

COLONY DYNAMICS AND PECTIN DEGRADATION EFFICIENCY OF PECTINOLYTIC BACTERIA INVOLVED IN JUTE RETTING DURING STORAGE IN SUGARCANE BAGASSE

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ABSTRACT

The present study evaluated colony population dynamics and pectin degradation efficiency of six bacterial isolates (D_4N_{16} , D_5M_4 , D_5M_5 , D_5R_2 , D_5R_{15} , and MCG_4) over a 120-day storage period. Significant variations were observed among isolates and across storage durations. Colony numbers were highest at 15 DAS, with D_4N_{16} (294) and D_5R_2 (287) showing superior survival, while all isolates gradually declined with time, reaching lowest counts at 120 DAS (ranging from 28 in D_5M_4 to 108 in D_4N_{16}). Pectin degradation activity also showed a decreasing trend over time, with maximum activity recorded at 15 DAS (29.00 in D_5M_5), followed by a gradual reduction across isolates. Among tested strains, D_4N_{16} and D_5R_2 maintained relatively higher colony counts and degradation activity, suggesting their potential stability in sugarcane bagasse as a carrier system. These findings highlight sugarcane bagasse as a sustainable and effective medium for bacterial storage, with implications for its application in rapid jute retting.

Key words: Pectinolytic bacteria, storage period, sugarcane bagasse

Introduction

Pectinolytic bacteria play a vital role in the degradation of pectin, a major structural polysaccharide of plant cell walls, and are widely used in agriculture, food processing, textile, paper, and biofuel industries (Kashyap *et al.*, 2001; Jayani *et al.*, 2005). Their ability to hydrolyze complex pectin polymers into simpler molecules makes them suitable candidates for biotechnological applications such as retting of fibers, clarification of fruit juices, and composting of agricultural residues (Pedrolli *et al.*, 2009). However, the effectiveness of these bacterial strains largely depends on their viability and enzymatic activity during storage and delivery, which necessitates the use of efficient carrier materials. Sugarcane bagasse, a lignocellulosic by-product of the sugar industry, has recently gained attention as a low-cost and eco-friendly carrier material due to its high availability, porous structure, and organic composition (Pandey *et al.*, 2000; Rodríguez-Zúñiga *et al.*, 2011). Utilizing bagasse as a microbial carrier not only reduces environmental pollution caused by agro-industrial waste but also enhances the long-term survival of beneficial microbes by providing a protective microenvironment (Singh *et al.*, 2014). Carrier-based formulations are particularly important for pectinolytic bacteria intended for agricultural use, where their pectin-degrading activity can improve soil organic matter decomposition and facilitate nutrient cycling (Sharma and Satyanarayana, 2016). Despite the potential, bacterial survival and enzymatic efficiency often decline during extended storage due to nutrient depletion, desiccation, and metabolic stress (Vassilev *et al.*, 2015). Therefore, understanding colony dynamics and pectin degradation patterns during storage is essential for identifying stable and efficient strains. In this context, the present study was conducted to evaluate the survival and pectinolytic activity of different bacterial isolates using sugarcane bagasse as a carrier material over a 120-day storage period.

Materials and Methods

The experiment was conducted at Basic and Applied Research on Jute (BARJ) Project, Bangladesh Jute Research Institute, Dhaka during January to June, 2023. Six pectinolytic bacterial isolates, namely

D₄N₁₆, D₅M₄, D₅M₅, D₅R₂, D₅R₁₅, and MCG₄ were evaluated using sugarcane bagasse as a carrier material under ambient storage conditions. The bagasse was air-dried, sterilized, and used as a substrate for bacterial inoculation following the procedure of Singh *et al.* (2014). Inoculated samples were stored for 120 days, and bacterial viability was assessed at 15, 30, 45, 60, 90, and 120 days after storage (DAS). Colony-forming units (CFU) were determined using the standard serial dilution and plate count method on nutrient agar medium (Cappuccino and Sherman, 2014). Pectin degradation activity was evaluated at the same storage intervals by culturing isolates on pectin-supplemented medium, and the extent of degradation was quantified by measuring the clear zone diameter surrounding colonies, expressed in millimeters (Jayani *et al.*, 2005). The experiment was laid out in a completely randomized design (CRD) with three replications. Data were analyzed statistically using analysis of variance (ANOVA), and treatment means were compared using the least significant difference (LSD) test at the 5% level of significance. The coefficient of variation (CV) was also calculated to assess the precision of experimental results (Gomez and Gomez, 1984).

Results and Discussion

Colony dynamics of pectinolytic bacteria in sugarcane bagasse

The number of bacterial colonies declined progressively with storage duration across all isolates, though the extent varied (Table 1). At 15 DAS, the highest colony counts were recorded in D₄N₁₆ (294) and D₅R₂ (287), followed by MCG₄ (250), while the lowest was in D₅M₄ (62). Colony numbers decreased steadily up to 120 DAS, with final counts ranging from 108 in D₄N₁₆ to 28 in D₅M₄. The significant reduction over time (LSD = 19.294-7.2818 across periods) highlights the impact of prolonged storage on bacterial survival. Similar reductions in viability of microbial inoculants during storage have been reported previously, often due to nutrient depletion, desiccation stress, and loss of metabolic activity (Vassilev *et al.*, 2015; Herrmann and Lesueur, 2013). Among isolates, D₄N₁₆ and D₅R₂ demonstrated superior survival stability, maintaining >100 colonies even after 120 days. Such resilience may be attributed to strain-specific physiological adaptations or efficient utilization of bagasse-derived carbon sources (Rodríguez-Zúñiga *et al.*, 2011). Conversely, D₅M₄ and D₅R₁₅ exhibited sharp declines, suggesting limited storage compatibility in sugarcane bagasse. The coefficient of variation (CV) increased with storage time (5.39% at 15 DAS to 12.30% at 120 DAS), indicating greater heterogeneity in survival among isolates at later stages.

Table 1. Number of bacterial colonies of different pectinolytic bacteria at different storage period in Sugarcane bagasse as career material

Bacteria	Days after storage (DAS)					
	15 DAS	30 DAS	45 DAS	60 DAS	90 DAS	120 DAS
D ₄ N ₁₆	294 de	193h	145g	136de	120c	108a
D ₅ M ₄	62 n	48n	46op	42nop	37no	28kl
D ₅ M ₅	194j	180h	153fg	125e	102ef	73c
D ₅ R ₂	287def	187h	166ef	142d	115cd	84b
D ₅ R ₁₅	175j	157i	98jk	63hijk	29op	30jkl
MCG ₄	250h	127j	125h	108f	66ijk	54de
CV	5.39	5.09	5.95	8.45	9.36	12.30
LSD	19.294	14.930	13.085	12.764	10.438	7.2818

Note: D₄N₁₆: *Aeromonas veronii*, D₅M₄: *Bacillus megaterium*, D₅M₅: *Aeromonas jandaei*, D₅R₂: *Bacillus subtilis*, D₅R₁₅: *Bacillus megaterium*, MCG₄: *Bacillus cereus*

Pectin degradation activity during storage

Pectin degradation activity also followed a declining trend across storage periods (Table 2). At 15 DAS, the highest activity (29.00) was observed in D₅M₅, while the lowest (24.00) was in MCG₄. As storage

progressed, activities decreased significantly (LSD = 1.7117–1.4815), with the lowest activity (18.00) recorded in D5M5 at 120 DAS. D4N16 and D5R2 retained moderate activity levels (24.00 and 20.00, respectively) even at 120 DAS, reflecting their better functional stability compared to other isolates. The observed reduction in pectin degradation efficiency with time is consistent with earlier findings that microbial enzyme activity diminishes during long-term storage due to loss of cell viability and decline in enzyme secretion (Sharma and Satyanarayana, 2016; Vassilev *et al.*, 2015). Notably, D5M5, despite showing the highest initial pectinase activity, exhibited a steep decline, suggesting that high initial performance does not necessarily correlate with long-term stability. This aligns with the notion that strain selection for bioformulation should prioritize both initial activity and storage resilience (Singh *et al.*, 2014). Implications of sugarcane bagasse as a carrier material. Overall, sugarcane bagasse demonstrated considerable potential as a carrier for pectinolytic bacteria, sustaining viable colonies and functional activity for up to 120 days. Its porous structure and organic composition likely provided a protective microenvironment, slowing desiccation and nutrient loss (Pandey *et al.*, 2000).

Table 2. Pectin degradation of different pectinolytic bacteria at different storage period as carrier material

Bacteria	Days after storage (DAS)					
	15 DAS	30 DAS	45 DAS	60 DAS	90 DAS	120 DAS
D ₄ N ₁₆	27.00kl	27.00ij	26.00lm	26.00ij	25.00h	24.00e
D ₅ M ₄	28.00jk	25.00k	25.00mn	23.00lm	20.00k	22.00f
D ₅ M ₅	29.00ij	25.667jk	25.00mn	25.00jk	22.00ij	18.00h
D ₅ R ₂	27.00kl	27.00ij	26.00lm	24.00kl	23.00i	20.00g
D ₅ R ₁₅	26.00lm	25.00k	22.00p	21.00n	20.00k	20.00g
MCG ₄	24.00no	24.00kl	23.00op	23.00lm	23.00i	22.00f
CV	3.26	3.43	2.79	3.14	2.89	4.19
LSD	1.7117	1.7169	1.3059	1.3906	1.2116	1.4815

Note: D₄N₁₆: *Aeromonas veronii*, D₅M₄: *Bacillus megaterium*, D₅M₅: *Aeromonas jandaei*, D₅R₂: *Bacillus subtilis*, D₅R₁₅: *Bacillus megaterium*, MCG₄: *Bacillus cereus*

However, variability among isolates indicates that compatibility depends on bacterial physiology and metabolic adaptability. Similar conclusions were reported by Mishra and Arora (2012), who emphasized that lignocellulosic carriers improve microbial inoculant shelf life but require strain-specific optimization. These findings suggest that D4N16 and D5R2 are promising candidates for long-term storage and potential application in jute retting or agro-industrial processes (Herrmann and Lesueur, 2013).

Conclusion

Sugarcane bagasse proved to be an effective carrier material for pectinolytic bacteria, sustaining colony viability and pectin degradation activity for up to 120 days. Among the isolates, D₄N₁₆ and D₅R₂ exhibited superior stability.

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