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Comparative growth kinetics of functional bacterial isolates with and without protectants for enhanced bioinoculant formulation

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Abstract

The present investigation was carried out in College of Agriculture, Raichur, Department of Microbiology evaluated the growth kinetics of six efficient bacterial isolates involved in nitrogen fixation and mineral solubilization under controlled conditions with and without protectants. Growth was assessed over six days and expressed as log CFU ml⁻¹. All isolates exhibited a typical sigmoidal growth pattern with peak population on the fourth day. The inclusion of protectants significantly improved microbial growth, survival, and stability. Statistical analysis (ANOVA) indicated significant differences ($p < 0.05$) between treatments. The results highlight the importance of protectants in enhancing microbial viability and provide a basis for developing stable microbial consortia.

Keywords: PGPR, growth kinetics, protectants, bioinoculants, CFU, microbial consortium

1. Introduction

Sustainable agriculture increasingly relies on environmentally friendly approaches to enhance crop productivity while maintaining soil health. Among these, plant growth-promoting rhizobacteria (PGPR) have gained significant attention due to their ability to improve nutrient availability, stimulate plant growth, and mitigate abiotic stresses. Functional bacterial groups such as nitrogen-fixing bacteria and mineral-solubilizing microorganisms play a vital role in nutrient cycling by converting unavailable forms of nutrients into plant-accessible forms. These beneficial microbes are widely utilized as bioinoculants, offering an eco-friendly alternative to chemical fertilizers.

Despite their potential, the field performance of microbial inoculants often remains inconsistent. One of the primary challenges limiting their effectiveness is poor survival and stability during storage and after application in soil. Environmental stresses such as desiccation, temperature fluctuations, and osmotic imbalance significantly reduce microbial viability. Therefore, improving the shelf-life and functional efficiency of bioinoculants has become a critical area of research. In this context, the use of protectants—substances that enhance microbial survival by providing physical and physiological protection—has emerged as a promising strategy. Protectants such as glycerol, trehalose, skim milk, and polymer-based carriers help maintain cell integrity, reduce oxidative stress, and improve overall microbial stability.

Understanding microbial growth kinetics is fundamental for developing efficient bioinoculant formulations. Growth kinetics studies provide insights into the lag, exponential, stationary, and decline phases of microbial populations under specific conditions. These studies help determine the optimal incubation period for maximum cell density, which is essential for large-scale production and formulation of microbial inoculants. Typically, bacterial growth follows a sigmoidal pattern, characterized by an initial adaptation phase followed by rapid multiplication and eventual stabilization. Monitoring growth in terms of colony-forming units (CFU) expressed as log CFU ml⁻¹ allows for precise quantification and comparison of microbial performance under different treatments.

The incorporation of protectants during microbial cultivation and formulation can significantly influence growth kinetics by enhancing cell proliferation and delaying the decline phase. Protectants not only improve survival rates but also help maintain metabolic activity, thereby ensuring the functional efficacy of microbial isolates. Comparative studies evaluating growth with and without protectants are essential to identify the most suitable formulation strategies for bioinoculant development.

In recent years, the concept of microbial consortia—combinations of compatible and functionally diverse microorganisms—has gained importance for improving crop productivity. Such consortia often exhibit synergistic effects, leading to enhanced nutrient availability and stress tolerance in plants. However, maintaining the viability and stability of multiple microbial strains in a single formulation presents additional challenges, further emphasizing the need for optimized growth conditions and protective additives.

Therefore, the present study was undertaken to evaluate the comparative growth kinetics of selected efficient bacterial isolates involved in nitrogen fixation and mineral solubilization, under conditions with and without protectants. The findings aim to provide a scientific basis for improving bioinoculant formulation strategies and enhancing the field performance of beneficial microbial consortia.

2. Materials and Methods

2.1 Growth kinetics of the efficient bacterial isolates

Population of each microbial strain of three nitrogen fixing bacteria, phosphate solubilizing bacteria, potassium solubilizing bacteria, zinc solubilizing bacteria and silicon solubilizing bacteria was assessed at regular interval to know maximum population of each microbial strain in

specific medium. The growth media namely Waksman 77, Sodium malate, Beijerinckia specific medium, Pikovaskaya, Alekshandrov, TRIS minimal and Silicate solubilizing media broths were used for their respective organisms identified. To all the flasks containing specific growth medium of the respective microbial strain, a 24 hrs grown culture of microbial strain was inoculated separately each @ 5% (v/v). While one set of flask with the chosen medium without any inoculation served as control for respective microbial strains. The flasks were incubated under shaking condition at 28 ± 2 °C and growth was measured in terms of viable cell count.

2.2 Viable population of efficient bacterial isolates by the plate count method with and without protectants

One gram of sodium alginate beads or one ml of liquid formulation and 10 g of carrier based formulations were aseptically transferred separately to 99 ml and 90 ml sterile water blanks respectively and serially diluted up to 10^{-10} dilution. One ml from each of 10^{-8} , 10^{-9} and 10^{-10} dilutions were used for initial and first month count and 10^{-6} , 10^{-7} , 10^{-8} , 10^{-9} dilutions were taken from second month estimations and inoculated onto the plates. Three replicates for each dilution were maintained. Sterilized and cooled media of Waksman 77, Sodium malate, Pikovaskaya, Alekshandrov, TRIS minimal and Silicate solubilizing medium were poured into the respective labelled plates, and the plates were swirled clockwise and anticlockwise for uniform mixing of inoculum and were kept for solidification. After inoculation plates were kept in an incubator at 28 ± 2 °C for 2-6 days. The colony forming units on each plate of different microbial strains were counted and the population was calculated using the formula.

$$\text{Population per ml} = \frac{\text{Number of colony forming units (CFU)} \times \text{Dilution factor Viable}}{\text{Weight of sample taken (g)}}$$

3. Results and Discussion

3.1 Growth kinetics of the efficient bacterial isolate

3.1.1 Growth kinetics of the efficient bacterial isolates without protectants

All the seven bacteria were assessed for their growth in their respective specific broth medium AFS 12, NFB 11, MPSB 3, MKSB 13, MZSB 7 and MSSB 23. The growth was measured in terms of CFU/ml and is presented in table 1 to 6 and figure 1.

The growth kinetics of the efficient microbial isolates were monitored in their respective optimized broth media up to six days of incubation, and the viable cell counts were expressed as CFU ml⁻¹ and their corresponding log values (Table 15-20).

The isolate AFS 12 recorded a gradual increase in population from 1.53×10^6 CFU ml⁻¹ (log 6.18) on day 1 to a maximum of 7.24×10^{10} CFU ml⁻¹ (log 10.86) on day 4, after which a slight decline was observed. Similarly, NFB 11 reached its peak population of 8.3×10^{10} CFU ml⁻¹ (log 10.92) on day 4, later a marginal decrease on subsequent days.

In the case of MPSB 3, the growth increased progressively reaching 4.4×10^{10} CFU ml⁻¹ (log 10.64) on day 4 and showing a slight decline thereafter. MKSB 13 also exhibited similar growth kinetics, with maximum population of $4.7 \times$

10^{10} CFU ml⁻¹ (log 10.67) on day 4, followed by a gradual reduction in viable count.

The isolate MZSB 7 recorded its highest viable count on day 4 (5.2×10^{10} CFU ml⁻¹; log 10.72), indicating a typical stationary phase after 96 hours of incubation. Among all the isolates, MSSB 23 exhibited a slower growth rate attaining a peak of 7.4×10^8 CFU ml⁻¹ (log 9.54) on day 4 which declined slightly thereafter.

Overall, all isolates followed a similar growth pattern with a lag phase up to day 2, an exponential phase up to day 4 and a stationary or early decline phase beyond day 4. These results indicate that the fourth day of incubation represents the optimum growth period for all the tested isolates in their respective medium under unprotected conditions.

Table 1: Growth kinetics of AFS 12 in Waksman 77 broth without protectants

Number of days	CFU ml ⁻¹	Log Value
Day 1	1.53×10^6	6.18
Day 2	7.12×10^7	7.85
Day 3	8.73×10^9	9.94
Day 4	7.24×10^{10}	10.86
Day 5	5.43×10^{10}	10.73
Day 6	4.41×10^{10}	10.64

Table 2: Growth kinetics of NFB 11 in sodium malate broth without protectants

Number of days	CFU ml ⁻¹	Log Value
Day 1	3.2×10^7	7.51
Day 2	4.8×10^8	8.68
Day 3	5.0×10^9	9.70
Day 4	8.3×10^{10}	10.92
Day 5	4.81×10^{10}	10.68
Day 6	4.0×10^{10}	10.60

Table 3: Growth kinetics of MPSB 3 in Pikovskaya's broth without protectants

Number of days	CFU ml ⁻¹	Log Value
Day 1	2.50×10^6	6.40
Day 2	4.83×10^8	8.68
Day 3	7.90×10^8	8.90
Day 4	4.4×10^{10}	10.64
Day 5	3.2×10^{10}	10.51
Day 6	2.4×10^{10}	10.38

Table 4: Growth kinetics of MKSB 13 in Aleksandrov broth without protectants

Number of days	CFU ml ⁻¹	Log Value
Day 1	2.15×10^6	6.33
Day 2	4.58×10^9	9.66
Day 3	3.20×10^{10}	10.51
Day 4	4.7×10^{10}	10.67
Day 5	3.6×10^{10}	10.56
Day 6	2.0×10^{10}	10.30

Table 5: Growth kinetics of MZSB 7 in TRIS minimal broth without protectants

Number of days	CFU ml ⁻¹	Log Value
Day 1	3.4×10^6	6.53
Day 2	7.8×10^6	6.89
Day 3	4.1×10^7	7.61
Day 4	5.2×10^{10}	10.72
Day 5	3.55×10^{10}	10.55

The growth kinetics of all tested bacterial isolates under unprotected conditions followed a typical sigmoidal growth pattern, consisting of lag, exponential, and stationary phases. The lag phase was observed during the initial incubation period (day 1-2), followed by a rapid increase in population up to the fourth day, indicating active exponential growth. Thereafter, a decline or stabilization in growth was observed, suggesting the onset of stationary and decline phases due to nutrient depletion and accumulation of inhibitory metabolites.

Among the isolates, NFB 11 exhibited the highest growth (log CFU ~10.92), indicating its superior metabolic efficiency and adaptability under *in vitro* conditions. Other isolates such as AFS 12, MPSB 3, and MKSB 13 also showed substantial growth, although comparatively lower. The reduction in population after the fourth day is consistent with classical microbial growth dynamics, where environmental limitations restrict further proliferation (Dixon & Kahn, 2004) [2].

The observed growth pattern aligns with earlier findings on plant growth-promoting bacteria, where maximum microbial activity is achieved within a limited incubation period before entering the stationary phase (Bhattacharyya & Jha, 2012) [1]. The variability among isolates suggests differences in physiological efficiency, nutrient utilization, and

tolerance to stress conditions, which are important traits for selecting efficient bioinoculant strains (Vessey, 2003) [3].

3.1.2 Growth kinetics of the efficient bacterial isolates with protectants

All the seven bacteria (AFS 12, NFB 11, MPSB 3, MKSB 13, MZSB 7 and MSSB 23) were assessed for their growth with additives, adjuvants and surfactants in their respective specific broth medium. The growth was measured in terms of CFU/ml and is presented in table 7 to 12 and Figure 2.

The AFS 12 (N₂ fixer) grown in Waksman 77 methanol medium with protectants exhibited a rapid multiplication from 2.1×10^6 CFU ml⁻¹ (log 6.32) on day 1 to a maximum of 8.3×10^{10} CFU ml⁻¹ (log 10.92) on day 4, maintaining a comparatively higher population even on day 6 (5.21×10^{10} CFU ml⁻¹).

Similarly, NFB 11 in sodium malate broth reached its peak population of 1.01×10^{11} CFU ml⁻¹ (log 11.00) on day 4, which was slightly higher than its growth without protectants indicating the positive effect of the stabilizers on cell viability.

The isolate MPSB 3, cultured in Pikovskaya's medium, achieved a maximum count of 6.3×10^{10} CFU ml⁻¹ (log 10.80) on day 4, with only a minimal decline thereafter, suggesting effective protection and nutrient utilization.

MKSB 13 in Aleksandrov medium showed a similar trend, recording a peak viable count of 5.2×10^{10} CFU ml⁻¹ (log 10.72) on day 4, while MZSB 7 in TRIS minimal medium attained its highest growth (6.4×10^{10} CFU ml⁻¹; log 10.81) at the same time point.

Among all the isolates MSSB 23 in silicate solubilizing broth exhibited a relatively slower growth rate but still reached its maximum population (8.3×10^8 CFU ml⁻¹; log 8.92) on day 4 with a moderate decline on subsequent days. The addition of protectants significantly enhanced the growth performance of all bacterial isolates, resulting in higher viable counts and prolonged survival compared to untreated controls. All isolates exhibited improved growth, with a marked increase in population during the exponential phase and a slower decline during the stationary phase.

The isolate NFB 11 recorded the highest population (log CFU ~11.00) under protectant conditions, followed by AFS 12 and MPSB 3, indicating that protectants effectively improved microbial viability and metabolic activity. The enhanced growth can be attributed to the role of protectants in stabilizing cell membranes, reducing osmotic stress, and protecting microbial cells from adverse environmental conditions.

These findings are in agreement with previous studies reporting that protectants improve microbial survival, shelf life, and functional efficiency in bioinoculant formulations (Bashan *et al.*, 2004) [4]. Similarly, enhanced growth and stability of Azospirillum and other PGPR strains in the presence of protective agents have been documented (Hungria *et al.*, 2010) [5].

The slower decline phase observed in treated cultures indicates sustained metabolic activity and improved resistance to stress conditions, which are critical for successful field application. The ability of protectants to maintain higher microbial populations over time enhances the effectiveness of biofertilizers and supports their role in sustainable agriculture (Vessey, 2003) [3].

Table 6: Growth kinetics of MSSB 23 in silicate solubilizing broth without protectants

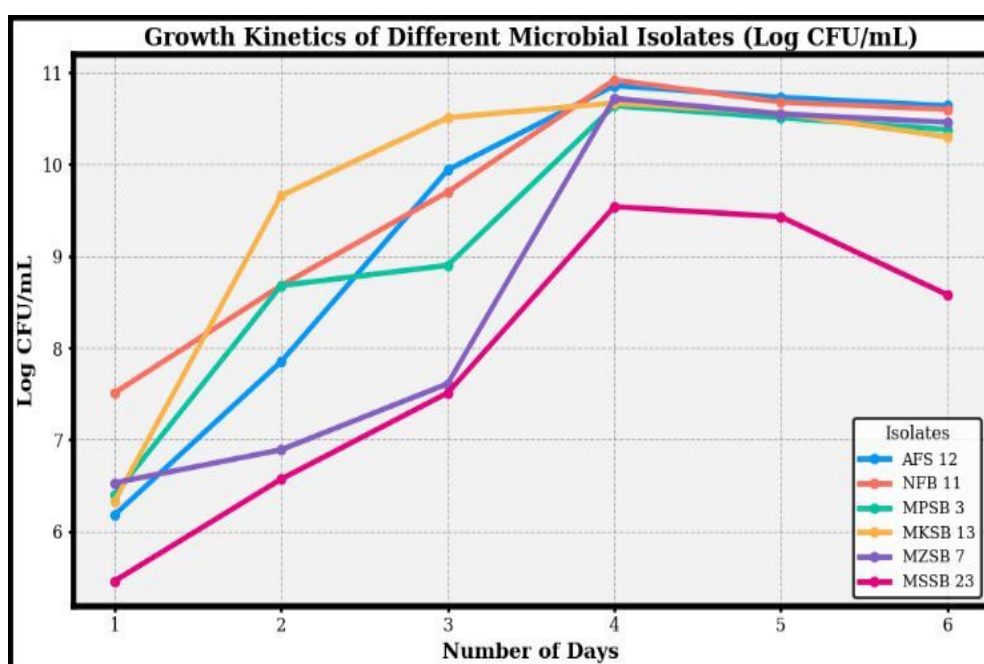
Number of days	CFU ml ⁻¹	Log Value
Day 1	2.90×10 ⁵	5.46
Day 2	3.70×10 ⁶	6.57
Day 3	3.2×10 ⁷	7.51
Day 4	7.4 ×10 ⁸	9.54
Day 5	2.7×10 ⁸	9.43
Day 6	4.1×10 ⁷	8.58

Table 7: Growth kinetics of AFS 12 with protectants in Waksman 77 broth

Number of days	CFU ml ⁻¹	Log Value
Day 1	2.1×10 ⁶	6.32
Day 2	9×10 ⁷	7.95
Day 3	10.5×10 ⁹	10.02
Day 4	8.3×10 ¹⁰	10.92
Day 5	7×10 ¹⁰	10.85
Day 6	5.21×10 ¹⁰	10.72

Table 8: Growth kinetics of NFB 11 with protectants in sodium malate broth

Number of days	CFU ml ⁻¹	Log Value
Day 1	5.3×10 ⁷	7.72
Day 2	7.4×10 ⁸	8.87
Day 3	8.2×10 ⁹	9.91
Day 4	10.1×10 ¹⁰	11.00
Day 5	6.2×10 ¹⁰	10.79
Day 6	6×10 ¹⁰	10.78

**Fig 1:** Growth kinetics of efficient bacterial isolates without protectants**Table 9:** Growth kinetics of MPSB 3 with protectants in Pikovskaya's broth

Number of days	CFU ml ⁻¹	Log Value
Day 1	4.3×10 ⁶	6.63
Day 2	6.4×10 ⁸	8.81
Day 3	9.2×10 ⁸	8.96
Day 4	6.3×10 ¹⁰	10.80
Day 5	5.1×10 ¹⁰	10.71
Day 6	3.8×10 ¹⁰	10.58

Table 11: Growth kinetics of MZSB 7 with protectants in TRIS minimal broth

Number of days	CFU ml ⁻¹	Log Value
Day 1	4.3×10 ⁶	6.63
Day 2	8.1×10 ⁶	6.91
Day 3	6.2×10 ⁷	7.79
Day 4	6.4×10 ¹⁰	10.81
Day 5	4.6×10 ¹⁰	10.66
Day 6	3.3×10 ¹⁰	10.52

Table 10: Growth kinetics of MKSB 13 with protectants in Aleksandrov broth

Number of days	CFU ml ⁻¹	Log Value
Day 1	4.2×10 ⁶	6.62
Day 2	6.34×10 ⁹	9.80
Day 3	4.4×10 ¹⁰	10.64
Day 4	5.2×10 ¹⁰	10.72
Day 5	4.8×10 ¹⁰	10.68
Day 6	3.1×10 ¹⁰	10.49

Table 12: Growth kinetics of MSSB 23 with protectants in Silicate solubilizing broth

Number of days	CFU ml ⁻¹	Log Value
Day 1	5.4×10 ⁵	5.73
Day 2	6.5×10 ⁶	6.81
Day 3	4.1×10 ⁷	7.61
Day 4	8.3×10 ⁸	8.92
Day 5	3.6×10 ⁸	8.56
Day 6	6.2×10 ⁷	7.79

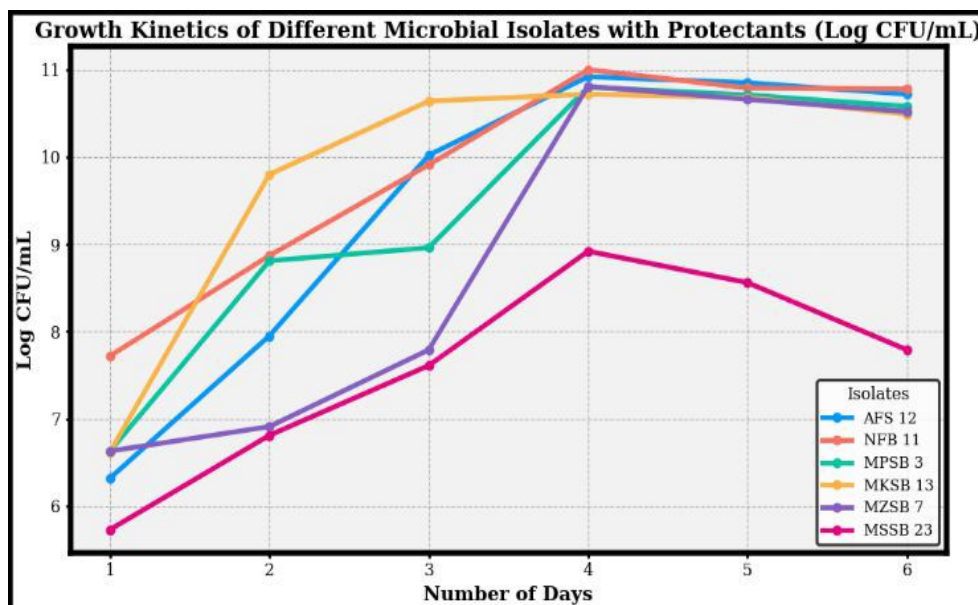


Fig. 2: Growth kinetics of efficient bacterial isolates with cell protectants

4. Conclusion

The present study clearly demonstrated that the growth kinetics of functional bacterial isolates involved in nitrogen fixation and nutrient solubilization follow a characteristic sigmoidal pattern, with the exponential phase occurring between the second and fourth day of incubation. Among the tested isolates, NFB 11 exhibited superior growth performance, followed by AFS 12 and MPSB 3, indicating their higher metabolic efficiency and adaptability under *in vitro* conditions.

Statistical analysis using two-way ANOVA revealed significant differences ($p \leq 0.05$) among isolates, incubation periods, and protectant treatments, confirming the reliability of the observed variations. The calculated Standard Error and low Coefficient of Variation further indicated good experimental precision. The incorporation of protectants significantly enhanced microbial growth, with noticeable increases in log CFU values, growth rate, and overall biomass production. The higher Area Under Growth Curve (AUGC) in treated cultures clearly demonstrated improved survival and sustained activity over time.

The enhanced performance of microbial isolates in the presence of protectants can be attributed to improved cellular stability, protection against environmental stress, and maintenance of metabolic activity. These findings are in agreement with earlier reports highlighting the role of protective substances in improving bioinoculant efficiency. The identification of efficient isolates such as NFB 11 and the demonstrated benefits of protectants provide a strong foundation for developing stable and effective microbial formulations. The study emphasizes the importance of optimizing growth conditions for large-scale biofertilizer production. Overall, this research contributes significantly to sustainable agriculture by promoting eco-friendly alternatives to chemical fertilizers and enhancing microbial-based nutrient management strategies.

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