|
| | 0.03 | 0.10 | 0.75 |
| 0 | 100 | 100 | 100 |
| 0.7 | 68 | 75 | 92 |
| 7.0 | 57 | 68 | 88 |

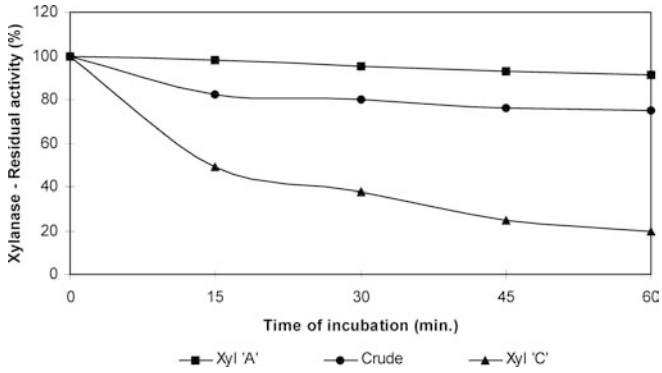


**Fig. 3** Comparison of purified xylanases A and C and crude broth from alkaliphilic *Bacillus* NCL (87-6-10), showing the compatibility of xylanases with Surf Excel

**Table 3** Compatability of purified alkaliphilic *Bacillus* xylanases with Alcalase

| Xylanase enzyme | Relative activity (%) | | |
|---|---|---|---|
| | 0 min | 30 min | 60 min |
| Crude broth | 100 | 96 | 80 |
| Xylanase A | 100 | 98 | 98 |
| Xylanase C | 100 | 70 | 68 |

was studied. *Conidiobolus* protease has been reported from this laboratory to be compatible with detergents [13]. All four alkaline xylanases evaluated showed high stability (80%) towards protease action (data not shown ). Proteases are generally added at levels from 0.0001% to 2.0% active enzyme by weight of the composition. The concentration of the *Conidiobolus* protease used in the present work, namely 3 Kunitz (equivalent to 0.01% active enzyme) falls well within the range [6, 7]. Similar observations were made by Shikata et al. [16] and Singh et al. [17] for alkaline cellulases from alkaliphilic *Bacillus* species.

## Effect on purified xylanases

Figure 3 shows the stability of the crude and purified xylanases A and C to detergents. Xylanase A was 100% active when incubated for 60 min with Surf Excel, while Xylanase C was inactivated markedly within 60 min. Fifty percent of Xylanase C was lost in 15 min when it was incubated with Surf Excel. Xylanase A was also more stable to Alcalase than xylanase C (Table 3).

## Action of xylanases on soiled cloth

Figure 4 shows the effect of enzyme supplementation to Surf Excel in the cleaning efficiency of certain stains. In order to substantiate the results obtained with the crude culture broth, a homogenous preparation of xylanase A was also used to carry out the application trials. From

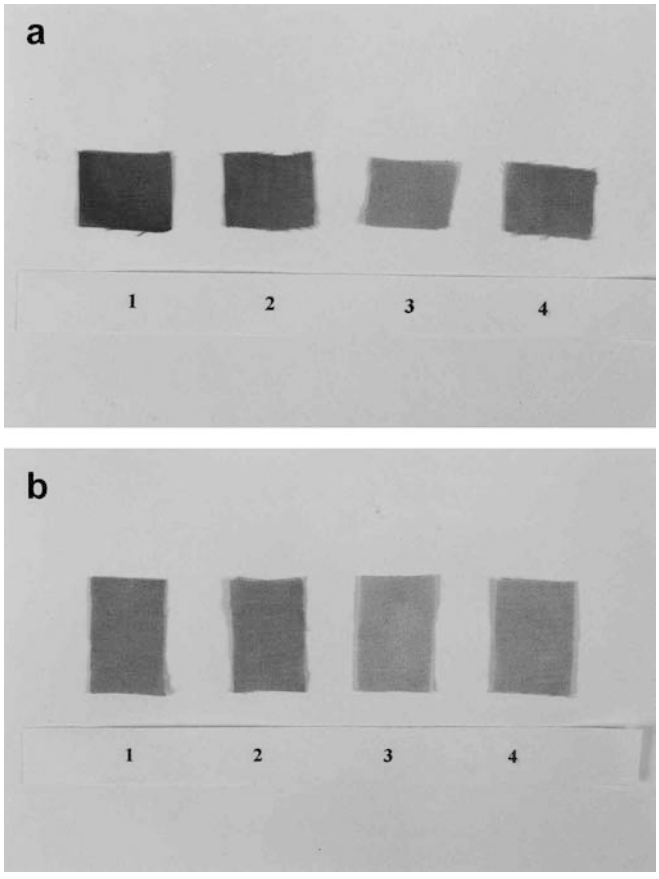


**Fig. 4** Washing performance of alkaline xylanases from alkaliphilic *Bacillus* NCL (87-6-10) in the presence of detergents on tea stains (**a**) and grass stains (**b**). *Sample 1* Untreated (control), *sample 2* washed with detergents, *sample 3* washed with detergents and crude xylanases, *sample 4* washed with detergents and pure xylanase A. Xylanase was added at 0.1 units $ml^{-1}$ in all cases

Fig. 4, it is evident that the component of the culture filtrate which gave a boost to the cleaning performance was the xylanase enzyme.

In conclusion, our studies show that the xylanases from alkaliphilic *Bacillus* sp. NCL (87-6-10) are not only compatible with a wide variety of commercial detergents of different formulations, but can also be used as a detergent additive.

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