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Ge-NOSE: Electronic Nose for Sniffing Food-Borne Bacteria

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Abstract: Gastronomy practice has become major attraction in tourism also promote food importation globally. So, controlling bacterial contamination to comply biosecurity regulations is one of imperative task for quarantine services. However detection method of bacteria causing food poisoning is laborious. Electronic nose technology has ability to recognise volatile compounds (VOCs) emitted by biological materials. Recently, the *Elecetronic Nose* is one of the best choice since it does not need reagen, cheap and fast. To proof-of concept, an investigation was carried out employing Ge-Nose (Universitas Gadjah Mada, Indonesia) to captured volatile emission of four food-borne bacteria: *E.coli* (ATCC 25922), *S.thypimurium* (ATCC 14028), *L.monocytogenes* 4b (ATCC 13932) and *B.cereus* (ATCC 10876). All of sample were then incubated at 37°C for 2, 8, 16, 24, 32, 40, and 48 hours then analysed using different methods such as *Linear Discriminan Analysis* (*LDA*), *Quadratic Discriminant Analysis* (*QDA*), and *SupportVectorMachine*(*SVM*). The result showed, using LDA methods, accuracy value of *E.coli was* 97.80±2.20%; *S.thypimurium:* 94.60±5.40%*: ; L.monocytogenes* 98.00±2.00%*:* and *B.cereus* 95.00±5.00%. Using *QDA methods, the accuarcy value of E.coli was* 94.80±5.20%; *S.thypimurium:* 95.60±4.40%*: ; L.monocytogenes* 92.00±8.00%*:* and *B.cereus* 95.00±5.00%; whereas SVM methods, it has been showed: *E.coli was* 97.00±3.00%; *S.thypimurium:* 92.40±7.60%*: ; L.monocytogenes* 89.00±11.00%*:* and *B.cereus* 89.00±11.00%. Highest accuracy classification average (98%) was achieved. Therefore, Ge-NOSE's discriminate power is able to deliver faster, accurate yet simple and inexpensive diagnostic result.

Keywords: Electronic Nose, *E.coli*, *S.thypimurium*, *L.monocytogenes*, *B.cereus*

Introduction

Food-borne disease has become serious issue for public health and food safety. However identification and detection of pathogen bacteria from clinical samples, environment or food is time-consuming (Gates, 2011). Other detection method such as Gas chromatography Nuclear Magnetic Resonance (NMR), Spectroscopy and Fourier Transform Infrared (FTIR) is high-cost besides required trained operator (Adley, 2006;Tian *et al*., 2013). Test that is rapid yet simple becoming increasingly important (Yu dan Zhao, 2012; Tait *et al*., 2014) and electronic nose (EN) provides promising features to overcome diagnosing limitations.

Electronic nose is odours-sensor-based device featuring beneficial attributes to help delivering faster, accurate, simple and inexpensive diagnostic result. The EN has been used in veterinary field to detect oestrus cycle in cow and recognise species of animal in mincemeat. However this discriminate power of EN has not been explore to identify food-borne bacteria. Therefore, this research is carried out to detect four significant bacteria causing food poisoning: *Escherichia coli, Salmonella thypimurium, Listeria monocytogenes*, and *Bacillus cereus*. The total number of those bacteria must comply strict regulations of Indonesian National Standard (SNI).

Bacteria are producing volatile organics compounds (VOCs) and gas specifically to each species, which able to be sniffed by EN sensors. Captured VOCs then analysed by pattern recognition chemomatric (PARC) (Capone *et al.*, 2001, Evans *et al.*, 2000, Haugen and Kvaal, 1998) and classified.

Material and Methods

Four ATCCs bacteria: *E.coli* (ATCC 25922), *S.typhimurium* (ATCC 14028), *L.monocytogenes* (ATCC 13932) and *B.cereus* (ATCC 10876) were cultured in medium at 37°C for 2, 8, 16, 24, 32, 40, 48 hours. Electronic nose Ge-NOSE 4th Generation (Fismatel, Physics Department, Faculty of Mathematics and Natural Sciences, Universitas Gadjah Mada, Indonesia) was employed to captured emitted VOCs. The Ge-NOSE was equiped with TGS 2600 (Air Quality Sensor), TGS 2603 (Odorous Gas Sensor), TGS 2612 (Methane and Liquefied Petroleum Gas Sensor), TGS 2620 (Solvent Vapors Sensor), TGS 813 (Combustible Gas Sensor), TGS 822 (Organic Solvent Vapors Sensor), TGS 826 (Ammonia Sensor), and TGS 832 (Chlorofluorocarbon Sensor).

To start, Ge-NOSE was undergone initial flushing for 10 minutes. Samples were warmed up between 37-47^{0}C for 70 seconds to produce maximum bacteria metabolites allowing maximum captured by the array sensors at 0.1 s/data speed. Each sample was sniffed for 1 minute and each sampling lasted 10 seconds. Flushing for 1 minute was applied between sample measurements.

Captured VOCs pattern was analysed by pattern recognition (PARC) then classified by Linear Discriminant Analysis (LDA), Quadratic Discriminant Analysis (QDA) and Support Vector Machine (SVM)

Ethics Statement

The research is exempt from full ethical clearance process based on no use of animals.

Result

In order to equally comparing the data, VOCs captured by each sensors were normalised prior analysis (Figure.1). Classification analysis by LDA (Figure.2A) showed clear clustering of contaminated medium by single bacteria (blue) compared to non-contaminated medium (red).

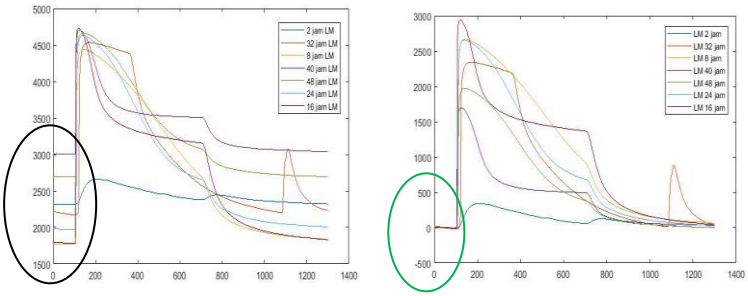

Figure 1. A. Sensor TGS 822 response to *L.monocytogenes* incubated at 2, 8, 16, 24, 32, 40 and 48 hours prior baseline normalisation (black circle), B. After baseline normalisation (green circle) should be started from 0 (source: Astantri. 2019)

The existance of *E.coli, S.typhy, L.monocytogenes*, and *B.cereus* in the medium was detected by all equipped sensors in Ge-NOSE. Chemomatric analysis of LDA, QDA and SVM was able to discriminate non-contaminated and contaminated medium either with single or multiple bacteria under different level of accuracy (Table 1). LDA classification showed highest average accuracy of 98% in comparisson to QDA and SVM, in distinguish non-contaminated and single-bacteri contaminated media. Monocytogenes was showing highest accuracy (98%) whilst Thypimurium the lowest (94.6%).

Table 1. Accuracy level between LDA, QDA and SVM method in classifying non-contaminated and contaminated medium (Prakoso., 2019; Astantri., 2019)

Group	*LDA*		*QDA*		*SVM*	
	Accuracy (%)	Std	Accuracy (%)	Std	Accuracy (%)	Std
N vs E	97.80	2.20	94.80	5.20	97.00	3.00
N vs S	94.60	5.40	95.60	4.40	92.40	7.60
N vs L	98.00	2.00	92.00	8.00	89.00	11.00
N vs B	95.00	5.00	95.00	5.00	89.00	11.00

Note: N: Negative control, E: *Escherichia coli*, S: *Salmonella thypimurium*, L: *Listeria monocytogenes*, B: *Bacillus cereus*. Std: Standart deviation

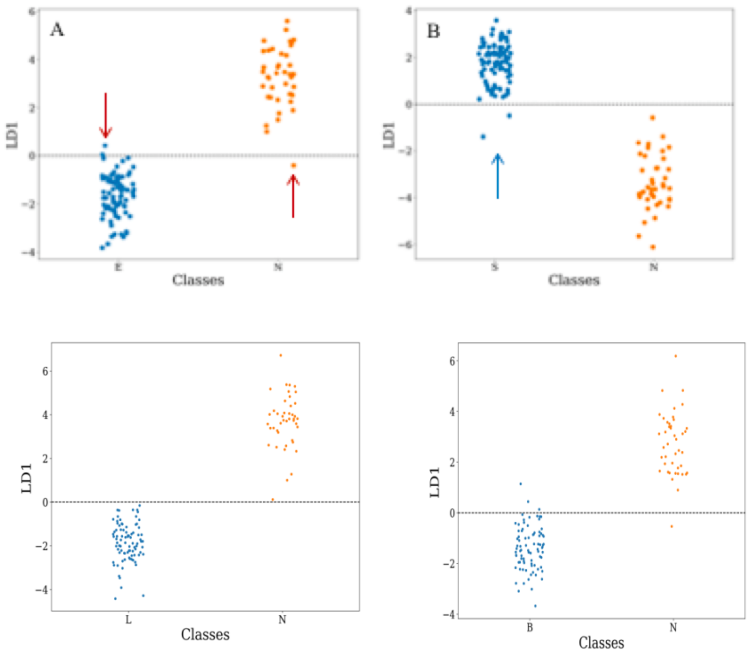

Figure 2. A. Clustered of *E coli* bacteria (blue) and clustered of non-contaminated medkum (red) with 97.8% of accuracy level. B. Clustered of *S. thypimurium* bacteria (blue) and clustered of non-contaminated medkum (red) with 94.6% of accuracy level (source: modified Prakoso., 2019)

Discussion

L.monocytogenes produced alcohol, aldehyde, ketone and alkane (Yu *et al.*, 2015) with predominance VOCs 3-methyl-butanal (Arnold & Senter, 2012), 2-nitrophenol and 3-fluoroaniline (Tait, 2014) whereas *B.cereus* produced 3-methyl-1-butanol, aldehyde, acetyl acid, and ethanol (Yu *et al*.,, 2015). These VOCs produce by the bacteria will enable application of EN for detection.

The level of accuracy between chemomatrics analysis is showing the influence of bacteria type and growth time, time of storage and chemomatrics method used. Rosyad and Lelono (2016) reported highest response of electronic nose sensors to VOCs at 16 hours of incubation. Bacteria VOCs emission may be reduce during supressed growing phase due to poor quality of media, altered medium pH, metabolite waste accumulation and overgrowth (Dwidjoseputro, 1998).

Therefore the result of this study is clearly indicating the accuracy of Ge-NOSE in detecting *Escherichia coli, Salmonella thypimurium*, *Listeria monocytogenes* and *Bacillus cereus* in medium.

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