Study of The Applications of Alkaline Protease Produced by Soil-Isolated *Bacillus cereus* FT 11

Asha B [1*, 2], Palaniswamy M[1]

Abstract

Background: Proteases, enzymes widely utilized in industries such as food, pharmaceuticals, detergents, leather, among others, represent the highest-selling enzyme category globally. Methods: This study investigates the industrial applications of an alkaline protease enzyme derived from *Bacillus cereus* FT11, isolated from soil. The enzyme, after partial purification, demonstrated significant keratinolytic activity by degrading human hair and chicken feathers. It effectively degraded various natural proteins including blood clots, meat, and coagulated egg albumin. The enzyme exhibited the capability to degrade gelatin extracted from used X-ray films, facilitating the recovery of silver, and it successfully de-haired goat skin without causing visible damage. In addition to its proteolytic properties, the enzyme proved adept at removing stubborn stains such as blood, turmeric, and plant extracts from fabrics. It displayed antibacterial activity against clinical pathogens, underscoring its potential for diverse industrial applications. Results: Collectively, these findings highlight the enzyme's suitability for a broad array of industrial processes. Its ability to degrade complex proteins, recover valuable materials, and exhibit antimicrobial properties suggests promising practical implications. Conclusion: This research underscores the enzyme's potential as a versatile tool in various industrial sectors, emphasizing its role in enhancing efficiency and sustainability in processes ranging from waste management to textile and pharmaceutical production.

Keywords: Alkaline protease, *Bacillus cereus* FT11, industrial applications, enzymatic degradation, antibacterial activity.

Significance | The study explores Bacillus cereus FT11's alkaline protease for diverse industrial applications, including keratin degradation, protein lysis, silver recovery, dehairing, stain removal, and antimicrobial activity.

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Introduction

Microbial proteases are pivotal enzymes in industrial biotechnology, prized for their diverse applications across various sectors. These enzymes, which facilitate the breakdown of proteins, represent a significant portion of the global enzyme market, with estimates suggesting they account for approximately 60% of the total market share (Gupta, Beg, & Lorenz, 2002; Rao, Tanksale, Ghatge, & Deshpande, 1998). The versatility of proteases, particularly alkaline proteases, underlies their widespread industrial use in fields such as food processing, pharmaceuticals, detergents, leather production, and waste management (Singh, Mittal, Kumar, & Mehta, 2016).

Alkaline proteases are especially valued for their stability and activity in basic conditions, making them suitable for various industrial processes. They enhance the efficiency of stain removal in detergents, facilitate hair removal in leather processing, and assist in the recovery of valuable metals from photographic and X-ray films by degrading gelatin (Govardhan & Margolin, 1995; Chellappan et al., 2011). Furthermore, these enzymes demonstrate significant potential in waste management by degrading complex protein waste materials (Sen & Satyanarayana, 1993). Among the many microbial sources of alkaline proteases, Bacillus species are prominent due to their ability to produce enzymes with desirable industrial properties. Several studies have highlighted the efficacy of Bacillus strains in producing robust alkaline proteases with

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applications ranging from waste degradation to industrial processes (Maal, Emtiazi, & Nahvi, 2009; Mothe & Reddy, 2016). The adaptability of these enzymes to extreme conditions, including high temperatures and alkaline pH, further enhances their utility (Hawumba, Theron, & Brozel, 2002; Takami, Nakamura, Aono, & Horikoshi, 1992).

This study focuses on the alkaline protease produced by *Bacillus cereus* FT11, which was isolated from soil. The objective is to evaluate the enzyme's potential in various industrial applications, including keratinolysis (the breakdown of keratin in human hair and chicken feathers), protein degradation (such as blood clots, meat, and coagulated egg albumin), gelatin degradation (from used X-ray films), dehairing of goat skin, stain removal from fabrics, and its antibacterial properties (Asha & Palaniswamy, 2018; Ahmed, Zia, & Iqbal, 2011).

The *Bacillus cereus* FT11 strain was selected for this study due to its promising preliminary activity and the known robustness of Bacillus strains in enzyme production. The research aims to assess the enzyme's performance in these various applications, providing insights into its potential to enhance industrial processes and contribute to sustainable practices (Riffel & Brandelli, 2006; Toni, Richter, Chagas, & Termignoni, 2002).

The exploration of *Bacillus cereus* FT11's alkaline protease highlights its versatility and potential for diverse industrial applications. By investigating its ability to degrade complex proteins, recover valuable materials, and exhibit antimicrobial properties, this research underscores the enzyme's role in improving efficiency and sustainability across multiple industrial sectors (Venugopal & Saramma, 2006; Mukherjee, Adhikari, & Rai, 2008).

Materials and Methods

Enzyme Production and Partial Purification

The *Bacillus cereus* FT11 strain was cultured in a protease production medium under optimized conditions as described by Chellappan et al. (2011). After cultivation, the cell-free supernatant was collected. This supernatant was then partially purified through ammonium sulfate precipitation followed by dialysis, as outlined by Rao et al. (1998). The partially purified enzyme was subsequently used for application studies.

Preparation of Enzyme for Application Studies

For the application studies, the enzyme was prepared by mixing the partially purified alkaline protease with an equal volume of Tris-HCl buffer (pH 9) (Singh, Mittal, Kumar, & Mehta, 2016).

Degradation of Human Hair and Chicken Feathers

Black human hair and white chicken feathers were collected, cleaned thoroughly, dried, and then suspended (0.1 g) in 10 mL of the enzyme reaction mixture. Control samples were prepared by suspending the materials in distilled water. The mixtures were incubated at 37°C for 6 hours. Keratin degradation was monitored through periodic protease assays and confirmed by scanning electron microscopy (SEM), following methodologies detailed by Takami, Nakamura, Aono, and Horikoshi (1992) and Masui, Fujiwara, Takagi, and Imanaka (1999).

Degradation of Natural Proteins

Proteins such as coagulated egg, raw meat, and animal blood clots were incubated with the enzyme mixture at 37°C for 6 hours. Degradation was visually assessed to determine the extent of protein breakdown (Ahmed, Zia, & Iqbal, 2011; Kalpana, Sravani, Vigneshwari, & Devi Rajeswari, 2016).

Recovery of Silver from X-Ray Films by Decomposition of Gelatin Coating

X-ray films, cut into 1 × 1 cm pieces, were incubated with the enzyme mixture at 37°C for 4 hours. Gelatin degradation was assessed through protease assays and visual examination to confirm complete hydrolysis of the gelatin layer (Fujiwara, Tsumiya, Katada, Hosobuchi, & Yamamoto, 1989; Foda et al., 2013).

Dehairing of Skin

Goat skin, cut into 2 × 2 cm pieces, was treated with the enzyme mixture at 37°C for 12 hours. The effectiveness of hair removal was evaluated after the incubation period (Mukhtar & Ul-Haq, 2008; Venugopal & Saramma, 2006).

Removal of Different Stains

Clean cotton cloth pieces (2 × 2 cm) were stained with blood, turmeric, and leaf extract stains. The stains were fixed by immersing the cloth in 2% formaldehyde for 30 minutes, then thoroughly rinsed and dried. The stained pieces were incubated with three different solutions at 37°C for 2 hours: distilled water, a 1% detergent solution, and a mixture of 1% detergent solution and enzyme mixture. After incubation, each cloth piece was rinsed in water for 2 minutes and assessed for stain removal (Rajesh et al., 2010; Devi Rajeswari, Jayaraman, & Sridharan, 2012).

Antimicrobial Activity

The antimicrobial activity of the enzyme was tested against pathogenic strains including Escherichia coli, Pseudomonas, Klebsiella, Serratia, and Proteus using the well diffusion method. The organisms were cultured on agar plates, and wells (0.8 mm) were cut into the plates. Partially purified enzyme (100 µL) was aseptically added to the wells and incubated at 37°C overnight. The development of zones of inhibition around the wells was observed to evaluate antimicrobial activity (Najafi, Deobagkar, & Deobagkar, 2005).

Results and Discussion

The degradation of hair and feathers was indicated by an increase in enzyme activity at each hourly interval. Since keratin degrades more slowly than casein, the enzyme activity increased at a slower rate (Figure 1). The enzyme responsible for degrading keratin is

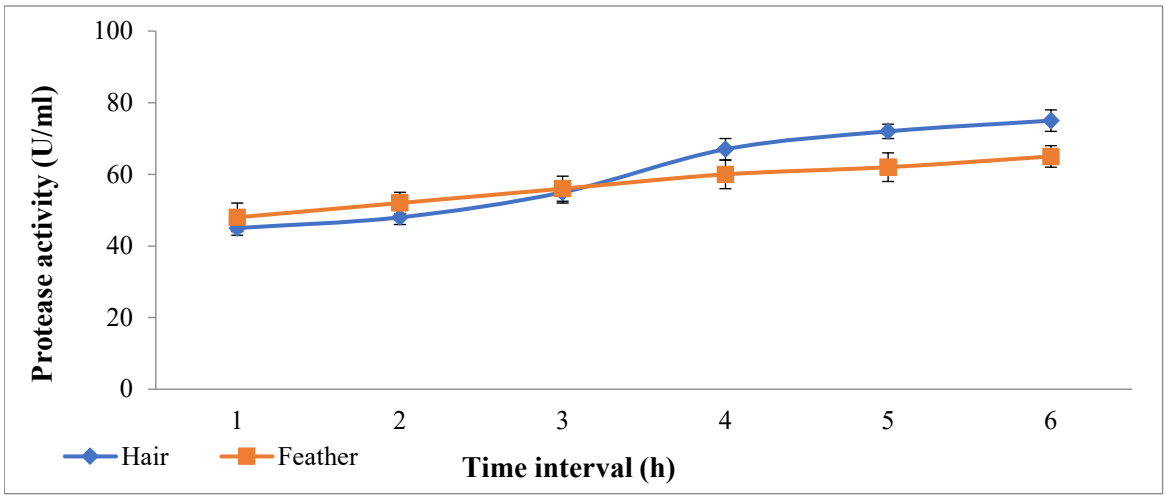

Figure 1. Enzymatic degradation of human hair and chicken feathers indicated by the increase in protease activity

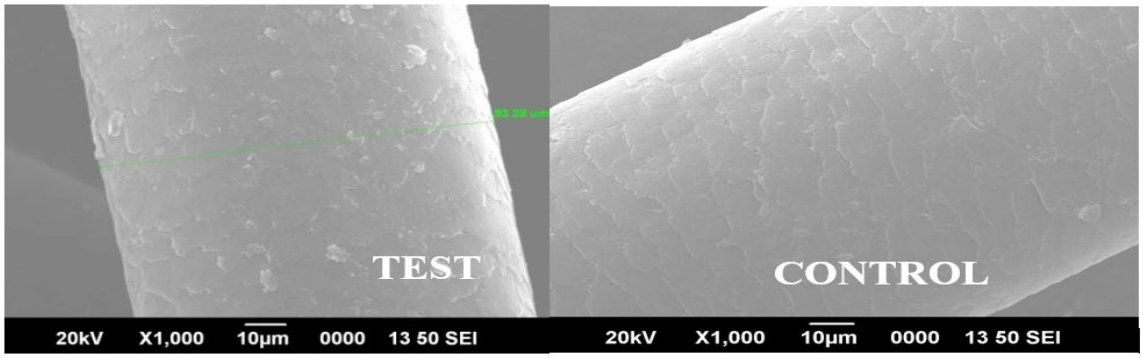

Figure 2. SEM of hair degradation

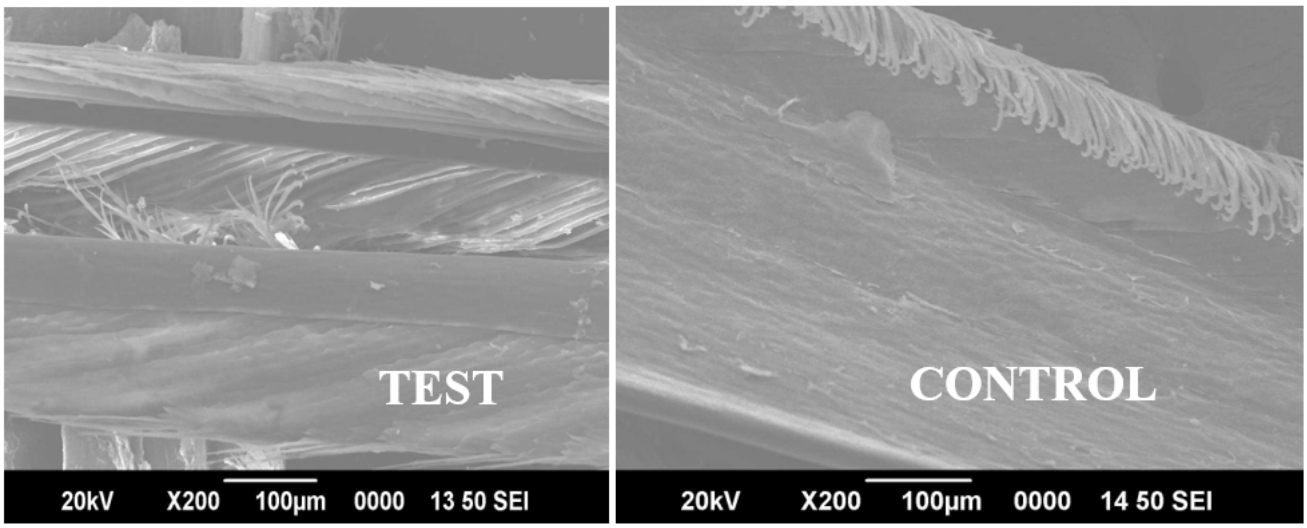

Figure 3. SEM of feather degradation

Figure 4. Lysis of blood clot

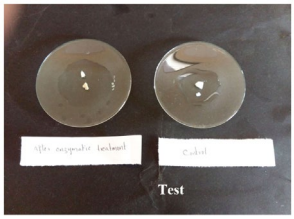

Figure 5. Degradation of coagulated egg albumin

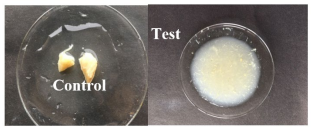

Figure 6. Degradation of meat

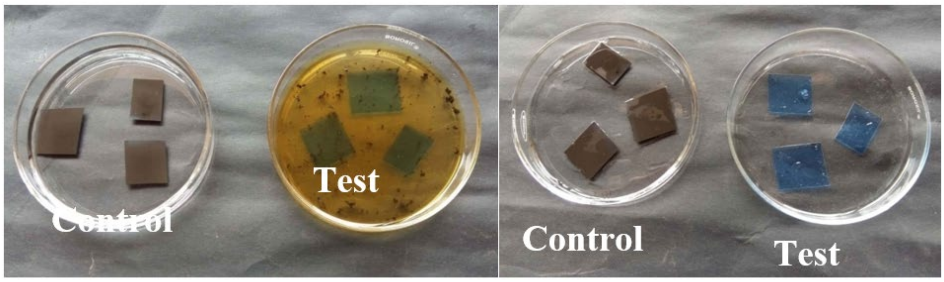

Figure 7. Enzymatic removal of surface layer of X-ray sheet

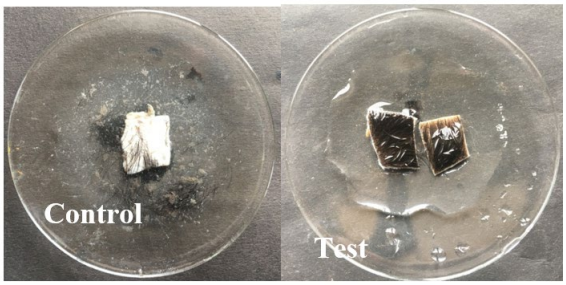

Figure 8. Dehairing of skin

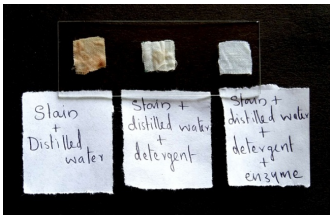

Figure 9. Blood stain removal test

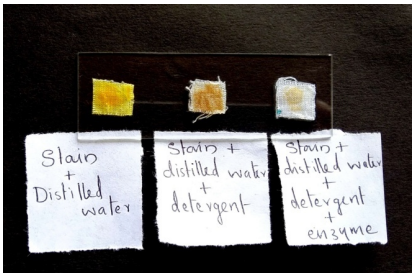

Figure 10. Turmeric stain removal test

Figure 11. Leaf extract stain removal test

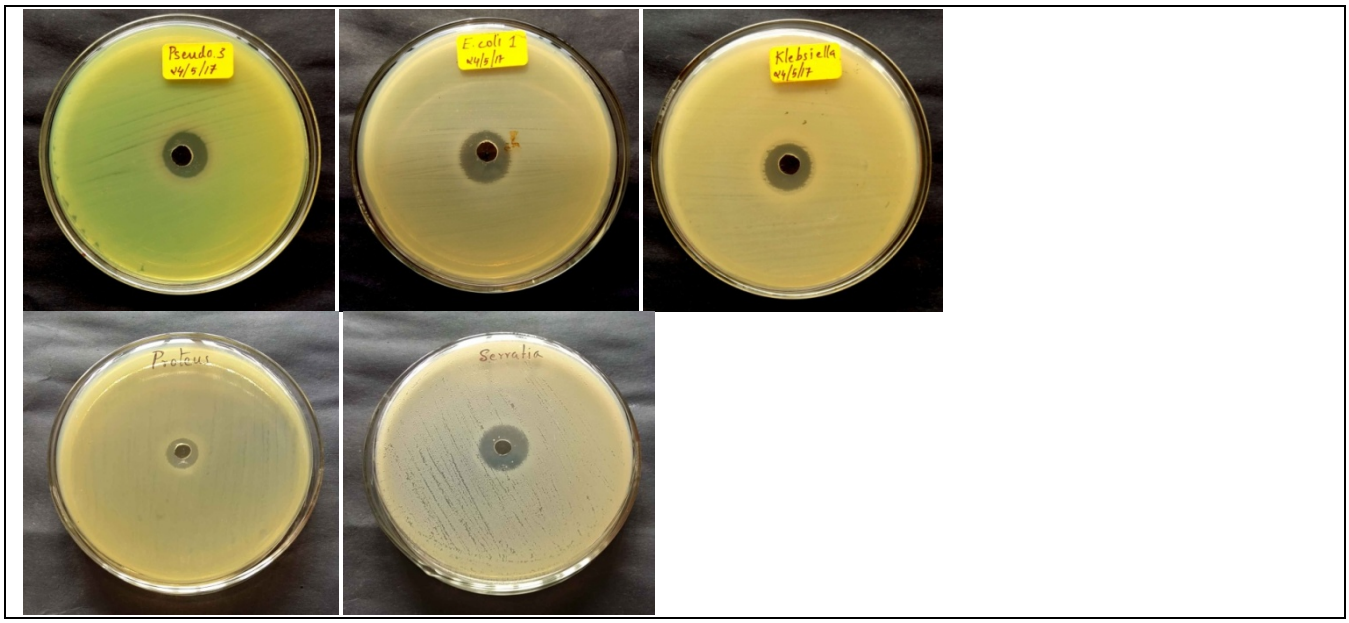

Figure 12. Bacterial strains showing sensitivity toward enzyme

keratinase, which has been produced by various microbial strains, including both Gram-positive and Gram-negative bacteria from diverse habitats. Notable keratinolytic bacteria, such as Burkholderia, Chryseobacterium, Pseudomonas, and Microbacterium, have been isolated from feather waste (Riffel & Brandelli, 2006). Additionally, Stenotrophomonas nitritireducens, isolated from soil containing deer fur, and Xanthomonas maltophila POA-1, isolated from waste feathers, have been reported for their keratinolytic activity (Toni, Richter, Chagas, & Termignoni, 2002; Yamamura et al., 2002).

Scanning electron microscopy (SEM) images showed evidence of keratin degradation. The cuticle layer of the hair shaft exhibited irregular degradation patterns compared to the control sample (Figure 2), while enzyme-treated feathers showed degradation of feather barbules compared to untreated feathers (Figure 3). Such observations of keratin degradation through SEM analysis have been previously documented and validated (Takami, Nakamura, Aono, & Horikoshi, 1992; El Zawahry et al., 2007).

Visual inspection of natural protein degradation revealed significant differences compared to control samples. Blood clots, coagulated egg, and meat showed signs of degradation within 6 hours (Figures 4, 5, 6). The enzyme effectively degraded the meat and blood clots, and although the coagulated egg was not completely degraded, signs of degradation were evident. This characteristic makes the enzyme a promising candidate for solid waste management. Similar results have been reported for various microbial strains, such as Clri strain 5468, which degraded tannery waste within 5 hours (Sreenivasagam & Rose, 2016), and Bacillus sp., which achieved complete degradation of blood clots and coagulated egg white within 20 minutes (Kalpana, Sravani, Vigneshwari, & Devi Rajeswari, 2016).

The silver in x-ray film is linked to gelatin in the emulsion layer and can be released by the action of proteolytic enzymes. The hydrolysis of gelatin in the enzyme mixture containing x-ray film was observed by the increase in turbidity in the enzyme solution. On completing the incubation period, the x-ray film was gently scratched with a toothpick and observed for the removal of the gelatin layer. The x-ray film pieces treated with enzyme showed easy removal of the layer, but the control samples retained the layer (Figure 7). The ability of gelatin layer degradation by protease enzymes with varying times of incubation are reported by many scientists. The gelatin degradation from x-ray film by the alkaline protease produced by Bacillus sp. occurred within 45 minutes (Masui, Fujiwara, Takagi, & Imanaka, 1999) and complete removal of gelatin by the alkaline protease produced by Bacillus sp. B21-1 occurred within 20 minutes (Fujiwara, Tsumiya, Katada, Hosobuchi, & Yamamoto, 1989). Alkaline protease from a Bacillus thuringiensis strain could remove the gelatin layer within 1 hour (Foda, Ali, Youssef, Kahil, Shata, & Roshdy, 2013).

After 12 hours of incubation, the skin treated with enzyme solution showed loosening of hair, which, on gentle rubbing, came out easily. But the control sample retained the hair on it (Figure 8). The test revealed the applicability of the enzyme in the leather industry for dehairing of animal skin. The same ability was reported for other bacterial proteases also. The alkaline protease from a strain of Bacillus subtilis dehaired goat skin within 12 hours of incubation, thus suggesting the applicability of the enzyme in the leather industry for the dehairing process (Mukhtar & Ul-Haq, 2008; Kalpana, Sravani, Vigneshwari, & Devi Rajeswari, 2016).

The stain removal test proved the ability of enzymes in improving the activity of detergents. The stained cloth pieces were removed from the solutions after 2 hours of incubation and washed thoroughly. The blood stain was completely removed from the cloth placed in a solution containing both detergent and enzyme, compared to the other pieces placed in detergent solution and distilled water (Figure 9). Although not completely removed, the turmeric stain and leaf extract stain were considerably lightened when placed in enzyme solution with detergent, compared to the cloth pieces placed in detergent solution and distilled water respectively (Figures 10 & 11). This ability of alkaline proteases in stain removal has been reported by multiple studies (Venugopal & Saramma, 2006; Mukherjee, Adhikari, & Rai, 2008; Ahmed, Rehman, Siddique, Hasan, Ali, & Hameed, 2016).

The alkaline protease produced by *Bacillus cereus* FT 11 showed antibacterial activity against the tested strains of Pseudomonas, E. coli, Klebsiella, Proteus, and Serratia (Figure 12). Previous research has reported the antibacterial properties of protease enzymes produced by microorganisms, including halotolerant proteases from Virgibacillus dokdonensis VIT P14 and alkaline proteases from Bacillus sp. against pathogenic strains such as E. coli, Staphylococcus aureus, and Pseudomonas aeruginosa (Devi Rajeswari, Jayaraman, & Sridharan, 2012; Kalpana, Sravani, Vigneshwari, & Devi Rajeswari, 2016).

Conclusion

Bacteria are important source of industrial enzymes. The alkaline protease produced by *Bacillus cereus* FT11 has got properties which can be made use in different industrial applications. It degraded hair and feather showing its keratin lytic activity. The enzyme also lysed blood clot, meat and egg albumin and actively recovered silver from used x-ray film by gelatin degradation. The enzyme could dehair goat skin and remove stains from cloth. It also showed antibacterial activity against clinical pathogens. Further purification of enzyme and modification of reaction steps can be followed to increase the effectiveness of the enzyme in industrial applications.

Author contributions

A.B., conceptualized and developed the methodology, P.M., prepared the original draft, collected and reviewed and edited the writing.

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Competing financial interests

The authors have no conflict of interest.

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