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Research Article

Isolation and Testing of Specialized-Bacteria Winogradsky Column and Food Spoilage Decomposer

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Abstract

Food spoilage remains a major challenge in food safety, leading to significant economic losses and health risks. This study investigates the isolation and characterization of specialized bacteria from sediments of Dhan Talav, Nargol (Gujarat), using Winogradsky columns to simulate natural sediment gradients and enrich metabolically diverse microbial communities. Over seven weeks of incubation, distinct aerobic, microaerophilic, and anaerobic zones developed within the columns, with black layers indicating active sulfate-reducing bacteria. Anaerobic sediments were selectively enriched in Lyndby medium, and bacterial isolates were purified and characterized using Gram staining, morphological observation, and biochemical assays. The isolates were Gram-positive rods with metabolic traits such as positive methyl red reactions and sugar fermentation. To assess spoilage potential, isolates were inoculated into nutrient broth containing fish muscle, and microbial growth was tracked over 48 hours using OD_{600} measurements. Fish samples inoculated with the isolates showed significantly elevated OD_{600} values compared to controls, confirming spoilage activity. This study demonstrates the utility of Winogradsky columns as an effective, low-cost enrichment technique for isolating food spoilage-associated bacteria from environmental samples. By bridging environmental and food microbiology, these findings offer valuable insights for developing sustainable spoilage monitoring methods and natural preservation strategies to improve food quality and safety.

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1. INTRODUCTION

Food spoilage remains a critical concern for food safety and quality management. Microbial spoilage processes degrade proteins, carbohydrates, and lipids, producing undesirable odors and textures that reduce shelf life and consumer acceptance. While traditional preservation methods like refrigeration and chemical additives are effective, there is increasing demand for natural, sustainable alternatives. A key step in developing these is understanding the microbes responsible for spoilage.

Estuarine mudflats and pond sediments are rich in metabolically diverse bacteria involved in nutrient cycling and organic matter decomposition. These include sulfate-reducing and fermentative species that produce hydrogen sulfide and volatile fatty acids, contributing to spoilage in anaerobic or vacuum-sealed food systems. However, these bacteria are underexplored in food microbiology.

The Winogradsky column is a classic enrichment system that mimics natural sediment gradients in a controlled setting, supporting stratified growth of aerobic, microaerophilic, and anaerobic microbial communities. This study uses Winogradsky columns prepared with sediment from Dhan Talav to isolate and characterize bacteria with spoilage potential in protein-rich food systems. By linking environmental microbiology and food safety research, the work supports sustainable spoilage assessment and preservation strategies**.**

2. LITERATURE REVIEW

A. National Status

In India, the Winogradsky column has been widely used as a simple, effective enrichment system for studying microbial diversity in aquatic and soil environments. Bhatt et al. (2016) [2] explored microbial diversity in the hypersaline Sambhar Salt Lake using Winogradsky columns, demonstrating enrichment of halophilic and sulfur-cycling bacteria. Abraham et al. (2004) [1] studied bacterial populations involved in nitrogen and sulfur cycles in shrimp culture ponds in Andhra Pradesh, highlighting the presence of sulfate-reducing and fermentative bacteria under aquaculture conditions.

Dabolkar and Kamat (2021) [6] used modified Winogradsky microcosms to isolate iron- and sulfur-oxidizing bacteria from coastal sediments in Goa, showcasing their potential for nanoparticle synthesis. Sridharan et al. (2021) [14] applied Winogradsky columns to study LDPE plastic biodegradation by native soil microbes in Tamil Nadu, demonstrating the utility of these columns for enriching specialized environmental bacteria with biotechnological relevance. These studies collectively demonstrate the adaptability of the Winogradsky column in India for isolating diverse functional bacterial groups from natural habitats. However, its application to food microbiology, particularly for isolating spoilage-associated bacteria from environmental sediments, remains largely unexplored.

B. International Status

Internationally, the Winogradsky column has long been used as a classic model for understanding microbial ecology and nutrient cycling. Winogradsky himself pioneered studies on sulfur bacteria in 1887, laying the foundation for enrichment culture techniques. Fenchel and Finlay (1995) [8] described the importance of stratified microbial communities in anoxic worlds, using columns to illustrate microbial succession and metabolic diversity.

Canfield et al. (2010) [4] highlighted the role of sulfur cycling in marine systems, demonstrating the environmental relevance of sulfate-reducing bacteria frequently enriched in Winogradsky columns. Moshynets et al. (2021) [11] emphasized the column's value as a microcosm for studying microbial succession, biofilm formation, and soil colonization under controlled gradients of oxygen and sulfur. Kharashqah (2002) [10] applied the column for depth-wise analysis of microbial stratification in freshwater lake sediments in Jordan, while Rundell et al. (2014) combined Winogradsky columns with high-throughput sequencing to study community shifts and the dominance of Proteobacteria, Bacteroidetes, and Firmicutes at different depths.

3. METHODOLOGY

All experimental procedures were conducted under aseptic and controlled laboratory conditions in a single primary phase comprising microbial isolation, screening, degradation testing, and biochemical characterization.

A. Sample Collection

Sediment and pond water samples were collected from Dhan Talav, Nargol, Gujarat. Surface sediment (1–2 cups) was taken from areas rich in decaying organic matter. Samples were stored submerged in pond water in covered containers to maintain native microbial integrity during transport (Abraham et al., 2004) [1].

Fig 1: Sample collection from a dhan talav.

B. Nutrient Matrix Preparation

A nutrient-rich slurry was prepared to support microbial growth in Winogradsky columns (Winogradsky, 1887; Fenchel & Finlay, 1995) [17, 8]:

- 2 cups dechlorinated water with 20 crushed antacid tablets (calcium carbonate source)
- 1 tbsp plaster of Paris (calcium sulfate)

- 1 tbsp non-fat dry milk (protein source)
- 3 tbsp high-phosphorus liquid fertilizer
- ¼ crushed multivitamin tablet (trace elements)

Three parts clean sand were added and mixed. The pH was adjusted to 7–8 using baking soda or vinegar as needed (Ray & Bhunia, 2013) [12].

Fig 2: Winogradsky Column Assembly

C. Winogradsky Column Assembly

Three parts of the nutrient slurry were mixed with one part sediment. Pond water was added gradually to form a thick, batter-like texture. Glass jars were packed incrementally to minimize air pockets, lined with moistened shredded paper (cellulose source), and labeled. A 1.5 cm layer of pond water was added above the sediment. The bottom third of each jar was wrapped with black paper to simulate anaerobic conditions. Jars were loosely covered to allow gas exchange while preventing contamination.

D. Incubation and Monitoring

Columns were incubated in a brightly lit area at room temperature (20–30 °C) for 4–8 weeks. Evaporated water was replenished with dechlorinated water. Weekly observations recorded stratification, color changes, and odor development indicative of sulfur cycling (Canfield et al., 2010) [4].

Fig 3: Incubation. And Monitoring

E. Enrichment of Specialized Bacteria

After incubation, black anaerobic sediment layers were sampled and inoculated into sterile Lyndby medium (Dworkin et al., 2006) [7] designed for sulfate-reducing bacteria. Cultures were incubated anaerobically at 30–37 °C for 7–10 days. For solid media isolation, 1.5% agar was added, and plates were incubated anaerobically using GasPak systems.

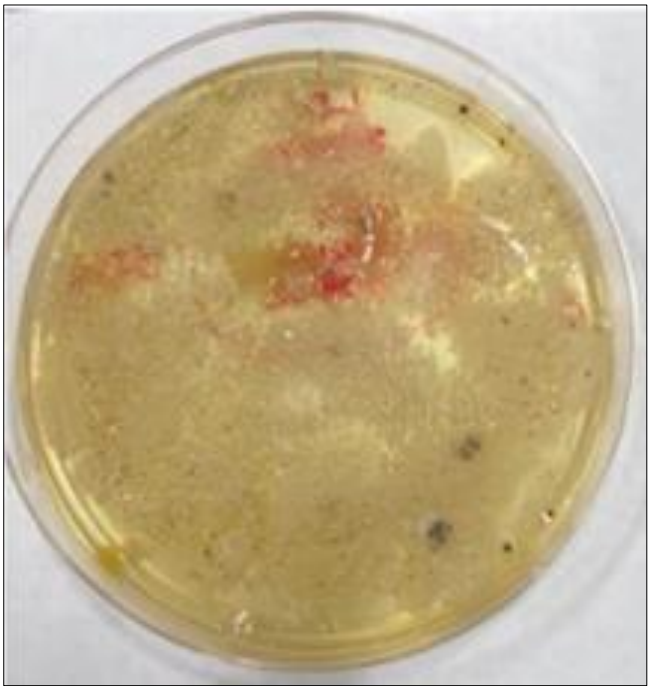

Fig 4: Screening of anaerobic bacteria

Table 1: Colony characters of isolate 1 and isolate 2.

Colony characters	Isolate 1	Isolate 2
Size	~2mm diameter	1mm
Shape	Round	Irregular
Elevation	Convex	Raised
Color	White	Cream
Opacity	Translucent	Opaque
Edge	Entire	Undulate

F. Morphological and Biochemical Characterization

Pure colonies were selected and characterized by morphology, Gram staining, and motility using standard methods (Cappuccino & Welsh, 2017) [5]. Biochemical tests (TSI, MR, Indole, VP) were performed following Bergey's Manual of Systematic Bacteriology (Brenner et al., 2005) [3].

G. Spoilage Potential Testing

Isolates were grown to $OD_{600} \approx 0.5$ in nutrient broth. Sterile fish muscle cubes were added to broth to simulate spoilage conditions. Test groups included controls and fish inoculated with isolates. Tubes were incubated at 30 °C for 48 hours. OD_{600} was measured at intervals to monitor microbial growth and spoilage (Gram & Dalgaard, 2002) [9].

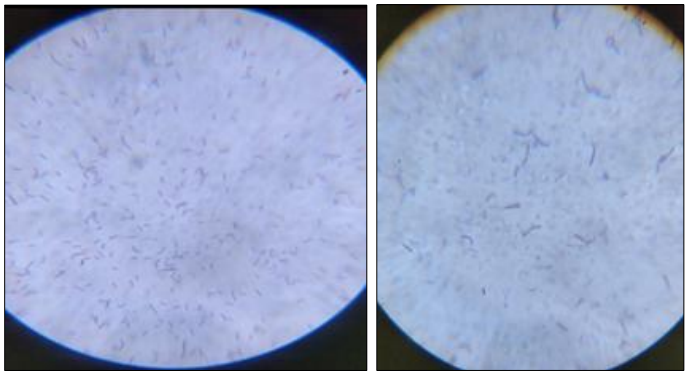

Fig 5: Gram nature of isolate 1 and 2

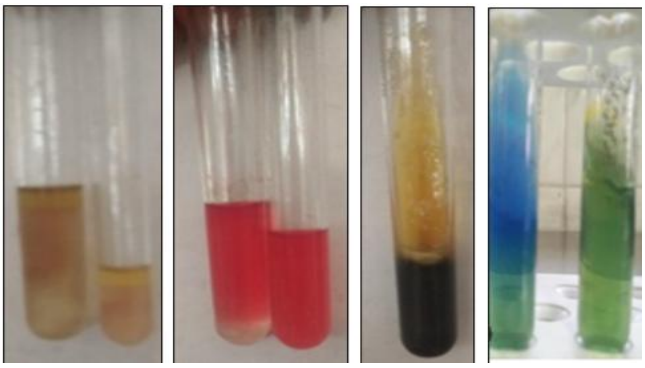

Fig 6: Biochemical test.

Table 2: Biochemical test results

Gram Staining	Positive	Positive
Indole Test	Negative	Negative
Methyl Red (MR) Test	Positive	Positive
Voges-Proskauer (VP) Test	Negative	Negative
Citrate utilization	Negative	Positive
TSI (Triple Sugar Iron)	H_2S production	No H2S production
Catalase	Negative	Positive

4. RESULTS

After 4–8 weeks of incubation, the Winogradsky columns developed clear vertical stratification, with green algal mats in the aerobic surface layers and black anaerobic zones at the bottom emitting a characteristic sulfurous odor indicative of sulfate-reducing bacterial activity. Anaerobic sediment from these black zones was successfully enriched in Lyndby medium, producing turbid cultures with strong sulfur odors. Subsequent anaerobic streaking on Lyndby agar yielded distinct colonies after 5–7 days of incubation at 30 °C.

Two dominant bacterial isolates were purified and exhibited Gram-positive rod morphology under oil immersion microscopy. Colony features included convex elevation, entire margins, and cream pigmentation. Biochemical tests showed both isolates were positive for Methyl Red, indicating acid production from glucose fermentation, while one isolate also tested positive on TSI slants, confirming its ability to ferment carbohydrates.

Table 3: OD600 - spoilage assessment

Time (hrs)	C (Broth only)	D1 (Broth + Isolate 1)	D2 (Broth + Isolate 2)	F (Broth + Fish)	F1 (Fish + Isolate 1)	F2 (Fish + Isolate 2)
0	0.04	0.05	0.05	0.06	0.05	0.05
112	0.05	0.39	0.35	0.15	0.47	0.43
24	0.09	0.60	0.56	0.24	0.75	0.71
36	0.09	0.65	0.60	0.27	0.78	0.74
448	0.09	0.66	0.60	0.27	0.78	0.74

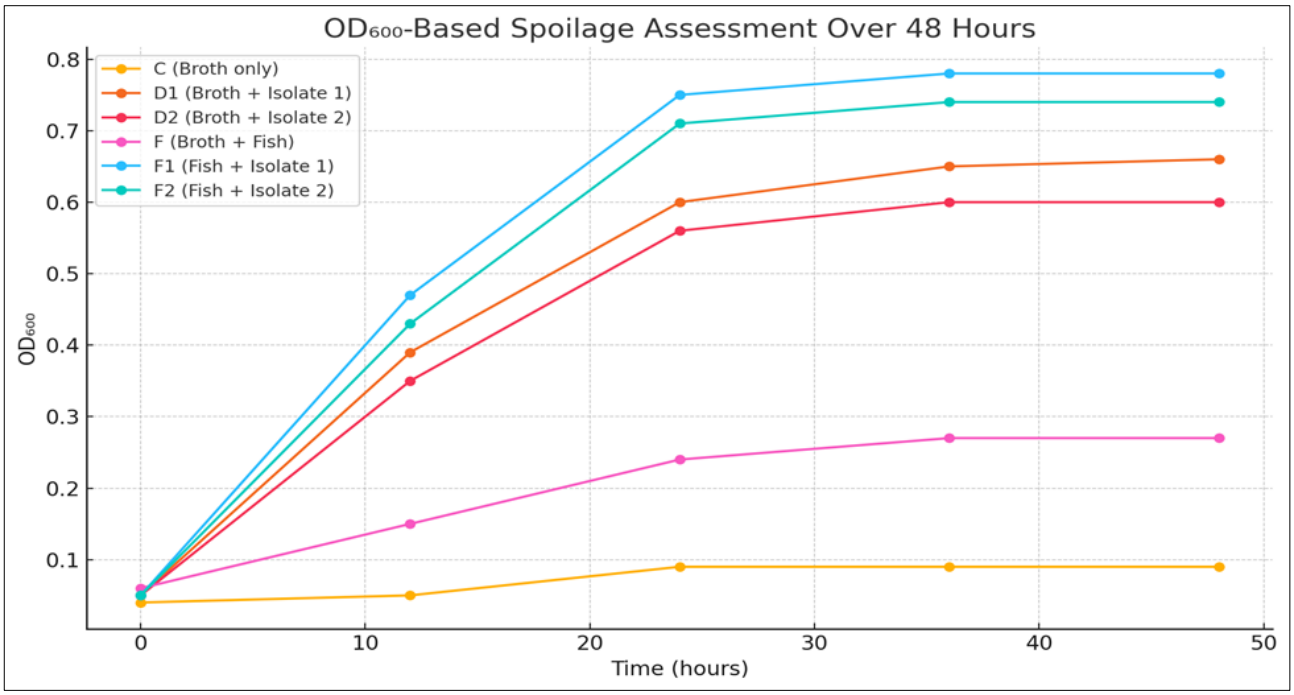

Fig 7: Graph of OD600 - spoilage assessment

Spoilage potential was assessed by inoculating the isolates into nutrient broth containing sterile fish muscle. OD_{600} measurements over 48 hours demonstrated clear microbial growth and spoilage activity. Fish samples inoculated with isolates showed significantly higher OD_{600} values (up to 0.78) compared to the fish-only control (0.27), confirming the capacity of these bacteria to decompose protein-rich food substrates under aerobic conditions.

5. CONCLUSION AND RECOMMENDATION

This study demonstrates that the Winogradsky column is an effective, affordable system for enriching and isolating specialized bacteria from pond sediments. By replicating natural environmental gradients, the columns selectively cultivated sulfate-reducing and fermentative bacteria capable of producing spoilage-associated metabolites.

The isolated strains showed clear spoilage potential when tested in fish muscle, confirming their ability to decompose protein-rich substrates under controlled conditions.

These results highlight the importance of environmental enrichment approaches in food microbiology for identifying natural spoilage agents. Understanding the ecology and metabolic capabilities of these bacteria can support the development of sustainable monitoring tools and preservation strategies, reducing reliance on synthetic additives and improving overall food safety and quality.

6. RECOMMENDATIONS

Future studies should consider applying molecular identification methods such as 16S rRNA gene sequencing to precisely classify the isolated bacteria and understand their phylogenetic relationships. This would improve the accuracy of spoilage assessments and facilitate the discovery of novel spoilage-associated species. N04 in real contaminated soils are recommended to validate laboratory findings.

Additionally, research should evaluate the behavior of these isolates under different food storage conditions, including refrigeration, vacuum packaging, and modified atmospheres. Understanding their growth dynamics in real-world settings can support the development of targeted, natural preservation strategies to improve food safety, reduce waste, and meet consumer demand for cleaner-label products.

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