



Invasive weed optimization for optimizing one-agar-for-all classification of bacterial colonies based on hyperspectral imaging

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ABSTRACT

Near-infrared hyperspectral imaging together with versatile chemometric algorithms including invasive weed optimization (IWO) were employed for optimizing fast classification of bacterial colonies on agar plates. Hyperspectral images of colonies from six strains of bacteria were collected, and classification models were established by applying partial least squares-discriminant analysis and support vector machine (SVM) on the original as well as difference spectra. The parameters of SVM models were optimized by comparing genetic algorithm, particle swarm optimization and the proposed IWO. The results showed that difference spectra amplified the variations among the spectra of the six strains thus potential for improving classification accuracy. The best full wavelength classification model was IWO-SVM model which produced overall correct classification rates (OCCRs) of 100.0% and 97.0% for calibration and prediction, respectively. Besides, competitive adaptive reweighted sampling (CARS), GA and successive projections algorithm (SPA) were utilized to select important wavelengths to establish simplified models. Among them, the simplified IWO-SVM model based on the feature wavelengths selected by CARS gave the best classification rates of 97.2% and 96.0% for calibration and prediction, respectively. The study demonstrated that IWO was a useful tool for optimizing calibration models thus potential for usage in many other applications.

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1. Introduction

Global concerns over food microbiological safety are growing with the increasingly reported cases of foodborne illness and broadened ranges of infections [1]. Bacterial pathogens, including *Escherichia coli*, *Listeria*, and *Staphylococcus* are three of the main species of microbes that can threaten public health. Enterohemorrhagic *Escherichia coli* cause various foodborne illnesses including bloody or non-bloody diarrhea and life-threatening hemolytic uremic syndrome (HUS) as well as thrombotic thrombocytopenic purpura (TTP) by producing potent Shiga toxins [2]. *Listeria* belongs to psychrophile and can grow well at refrigeration temperature. Listeriosis produced by *Listeria monocytogens* can lead to a mortality rate of ca. 30%, and immune-compromised people and pregnant women are the most possible victims [3]. Meanwhile, consuming food containing 100–200 ng of *Staphylococcal* toxins would also cause gastroenteritis to a healthy [4]. Therefore, it is urgently

needed to identify and classify bacteria strains to ensure public health. Current routine methods rely heavily on plating and culture methods which are recognized as well-established gold-standard. However, these methods are usually time-consuming, laborious and tedious thus not suitable for real-time detection and inspection. More recently, a variety of new methods based on immunology (i.e., enzyme-linked immunosorbent assay, ELISA) and molecular biology (e.g., polymerase chain reaction, PCR) were developed [5,6]. The application of these new methods has greatly enhanced the detection speed as well as accuracy if combined with culture-based methods. Nevertheless, the new methods may require high expertise and expensive instrument for the detection experiments.

As a novel technology, hyperspectral imaging (HSI) enables image and spectral assays in one single integrated system and has showed significant power in fast and nondestructive quality and safety measurement of food and agricultural products [7–10]. The application of HSI in microbial detection was also summarized [11]. The principle for bacterial classification based on hyperspectral imaging sensors relies on the utilization and analysis of chemical information within the bacterial colonies due to species or sub-species difference. Such variations are then captured and reflected in the hyperspectral images. Therefore, by investigating hyper-

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spectral image variations using multivariate analysis approaches, bacterial attributes can then be attained. With respect to bacterial colony classification, the feasibility of hyperspectral imaging for classification of *Campylobacter* against non-*Camp.* colonies grown on different agar plates was first confirmed [12,13]. The authors applied principal component analysis (PCA) to identify important wavelengths and a pattern classification algorithm together with Bhattacharyya distance for separation of *Camp.* colonies from non-*Camp.* colonies and agar background. The classification accuracy in species level as shown in these studies could both reach 99%. *Salmonella* serotypes were also classified with similar accuracy by using quadratic discriminant analysis [14]. Besides, Yu et al. [15] established both full and selected wavelength models to classify bacterial colonies into three species of *E.coli*, *Listeria* and *Staphylococcus*, where the best classification accuracy was between 95.92% and 100%.

For the identification of six different *E.coli* serogroups, Park et al. [16] employed a hyperspectral microscope imaging (HMI) system based on acousto-optic tunable filters (AOTF). In a later study for classification of six different non-O157 *E.coli* strain colonies, Yoon et al. [17] applied principal component analysis on the preprocessed pixel spectra and employed Mahalanobis distance classifier and k-nearest neighbor (kNN) classifier for class assignment. The overall classification accuracy was over 95% for both pixel- and colony-level classifications and the main contributor for such successful separation was the distinctive color information of colonies of each type. Therefore, RGB color information was subsequently extracted from the visible and near infrared hyperspectral images and subjected to PCA and kNN for classification of bacterial colonies where up to 92% accuracy was achieved [18]. Instead of Vis-NIR region (typically 400–1000 nm), Kammies et al. [19] employed hyperspectral imaging in longer wavelength of NIR region (1000–2500 nm) to classify *Bacillus cereus*, *Escherichia coli* and *Salmonella enteritidis* in species level and *Staphylococcus aureus* and *Staphylococcus epidermidis* in strain level. All of the above-mentioned studies demonstrated that hyperspectral imaging is a powerful tool for colony classifications. Nevertheless, it was noticed that the majority of the current studies focused only on specific bacteria that were grown on their preferred selective agars. For example, for classification of *E.coli* bacteria, rainbow agar and Sorbitol MacConkey agar may be utilized for bacterial cultivation. Though relevantly accurate in such a circumstance, the methodologies adopted above may require intensive usage of miscellaneous selective agars for the classification of bacteria if their background information is absent. In other words, more agars should be involved to determine species attributes of the bacteria under study before their serotyping. By doing this, the experimental complexity and burdens will be substantially increased making the whole analytical process less cost-effective. Therefore, it is very important to use one-agar-for-all protocol in order to include variability due to other factors than bacteria as well as to classify bacteria in strain/subspecies level while assuming their species information is unknown, that is, in a blind test. Moreover, bacterial classification based on hyperspectral imaging relies largely on the chemometric algorithms exploited, thus optimization of modeling parameters becomes an important issue. Meanwhile, due to the intrinsic disadvantage of hyperspectral imaging where massive and perhaps redundant information is present, selection of feature wavelengths for simplification of computing and reduction of system cost is preferred. Therefore, taking *E.coli*, *Listeria* and *Staphylococcus* for example, this study aimed to verify the feasibility of one-agar-for-all classification of bacteria in the subspecies level by employing Vis-NIR hyperspectral imaging. The sub-objectives were (1) to verify the efficiency of difference spectra for classification; (2) to enhance calibration models by applying various intelligence

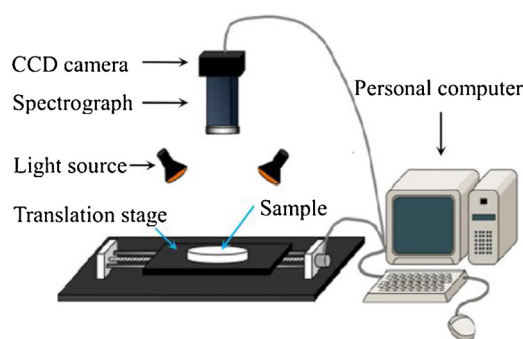


Fig. 1. Configurations of hyperspectral imaging system.

algorithms in SVM parameter optimization and (3) to optimize wavelength selection during the development of simplified models.

2. Materials and methods

2.1. Bacterial agar plates

Three strains of *Escherichia coli* bacteria (i.e., *E.coli* O8, O11 and O138) [20], two strains of *Listeria* (i.e., *L. monocytogenes* and *L. seeligeri*) and *Staphylococcus aureus* were used in this study. These bacteria were collected from State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University. Bacterial suspensions were prepared according to previously described methods [21]. Briefly, frozen stored bacteria (-80°C) were activated through incubation on tryptone soya agar (TSA, BD, USA) at 37°C for 22 ± 2 h. The activation process was repeated twice to ensure bacterial viability. One single typical colony was subsequently picked and inoculated into tryptone soya broth (TSB, BD, USA) for culture at 37°C for 18 ± 2 h. Serial dilutions were then made and bacterial agar plates were prepared by the pour-plate technique. After incubation at $36 \pm 1^{\circ}\text{C}$ for 24 ± 1 h, the plates were harvested and subjected to hyperspectral image acquisition. It should be noted that for all cultivation of all bacterial subspecies, only TSA (a commonly used type of agar for all bacteria) was used to testify the feasibility of the one-agar-for-all methodology for bacterial colony classification or identification.

2.2. Image acquisition and spectral extraction

The apparatus setup is illustrated in Fig. 1. As indicated, the reflectance hyperspectral imaging system is mainly composed of four parts, including a hyperspectral spectrograph (SPECIM, V10E, Finland), a CCD camera (Clara, Andor, UK), two halogen light sources (DECOSTAR51, MR16, OSRAM, Germany), a high-precision translation stage attached with a sample holder (Zolix, China) and a personal computer. The hyperspectral images were recorded in the wavelength range of 400–1000 nm with a spectral resolution of 2.8 nm and a spectral interval of 1.25 nm. To enhance the quality of captured images, the exposure time of the camera was set as 100 ms when the translation stage moved at a speed of 2 mm/s. Besides, the distance between camera and the plate surface was ca. 400 mm. All the parameters remained the same throughout the experiment.

The raw data (I_{Raw}) acquired were in radiance which may vary in different scans due to apparatus status variations. In order to make the data more meaningful and applicable for chemical interpretations, the following equation was employed to calibrate the raw data into reflectance unit [7].

$$R = \frac{I_{\text{Raw}} - I_{\text{Dark}}}{I_{\text{White}} - I_{\text{Dark}}} \quad (1)$$

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