HIERARCHICAL CLASSIFICATION OF BACTERIA FROM HYPERSPECTRAL VISIBLE-NIR IMAGING OF BACTERIAL CULTURES ON AGAR PETRI DISHES

Ana Herrero-Langreo[1], Anastasia Falkovskaya[1], Mariateresa Ferone[1], Amalia Scannell[2,3,4] Aoife Gowen[1]

[1] UCD School of Biosystems and Food Engineering, University College of Dublin (UCD), Belfield, Dublin 4 Ireland *ana.herrero-langreo@ucd.ie
[2] UCD Institute of Food and Health
[3] UCD Center for Food Safety
[4] UCD School of Agriculture and Food Science

Microbiological food spoilage is a worldwide issue, affecting public health and holding great social and economic repercussions. ISO standard methods for the identification of food borne bacteria are generally culture-based and include several microbial culturing steps on specific media. This process can take up to 7 days. This delay period constitutes a food safety hazard in the food production chain. Several works have recently focused on speeding up this process by using hyperspectral imaging for identifying food borne bacteria on generic culture media. Some of the main challenges encountered in these works include: interference due to sample presentation and restrictions imposed by the need to secure biological safety during the imaging process. In this respect, the recent development and affordability of portable hyperspectral imagers, opens-up the applicability of this technology for the detection of foodborne bacteria. In this context, the objective of this work is to test the ability of the portable Specim IQ camera system (400 – 1000 nm) to detect and discriminate between common foodborne bacteria. Eight bacterial strains, with different degrees of taxonomical proximity were considered: three Gram-negative, *E. coli (EC), C. sakazakii (CS)* and *P. fluorescens (PF)*; and four Gram-positive bacteria, *L.monocytogenes (LM)*, *B. subtilis (BS), B. amyloquefaciens (BA), B. cereus NCTC (BCN),* and *B. cereus DSM (BCD).* All bacterial cultures were grown on Luria Bertani agar and presented in Polystyrene (PS) petri plates with a glass lid. To control the robustness of the approach, six replicates of each bacterial strain were cultured, plated, and imaged in two independent trials. A total of 48 samples were used for calibration and 48 for validation, plus 4 sterile agar controls. Spectral Angle Mapper (SAM) distancewas used to identify pixels corresponding either to bacteria or agar for each sample. The mean reflectance spectra of the bacteria pixels for each plate were subsequently used for sample classification. A non-supervised hierarchical cluster analysis allowed correct segregation of 100% of calibration plates into four groups: two groups of Gram-positive (*LM-BS-BCN-BCD* and *BA*) and two groups of Gram-negative strains (*EC-PC* and *CS*). The robustness of this classification procedure was tested by assigning each mean spectrum from the validation dataset to a group. Using Mahalanobis distance as a metric, 100% of the validation samples were correctly classified into their corresponding group. This work proves the feasibility of using portable hyperspectral camera systems in the visible-nir range for the identification of groups of bacteria with similar spectral characteristics. It proposes a simple procedure allowing both to evaluate the spectral similarity between bacterial strains and to classify monocultured plates into previously identified groups of bacteria. This approach can be used as a rapid preliminary scanning approach for streamlining the identification of cultured bacteria. The groups identified by non-supervised clustering, based on mean spectra per sample, can also serve as an initial step for supervised hierarchical classification at a pixel level. The portable Specim IQ camera system was shown to be well suited for this application.

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***Key words*—** hyperspectral, portable, Specim IQ, polystyrene, petri, agar, hydrogel, microbiology.

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