Field Guide: Morphometric Visualization and Characterization of Selected Foodborne Pathogens Using Advanced Imaging Techniques

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This paper aims to present the phenotypic characteristics, such as length, width, circular diameter, volume, surface area to cross-sectional area ratio, surface uniformity, and surface texture, of the foodborne pathogens *Salmonella enterica* serovar Agona, *Salmonella enterica* serovar Enteritidis, *Listeria monocytogenes*, and *Campylobacter jejuni*. It is a novel “field-guide” presentation of the observable morphological characteristics of these four species in the form of a Dichotomous Key. Phenotypic values of the pathogens were measured using a high-accuracy, nano-level-resolution 3D laser confocal scanning microscope. All samples were fixed with glutaraldehyde and stained with methylene blue, ensuring morphological preservation. The images of the pathogens were captured under various imaging modes, including 3D, laser, and transmission electron microscopy resolutions. Results show that these pathogens exhibit distinct morphological and surface properties, with *Campylobacter jejuni* showing unique features. The dichotomous key shows a simplified way of classifying selected foodborne pathogens from their morphometric properties. The morphometric measurements, in many instances first reported in the literature, and images provide a readily observable way to identify and classify microorganisms, allowing researchers to potentially study evolutionary relationships, assess species diversity, and understand how organisms interact with their environment, especially when genetic information is limited or difficult to obtain.

foodborne pathogens phenotypes length width surface texture surface roughness bacteria TEM 3D laser confocal microscopy

Foodborne illnesses remain a pervasive public health issue, with the Centers for Disease Control and Prevention (CDC) estimating approximately 48 million cases annually in the United States, resulting in 128,000 hospitalizations and 3000 deaths [1]. Globally, the World Health Organization reports that foodborne diseases cause approximately 600 million illnesses and 420,000 deaths each year [2]. Among the most common culprits are *Salmonella*, *Listeria*, and *Campylobacter*, which have been linked to outbreaks in fresh produce, poultry, and dairy products [3]. These pathogens are increasingly studied due to their adaptability to food environments and resistance to control measures [4]. Phenotypic profiles of bacteria are important for identifying and classifying bacterial species, assessing their potential pathogenicity, and studying their adaptability to different environments, thereby enabling the for monitoring their behavior in diverse situations.

Recent studies suggest that certain phenotypic traits, such as surface roughness, cell size, and structural uniformity, can indicate a pathogen's ability to form biofilms, resist antimicrobials, or evade host immune defenses [5]. For instance, rough surface textures in fungus, such as *Candida albicans*, have been linked to increased biofilm formation, a known virulence factor that enhances persistence in food processing environments [6][7]. Additionally, the ability of *Salmonella* to modify its cell surface under stressful conditions has been shown to enhance its survival in acidic environments, such as the stomach, thereby facilitating host colonization [8]. Thus, this paper presents phenotypic measurements and images of the foodborne pathogens *Salmonella enterica* serovar Agona, *Salmonella enterica* serovar Enteritidis, *Listeria monocytogenes*, and *Campylobacter jejuni*. The phenotypic profiles will enhance the scientific understanding of these pathogens and support the development of targeted mitigation strategies, especially given the increasing burden of foodborne illness and the need for improved surveillance and intervention tools.

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