

# Characterization and Semi-Quantitation of Microorganisms Present in the Partially Spoiled Tomatoes

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## Abstract:

**Introduction:** Tomatoes are considered a nutrient-rich and versatile food, that shields the cells against oxidative stress while reducing the likelihood of cancer and heart disease. Nevertheless, their high water content renders them susceptible to spoilage.

**Material and methods:** Thirty partially spoiled tomatoes underwent examination for bacterial and fungal proliferation. Each tomato was segmented into the spoiled section, the region beneath the spoiled portion, and the fresh lower segment. Samples from each segment were cultured to ascertain the types of microorganisms present. The microbial organisms were then identified using MALDI-TOF.

**Results:** Twenty-seven microorganisms were detected, with one organism remaining unidentified. Out of these, ten were identified as pathogenic, six as opportunistic, and ten as non-pathogenic or beneficial organisms. The majority of pathogenic microbes were *Klebsiella* (39%), followed by *Staphylococcus* species (17.5%), *Enterobacter* (15%), and *Candida* (14%). The microbial composition remained consistent across all three regions of the decayed tomato.

**Conclusion:** There is limited research available regarding the safety implications of consuming partially spoiled fruit and its impact on human health. Published studies on this topic are scarce. It is strongly advised to avoid consuming spoiled tomatoes, particularly in their raw form or as juice.

**Keywords:** MALDI-TOF, Food Safety, Food-borne Pathogens

## Introduction

A man can hardly do without consuming tomatoes, either as a fruit or as a vegetable in his daily meal. Renowned experts assert that tomatoes stand as a paramount food source of antioxidants, pivotal in shielding against cellular damage [1]. Additionally, lycopene in tomatoes contributes to mitigating the risks associated with heart disease and cancer [2]. Tomato fruits are highly perishable due to their high water content, which makes them susceptible substrates for the growth of microorganisms and results in subsequent spoilage by these microbes [3]. Research has revealed that microorganisms isolated from

rotting tomatoes encompassed, *Bacillus subtilis*, *Bacillus cereus*, *Listeria monocytogenes*, *Pseudomonas*, *Aspergillus niger*, and *Fusarium* species [4,5]. It is speculated that the microbes responsible for spoilage gain entry into tomatoes during their cultivation, harvesting, and packaging processes [6]. The proliferation of microorganisms throughout the most rotting tomatoes could be potentially dangerous to human health, if such contaminated tomatoes are ingested in inadequately prepared dishes or eaten raw, resulting in food poisoning [7]. Regardless, to save costs, people often buy tomatoes of inferior quality in the markets, i.e., "partially spoiled or perishable tomatoes," from which they cut off the rotten portion and use them. Nevertheless, eliminating the rotten portion and utilizing the remaining portions may not be sufficient to eliminate pathogens. The primary objective of the present research is to identify and isolate microorganisms from different portions of the partially rotten tomato. In contrast, little study has been done to identify and define the fungus found on rotting tomatoes. This study has been carried out to exemplify the presence of a wide range of microorganisms present in the various portions of spoiled tomatoes, with a particular emphasis on human pathogens. Furthermore, to demonstrate that cutting off the spoiled portion and using the unspoiled portion, while presuming that the fresh portion does not completely eradicate them. Morphological and biochemical characterization is used to characterize the isolated microorganisms, and those isolates that elude identification undergo MALDI-TOF MS analysis since this technique enables the unbiased identification of microorganisms within a few minutes. MALDI-TOF MS, or Matrix-Assisted Laser Desorption/Ionization coupled to Time-of-Flight Mass Spectrometry, serves various purposes such as protein sequencing, mapping biomolecules in tissues, microbial identification, and high-throughput analysis of numerous biochemical assays. The TOF mass analyzer then detects and measures these ions, providing valuable insights into the composition of the analyzed sample [8].

## Methodology

### Materials

The sample comprised 30 partially spoiled tomatoes collected from three distinct markets situated in Mangalore. One store specialized in the sale of organic tomatoes, while the remaining two provided conventionally cultivated, inorganic tomatoes.

### Primary Isolation

1. To extract the juice, 1 gram of a tomato segment was procured from three discrete regions of the same partially spoiled tomato. The initial portion was derived from the spoiled or rotten region – “A” (Spoiled Portion), the second from beneath the deteriorated portion - “B” (Middle Portion), and the third from the contiguous, untarnished section - “C” (Third Portion/fresh portion). Each of these three parts was individually squashed within sterile test tubes, facilitated by a sterile glass rod.
2. For the dilution process, 0.5 ml of undiluted tomato juice extract was added to 4.5 ml of nutrient broth, maintaining a 1:10 dilution ratio. This dilution method was followed for each of the three separate tomato juice samples from the different sections.
3. Isolation of Bacteria: each of the three dilutions was streaked on Blood Agar and MacConkey plates using a semi-quantitative streaking technique. The plates were then incubated at 32°C for the subsequent 24 hours.
4. Isolation of Fungi: each of the three dilutions was streaked on Sabarose Dextrose Agar using a semi-quantitative streaking technique and incubated at room temperature for at least a week

**Characterization of Microbes using MALDI-TOF by Extended Direct Transfer**

1. A layer of an isolated colony was smeared directly onto an unoccupied spot on the MALDI target plate. Minimal amount of biological material was utilized for the direct transfer. The biological material was overlaid with 1.0 µL of 70% formic acid and allowed it to dry naturally at room temperature.
2. Within 30 minutes of the drying process, the substance was covered with 1.0 µL of α-cyano-4-hydroxy-cinnamic acid solution (HCCA) and allowed to air-dry at room temperature.
3. The MALDI target plate was loaded inside the MALDI-TOF apparatus. Following the acquisition of spectra by aggregating laser shots, the instrument's software automatically processed the data. Subsequently, the spectra were compared against reference libraries for microbial identification to determine the closest match.

**Results**

After characterizing the microorganisms from each part of the tomato sample, **Table 1** illustrates the semi-quantitative growth of the isolated microbes in relation to the total sample size. “A” denotes the rotten part of the partially spoiled tomato. “B” denotes the Middle Portion (Area beneath the spoiled part) of the partially spoiled tomato. “C” denotes the fresh lower segment of the partially spoiled tomato.

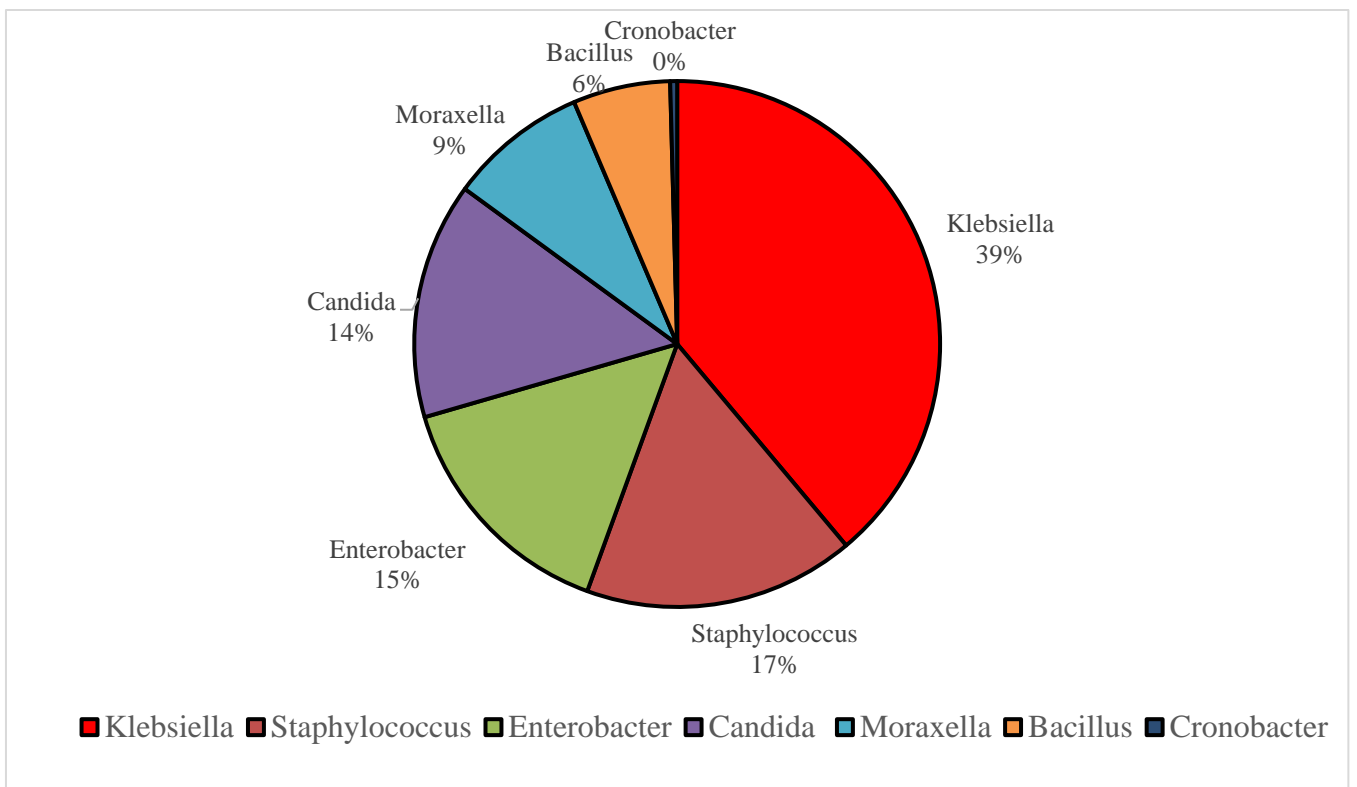
**Figure 1** delineates the presence of various Pathogenic microbial species in the sample. **Figure 2** compares the pathogenic microbes against the spoiled portion (A), middle portion (B), and fresh portion (C) of the tomato sample.

**Table 1: The growth of the isolated microbes was observed in comparison to the total sample size of 30.**

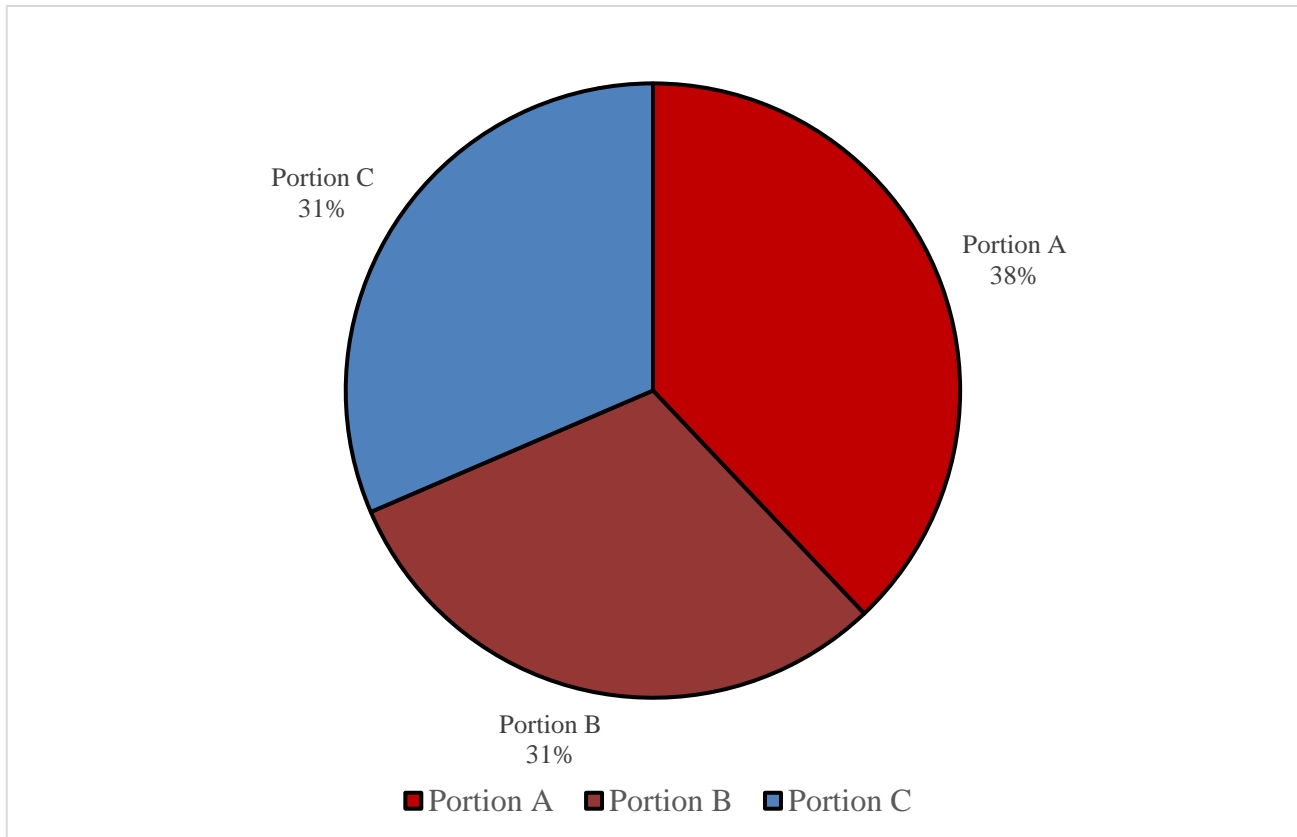
Microorganisms in partially rotten Samples							
	Microorganism	Organic Samples			Conventional Samples		
		A	B	C	A	B	C
Highly Pathogenic	Klebsiella oxytoca	8	8	10	11	11	10
	Klebsiella varicola	5	6	5	6	5	6
	Staphylococcus succinus	5	2	4	2	0	1
	Staphylococcus sciuri	3	2	3	2	3	2
	Staphylococcus xylosus	2	2	2	3	1	0
	Enterobacter bugandensis	7	6	6	6	5	5
	Candida parapsilosis	5	4	4	9	6	6
	Moraxella osloensis	2	2	2	6	3	3
	Bacillus cereus	3	3	2	2	2	2
	Cronobacter spp	1	0	0	0	0	0
Opportunistic Pathogen	Acetobacter indonesinesis	5	4	2	6	5	6
	Leuconostoc citreum	3	3	2	10	9	8
	Weissella cibaria	6	5	1	7	4	3
	Weissella paramesenteroides	1	0	1	0	0	0
	Pichia kudriavzevii	3	2	2	5	3	2

	Kosakonia cowanii	1	0	0	0	0	0
Non- Pathogenic	Acetobacter orientalis	9	10	4	8	3	3
	Lactobacillus plantarum	2	6	8	6	9	9
	Lactobacillus mali	0	1	6	1	0	4
	Lactobacillus amylovarus	2	1	3	5	5	3
	Lactobacillus fermentum	1	1	1	0	0	0
	Aromatoleum terpenicum	6	4	4	4	2	4
	Kurthia gibsonii	7	5	4	2	2	1
	Hanseniasspora opuntiae	3	2	0	7	5	1
	Corynebacterium flavesceens	2	1	1	2	1	1
	Bacillus subtilis	0	1	3	0	0	0
Unidentified	Water Bubble	9	10	6	0	0	0

**Figure 1: Pathogenic Microbial Spectrum**



**Figure 2: Juxtaposition of pathogenic microbes against the spoiled portion (A), Middle portion (B), and Fresh portion(C).**



### Discussion

Microbial growth in the majority of deteriorating tomatoes poses a considerable risk to individuals, potentially triggering food-borne disease. The microbes that cause human sickness were identified and investigated.


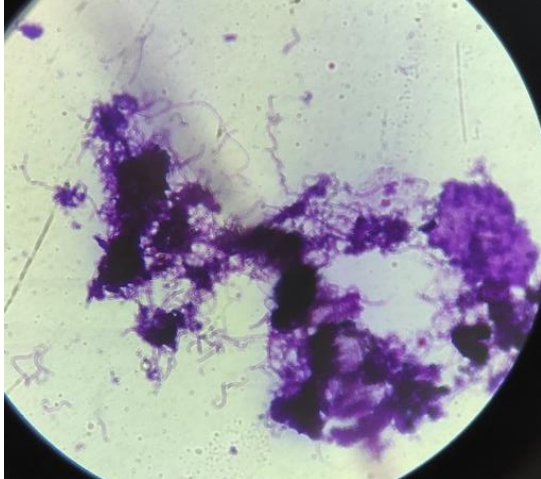
Following the findings depicted in **Table 1**, the semi-quantitative growth rate of the isolated microbes relative to the total sample size determined that the predominant microbial species were highly pathogenic, followed by species classified as opportunistic or emerging pathogens, with some species being non-pathogenic. Upon thorough investigation, these bacterial strains displayed a heightened presence not only within the decayed section but also extended beneath the spoiled area, eventually reaching the adjacent fresh portion. This observation typically suggests that the dissemination of microbes in decaying areas inevitably leads to their spread into the internal segments of the tomato. The findings underscore the potential for microbial contamination to permeate the entire fruit.

**Figure 1** exhibits the distribution of Pathogenic microbial species as a percentage across overall samples. *Klebsiella* species were notably more prevalent in higher proportions in partially decayed tomatoes. *Klebsiella oxytoca* and *Klebsiella varicolla* are implicated in a broad spectrum of healthcare-associated and community-acquired infections, causing bronchopneumonia, urinary tract infections, and septicemia upon Ingestion [9]. *Staphylococcus* species accounted for about 18% of the samples. *Staphylococcus succinus*, *Staphylococcus sciuri*, and *Staphylococcus xylosus*, a significant pathogen, potentially cause systemic infections. *Staphylococcus xylosus* is associated with fecal matter. *Enterobacter Bugandensis*, found in approximately 15% of the samples, is extremely pathogenic, causing severe neonatal sepsis, as

well as urinary, respiratory, and bloodstream infections, with severe outcomes even after proper treatment [15]. *Candida parapsilosis*, identified in 14% of total samples, is a significant human fungal pathogen. It poses a substantial risk if it invades organs or the bloodstream, potentially causing severe illness, prolonged hospitalization, and death [19]. *Moraxella Osloensis*, detected in 9% of samples, is associated with various human ailments like meningitis and bacteremia. *Bacillus cereus*, found in 6% of samples, commonly triggers gastrointestinal discomfort. *Cronobacter spp.*, found in one spoiled organic sample, is connected to several infections, including bacteremia and urinary tract infections [18].

In addition to the pathogenic organisms identified, **Table 1** also delineates the presence of opportunistic pathogens, which present significant risks to immunocompromised patients, along with some potential non-pathogenic microbes or fermenting organisms. For example, *Lactobacillus* species, prevalent in the spoiled section as a fundamental fermenting bacterium, may not pose a threat to healthy individuals but could endanger those with compromised immune systems. Moreover, rare microbial species were also detected, which could potentially pose risks to immunocompromised individuals.

The unidentified microbe, produced as a water-bubble-like colony exhibited a distinctive shiny, mucoid exclusively observed only on the SDA plates solely in the organic samples, not the conventional ones. Its growth was more prominent in Potions A and B, with some presence still noticeable in Potion C. The colony characteristics displayed a translucent, shiny appearance resembling water bubbles, growing both individually and densely layered (**Figure 3**). One notable aspect of this colony was its initial growth, which outpaced other microbial colonies, suggesting inhibition of growth in surrounding microbes. Gram stain morphology suggested Gram-positive thin filaments (**Figure 4**), indicating a *Bacillus* species. Identification proved challenging, as subculture growth in one organic sample took over a month with no growth observed. MALDI-TOF analysis reported "No peaks found," indicating a lack of identification in the Bruker Library due to the old culture. Web search about this microorganism based on the colony characteristic, the likely match that emerged was *Bacillus amyloliquefacien*. This soil bacterium is esteemed for its ability to colonize roots and combat certain plant root pathogens in agriculture [21,22]. However, it remains uncertain whether this unidentified microbe can definitively be classified as *Bacillus amyloliquefacien*.

<p>Figure 3: Subculture of “Unidentified” microorganism isolated from an Organic Tomato Sample on SDA Plate</p>	<p>Figure 4: Microscopic Analysis of the Unidentified Microorganism.</p>
	



According to the data presented in **Figure 2**, and the analysis provided in **Table 1**, it is evident that the decayed portion, denoted as “portion A”, contained in total of 38% of human pathogenic microorganisms. This suggests that the spoiled portion typically harbors a diverse range of pathogenic microbes alongside the fermenting or non-pathogenic spoilage-causing microbes. Moving to the region beneath the spoiled part, which was denoted as “portion B” it was found to contain 31% of the pathogenic microbes that initially originated from the spoiled portion. This indicates the propensity of microbes to spread and colonize the area beneath the spoiled portion quite easily. As for the fresh lower segment, which is referred to as “Portion C” one might assume that microbes wouldn't have spread to this part due to its freshness. However, surprisingly, 31% of the total pathogenic microbes were found to have spread to this section as well. **Figure 2** provides a clear visual representation of the microbial spread throughout the fruit, irrespective of its state of spoilage or freshness.

The objective of this investigation is twofold: to demonstrate the presence of pathogenic or rare opportunistic microorganisms within a partially decayed sample, and to provide a semi-quantitative assessment of the microbial population, particularly those with human pathogenic attributes. The underlying assumption in this research is that remnants of pathogenic organisms may endure within the fresh segment of deteriorated tomatoes. Despite the outward appearance of freshness and absence of visible infection in the fresh segment of partially rotten tomato, microbes can infiltrate extensively throughout the fruit, rendering it more vulnerable and potentially jeopardizing human health concerns.

## Conclusion

Scarce literature aligns with the corresponds to the core idea of this research concept. Limited or negligible research exists on the safety of consuming partially spoiled fruits and its potential impact on human health. Despite the paucity of published studies, various online sources caution against the consumption of partially spoiled fruits due to the risk of inducing food poisoning. Since agricultural practices are constantly evolving over time, there is a possibility for the emergence of numerous distinct species, some of which might which could have notable significant health effects on individuals. When tomatoes are consumed raw, there's a chance that if pathogens are present, it could pose a potential risk to human health. However, cooking tomatoes by boiling them may lower the risk due to the heat involved in the process.

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