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ENZYMATIC POTENTIAL OF *FUSARIUM* SPECIES WITH EMPHASIS ON AMYLASE, CELLULASE, LIPASE AND PROTEASE PRODUCTION

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ABSTRACT

Fusarium is a diverse genus of filamentous fungi comprising several economically important plant pathogens. The production of extracellular hydrolytic enzymes plays an important role in fungal nutrient acquisition, substrate degradation. The present study was to evaluate the enzymatic potential of different *Fusarium* species with emphasis on four major hydrolytic enzymes, such as amylase, cellulase, lipase and protease. Different *Fusarium* species were screened for enzyme production using suitable substrate and non-substrate media. Enzymatic activity was initially assessed by observing hydrolysis zones around fungal colonies and subsequently evaluated using appropriate quantitative assays. Considerable variation in enzyme production was observed among the tested *Fusarium* species. The isolates differed in their ability to produce amylolytic, cellulolytic, lipolytic and proteolytic enzymes, indicating considerable physiological and metabolic diversity. Such variations may influence substrate utilization, ecological adaptation and pathogenic potential. The study highlights the importance of hydrolytic enzyme production in understanding the biochemical characteristics of *Fusarium* species. The enzyme-producing potential of selected isolates may also provide opportunities for further studies on enzyme characterization, optimization and potential biotechnological applications.

Keywords: *Fusarium*, Hydrolytic Enzymes, Amylase, Cellulase, Lipase, Protease, Enzyme Production.

1 INTRODUCTION

Fusarium is a large and diverse genus of filamentous fungi that occurs widely in soil, plant residues, water and association with plants as pathogens, endophytes and saprophytes. Several *Fusarium* species are economically important because of their ability to cause diseases such as vascular wilt, root rot, crown rot and fruit rot in a wide range of agricultural and horticultural crops [1]. The successful colonization of plant tissues by phytopathogenic fungi involves a complex interaction between fungal virulence factors and host defense mechanisms. Among the important fungal

factors associated with degradation and modification of host tissues are extracellular hydrolytic enzymes, which facilitate the breakdown of complex plant polymers into simpler compounds that can be utilized by the fungus [2], [3].

Plant cell walls are primarily composed of cellulose, hemicellulose, pectin and lignin, whereas storage tissues and cellular components contain starch, proteins and lipids. Fungi produce a wide range of extracellular enzymes to utilize these polymers as sources of carbon and energy. Cell wall-degrading enzymes, including cellulases, hemicellulases and pectinases, are particularly important in fungal nutrition and plant–fungus interactions [2], [4]. In addition, amylases hydrolyze starch into smaller carbohydrates, proteases degrade proteins into peptides and amino acids, while lipases catalyze the hydrolysis of triglycerides into glycerol and fatty acids. The production of these enzymes therefore reflects the metabolic versatility of fungi and their ability to exploit complex organic substrates. Amylases constitute an important group of hydrolytic enzymes that catalyze the degradation of starch and related polysaccharides. Fungal amylases have attracted considerable attention because of their extracellular secretion, relatively simple production systems and potential applications in food, textile, fermentation and other industrial processes [5]. Similarly, cellulases are a group of synergistically acting enzymes responsible for the hydrolysis of cellulose into soluble sugars. Fungal cellulolytic systems generally involve endoglucanases, cellobiohydrolases and β-glucosidases, which collectively contribute to cellulose degradation [6], [7]. *Fusarium* species have been reported to possess cellulolytic capabilities, reflecting their capacity to utilize plant-derived cellulosic materials. Lipases are another important class of hydrolytic enzymes produced by microorganisms. These enzymes hydrolyze ester bonds in water-insoluble lipids and have significant applications in food processing, detergents, pharmaceuticals, biodiesel production and biocatalysis [8], [9]. Fungal lipases are particularly attractive because many filamentous fungi can secrete substantial quantities of extracellular enzymes into the culture medium. Proteases, meanwhile, represent one of the most extensively studied groups of industrial enzymes and catalyze the hydrolysis of peptide bonds in proteins. Microbial proteases account for a major proportion of the commercially important enzyme market and are widely used in detergent, food, leather, pharmaceutical and biotechnology industries [10], [11].

The production of hydrolytic enzymes by *Fusarium* may vary considerably among species and isolates because enzyme synthesis is influenced by genetic characteristics as well as environmental and nutritional factors. Carbon and nitrogen sources, pH, temperature, incubation period, water availability and the composition of the growth substrate can substantially affect extracellular enzyme production [12], [13], Such interspecific variation is particularly important because closely related *Fusarium* species may differ in their nutritional requirements, ecological adaptations and pathogenic behavior. Therefore, comparative evaluation of enzyme production can provide useful information regarding the physiological and metabolic diversity of different *Fusarium* species. Hydrolytic enzymes also have considerable significance in plant pathology. The degradation of structural and storage components of plant tissues can facilitate fungal penetration, colonization and nutrient acquisition. However, the contribution of individual enzymes to pathogenicity is complex and may differ according to the pathogen, host and infection stage. Some extracellular enzymes function primarily in nutrient acquisition, whereas others may contribute directly or indirectly to tissue maceration and disease development [3], [4]. Consequently, studying enzyme production in different *Fusarium* species can provide insights into their biochemical capabilities and may help explain differences in their ecological fitness and pathogenic potential.

In recent years, fungal hydrolytic enzymes have also gained importance from a biotechnological perspective because they can be used for the conversion of renewable biomass into value-added products. The enzymatic degradation of agricultural residues and other lignocellulosic materials is an important component of sustainable bioprocessing and biofuel production [14]. Filamentous fungi are considered promising producers of extracellular enzymes because of their efficient secretion systems and ability to grow on inexpensive agricultural substrates. In this context, *Fusarium* species represent a potentially useful but comparatively less explored source of diverse hydrolytic enzymes. Therefore, the present study was undertaken to evaluate the enzymatic potential of different *Fusarium* species, with particular emphasis on the production of amylase, cellulase, lipase and protease. Comparative assessment of these enzymatic activities may provide valuable information on the biochemical diversity of *Fusarium* species and their ability to produce extracellular hydrolytic enzymes. The findings may contribute to a better understanding of the physiological characteristics of these fungi and may also identify promising *Fusarium* isolates for further studies related to enzyme characterization and biotechnological applications.

2 MATERIAL AND METHODS

2.1 Area of Study

The present investigation was conducted at the Seed Pathology and Fungal Biotechnology Laboratory, Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Chhatrapati Sambhajinagar. This chapter provides a detailed description of the field and laboratory procedures adopted during the course of the present investigation.

2.2 Isolation, purification and Identification of the *Fusarium* species

Fusarium species were predominantly isolated from the roots, stems, and wilted plants of various vegetable crops. A total of 10 *Fusarium* species were obtained during the isolation studies. Fungal growth was observed in all isolates within 5–7 days of incubation at 30 °C. All *Fusarium* isolates were purified using the single-spore isolation method [15]. Adequate precautions were taken to maintain the purity and viability of the isolates throughout the study.

Following the development of *Fusarium* colonies on Petri plates, macroscopic and microscopic observations were recorded. Macroscopic characteristics, including colony morphology, texture, colour, growth rate, and other external features, were examined. Microscopic characteristics, particularly the morphology and arrangement of conidia, were also studied. The combined assessment of macroscopic and microscopic characteristics was used for the preliminary and accurate identification of *Fusarium* isolates. The identification of *Fusarium* fungi was carried out with reference to relevant research papers, monographs, identification manuals, and other taxonomic literature, including Manual of Soil Fungi [16], Handbook of Soil Fungi [17], *Fusarium* Species: An Illustrated Manual for Identification [18], The *Fusarium* Laboratory Manual [1], Fuskey: *Fusarium* Interactive Key [19], and The Illustration of Fungi [20].

2.3 Hydrolytic enzymes production of *Fusarium* species (Cup-plate assay method)

2.3.1 Amylase

Amylase activity was detected using the cup-plate method [21]. For the assay, 20 mL of starch agar medium was prepared using soluble starch (10 g), disodium hydrogen phosphate (Na_2HPO_4) (2.84 g), sodium chloride (NaCl) (0.35 g), agar-agar (20 g), and distilled water (1000 mL), with the pH adjusted to 6.9. The prepared medium was dispensed into sterile Petri plates and allowed to solidify. After solidification, an 8 mm diameter well was made at the center of each plate using a No. 06 cork borer. Subsequently, 1 mL of culture filtrate (crude enzyme preparation) obtained from the respective test *Fusarium* species was aseptically transferred into the well. The inoculated plates were incubated at 28 °C for 24 h. Following incubation, the plates were flooded with Lugol's iodine solution as an indicator. Amylase activity was indicated by the formation of a distinct, clear, non-blue zone surrounding the well against the blue-coloured starch medium. The diameter of the clear zone was measured in millimeters and recorded as the amylase activity zone. A control was maintained under identical conditions, except that the culture filtrate containing the crude enzyme preparation from the test *Fusarium* isolate was not added.

2.3.2 Cellulase

Cellulase production was carried out by cultivating *Fusarium* fungi in a liquid medium containing carboxymethyl cellulose (CMC) (10 g), potassium nitrate (KNO_3) (2.5 g), potassium dihydrogen phosphate (KH_2PO_4) (1.0 g), magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$) (0.5 g), and distilled water (1000 mL), with the pH adjusted to 5.0. Fifty milliliters of the prepared medium were dispensed into 100 mL Erlenmeyer flasks and sterilized by autoclaving at 15 psi for 15–20 min. After cooling, each flask was inoculated aseptically with 1 mL of spore suspension prepared from the respective *Fusarium* species. The spore suspension was obtained from 7-day-old cultures grown on Potato Dextrose Agar (PDA) medium. The inoculated flasks were incubated at 28 °C for seven days under a diurnal light cycle. After seven days of incubation, the contents of each flask were harvested by filtration through Whatman No. 1 filter paper. The resulting filtrate was collected in pre-sterilized conical flasks and used as the crude enzyme preparation. Cellulase activity was detected using the cup-plate method [22]. The assay medium consisted of carboxymethyl cellulose (CMC) (10 g) and Difco agar (20 g). Approximately 20 mL of the medium was poured into sterile Petri plates and allowed to solidify. An 8 mm diameter well was subsequently made at the center of each plate using a pre-sterilized No. 06 cork borer. The well was filled with 0.1 mL of the culture filtrate and the plates were incubated at room temperature for 48 h. Following incubation, cellulase activity was visualized by flooding the plates with 3% lead acetate solution (10–15 mL per plate). A distinct white-coloured zone of enzymatic activity was observed around the well. The plates were subsequently washed with distilled water for approximately 30 min to remove excess lead acetate, and the diameter of the clear activity zone was measured in millimeters (mm). A control was maintained under identical conditions without the addition of culture filtrate obtained from the test *Fusarium* isolates. The observations obtained from the test plates were compared with those of the control to confirm cellulase activity.

2.3.3 Lipase

Lipase activity was assessed using the cup-plate [21], [24]. The assay medium consisted of Difco peptone (10 g), sodium chloride (NaCl) (5 g), calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$) (1.0 g), agar-agar (20 g), and 10 mL of the lipid substrate sorbitan monolaurate (Tween-20), with distilled water added to make the final volume up to 1000 mL. The pH of the medium was adjusted to 6.0. The prepared medium was dispensed into sterile Petri plates and allowed to solidify. After solidification, an 8 mm diameter well was made at the center of each plate using a No. 06 cork borer. Subsequently, 0.1 mL of the culture filtrate (crude enzyme preparation) was aseptically added to each well. The plates were incubated at 30 °C for 24 h. Following incubation, lipase activity was determined by observing the formation of a distinct clear zone surrounding the well. The diameter of the clear zone was measured in millimeters (mm) and recorded as an indicator

of lipase activity. A control was maintained under identical experimental conditions, except that the culture filtrate obtained from the test *Fusarium* isolates was not supplemented with the crude enzyme preparation. The observations from the test plates were compared with those of the control to confirm lipase activity.

2.3.4 Protease

Protease activity was assessed using the cup-plate method [25]. The assay medium was prepared by incorporating 8% gelatin into Nutrient Agar (NA). The prepared medium was autoclaved and approximately 20 mL was dispensed into sterile Petri plates and allowed to solidify. After solidification, an 8 mm diameter well was made at the center of each plate using a pre-sterilized No. 06 cork borer. Subsequently, 1 mL of culture filtrate containing crude protease was aseptically added to the well. The plates were incubated at room temperature for 24 h. Following incubation, the plates were flooded with a saturated ammonium sulfate solution. A distinct zone of clearance developed around the well within approximately 10 min. The diameter of the zone of clearance was measured in millimeters (mm) and considered indicative of protease activity, with a larger zone representing greater enzymatic activity. A control was maintained under identical experimental conditions using the corresponding culture medium without the addition of the culture filtrate. The observations obtained from the test plates were compared with those of the control to confirm protease activity.

3 RESULTS AND DISCUSSION

3.1 Hydrolytic enzymes Production

3.1.1 Production of amylase

The effect of substrate and non-substrate media on amylase production by different *Fusarium* species was investigated, and the results are presented in Table 1. Among the *Fusarium* species studied, *Fusarium pseudocircinatum* exhibited abundant amylase production in the presence of the substrate. Similarly, *F. foetens* and *F. pernambucanum* showed maximum amylase production on substrate-containing medium. In contrast, *F. concentricum, F. equiseti, F. fujikuroi,* and *F. hainanense* produced comparatively similar levels of amylase in the presence of the substrate. However, amylase production by these fungi was reduced in the absence of the substrate. In non-substrate medium, *F. pseudocircinatum* exhibited the highest amylase production compared with the other *Fusarium* species studied (Fig. 1).

3.1.2 Production of cellulase

The effect of substrate and non-substrate media on cellulase production by different *Fusarium* species was investigated, and the results are presented in Table 1. Among the species studied, *F. pernambucanum, F. incarnatum*, and *F. chlamydosporum* exhibited abundant cellulase production in the presence of the substrate. *F. fujikuroi* showed the highest cellulase production on the substrate-containing medium. In comparison, *F. annulatum, F. equiseti, F. foetens*, and *F. pseudocircinatum* exhibited similar levels of cellulase production. However, the absence of the substrate resulted in a reduction in cellulase production in most of the *Fusarium* species. In the non-substrate medium, *F. incarnatum* and *F. pseudocircinatum* exhibited comparatively higher cellulase production than the other *Fusarium* species studied (Fig. 1).

3.1.3 Production of lipase

The effect of substrate and non-substrate media on lipase production by different *Fusarium* species was investigated, and the results are summarized in Table 1. Among the species studied, *F. equiseti* exhibited abundant lipase production in the presence of the substrate. *F. chlamydosporum, F. incarnatum*, and *F. foetens* showed comparatively higher lipase production under substrate-containing conditions. Similarly, *F. annulatum, F. fujikuroi, F. hainanense,* and *F. pernambucanum* exhibited lipase production in the presence of the substrate. In the absence of the substrate, *F. chlamydosporum* recorded the highest lipase production, whereas lipase production was reduced in the other Fusarium species. The results are presented in Fig. 1.

3.1.4 Production of protease

Different *Fusarium* species were grown on substrate and non-substrate media to evaluate their protease production. Among the species studied, *F. concentricum* and *F. incarnatum* exhibited abundant protease production in the presence of the substrate compared with the other *Fusarium* species. The highest protease production was recorded in *F. chlamydosporum, F. foetens,* and *F. pseudocircinatum* when grown on substrate-containing medium. *F. equiseti* and *F. pernambucanum* showed comparable levels of protease production in the presence of the substrate. In contrast, in the absence of the substrate, *F. pernambucanum* exhibited the highest protease production, whereas protease production was reduced in the other *Fusarium* species. The results are presented in Table 1 and Fig. 1.

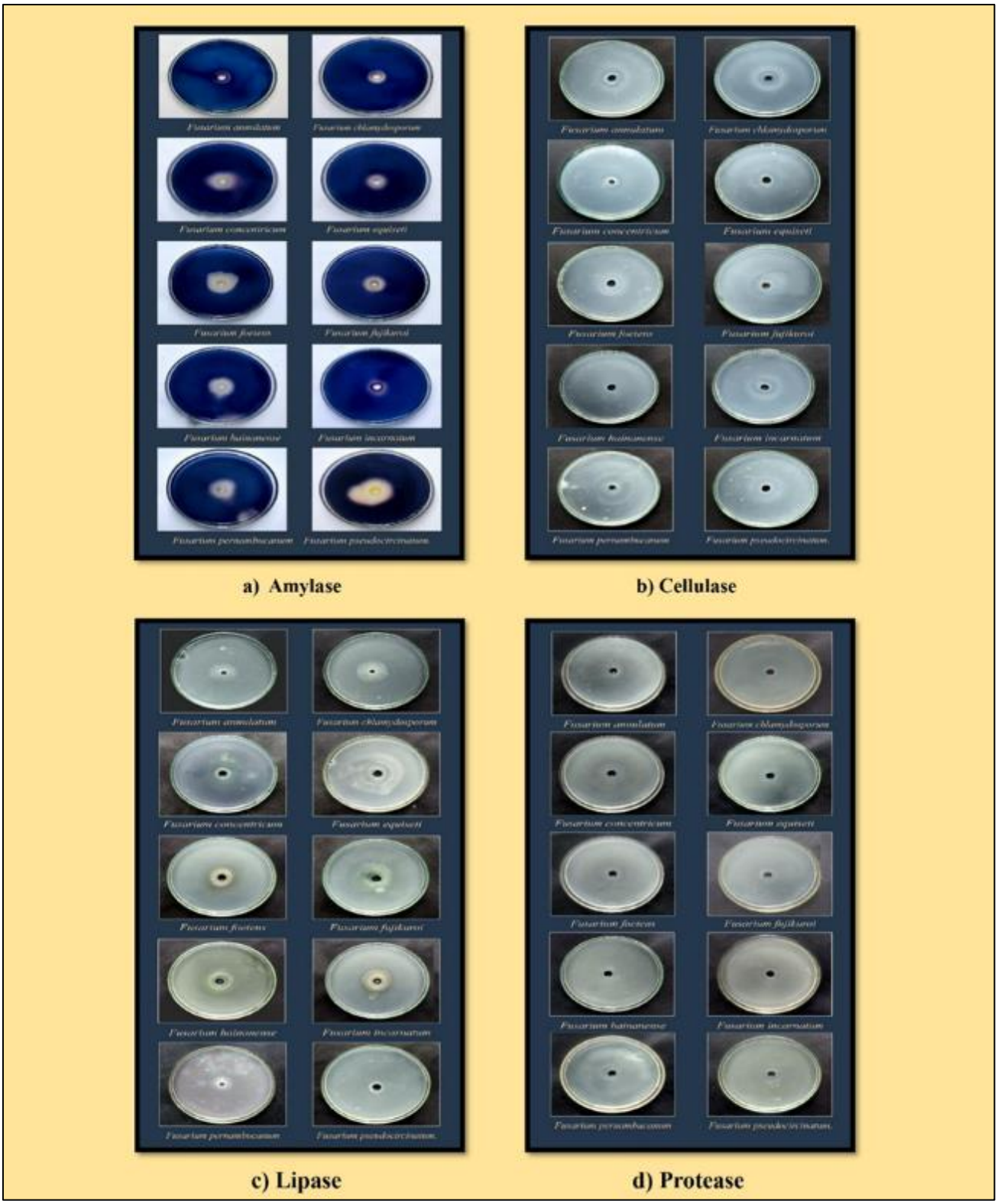

Figure 1: Production of Amylase, Cellulase, Lipase and Protease Enzyme of different Fusarium species.

Table 1: Effect of substrate and non-substrate media on hydrolytic enzyme production

Sr. No.	Name of *Fusarium* species	Amylase		Cellulase		Protease		Lipase	
		Sub	Non-sub	Sub	Non-sub	Sub	Non-sub	Sub	Non-sub
1	*F. annulatum*	13	10	15	10	12	06	12	08
2	*F. chlamydosporum*	12	08	18	12	14	08	16	12
3	*F. concentricum*	15	11	12	08	15	10	11	08
4	*F. equiseti*	14	12	15	09	11	08	20	11
5	*F. fujikuroi*	14	10	17	12	10	07	13	10
6	*F. foetens*	17	14	16	11	13	08	14	11
7	*F. hainanense*	15	11	14	10	12	07	13	10
8	*F. incarnatum*	10	06	19	13	15	06	16	11
9	*F. pernambucanum*	16	13	20	12	11	09	12	07
10	*F. pseudocircinatum*	19	15	16	13	13	08	10	06

4 CONCLUSION

The present study revealed considerable variation in the production of extracellular hydrolytic enzymes among the studied *Fusarium* species. The isolates exhibited differential abilities to produce amylase, cellulase, lipase and protease, reflecting their physiological and metabolic diversity. These enzymes may contribute to substrate degradation, nutrient acquisition and plant tissue colonization, thereby influencing the ecological adaptability and pathogenic potential of *Fusarium*. The findings demonstrate that enzymatic profiling can be useful for understanding the biochemical characteristics and functional diversity of *Fusarium* species. The results also suggest that selected high enzyme-producing isolates could serve as potential sources of extracellular enzymes for further investigation. Future studies should focus on purification and characterization of the enzymes, optimization of culture conditions, evaluation of enzyme stability and determination of their specific roles in plant–fungus interactions. Such investigations may contribute to a better understanding of *Fusarium* biology and may also reveal promising opportunities for the utilization of *Fusarium*-derived enzymes in agricultural, industrial and other biotechnological applications

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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