Evaluating the limit of early detection and diagnosis of human pathogenic bacteria using electronic tongue and multivariate data analysis

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Dedication

This research is passionately dedicated to my idolized parents Ali and Najah Abu Rumaila, whose inspiration was my motive to complete the whole journey and thought me the value of patience and hard work, whose support was endless either on the spiritual, moral, or even the emotional level.

It is also dedicated to my dear seven brothers and ten sisters, all by their name and value in my heart, especially to my sister Basima for her help that meant so much to me and my sincere friends. I dedicate it, to their kind and encouraging words and advice, and also to their energy to bear me during the hard time in the past period until the last day of this work.

Last but not least, this study is also dedicated to everyone with good vibes or who had spared their time to educate, motivate and find solutions to the problems I had. Also, to every challenging obstacle I had to handle throughout the past two and half years.

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List of Abbreviations

The abbreviation	Abbreviation description
˚C	Celsius
µg/mL	Microgram per milliliter
µL	Microliter
µM	Micromolar
Ag/AgCl	Silver-silver chloride reference electrode
ANN	Artificial neural network
ATCC	American Type Culture Collection
BLAST	Basic local alignment search tool
Bp	Base pairs
ca.	Circa
Cat #	Catalog number
CFU	Colony forming unit
ChemFETs	Chemical sensitive field effect transistor
Cm	Centimeter
D.W.	Distilled water
DFA	Discrimination function analysis
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
dNTPs	Deoxyribonucleotide triphosphates
EDTA	Ethylenediaminetetraacetic acid
ELIZA	Enzyme-linked immunosorbent assay
EN	Electronic nose
ET	Electronic tongue
G	Gram
HCA	Hierarchical cluster analysis
H	Hour/ hours
ISFET	Ion-sensitive field-effect transistor
KDa	Kilodalton
L	Liter
LDA	Linear discriminate analysis
LOD	Limit of detection
LT	Heat-labile

M	Molar
$MgCl_2$	Magnesium chloride
min	Minute/ minutes
mL	Milliliter
mm	Millimeter
mM	Millimolar
MVDA	Multivariate data analysis
NA	Nutrient agar
NB	Nutrient broth
NH_4SO_4	Ammonium sulfate
PCA	Principle component analysis
PCR	Polymerase chain reaction
PCs	Principal components
PLS	Partial least square
Psi	Pounds per square inch absolute
RNA	Ribonucleic acid
rpm	Round per minute
rRNA	Ribosomal RNA
RTU	Ready to use
Sec	Second
SEs	Staphylococcal enterotoxins
SIMCA	Soft independent model class analogy
ST	Heat-stable
Stx1	Shiga toxin 1
Stx2	Shiga toxin 2
SVMs	Support vector machines
TBE	Tris borate EDTA
TE	Tris EDTA
TRI reagent	TRIzol reagent
Tris	Tris(hydroxymethyl)aminomethane
Tris-HCl	Tris- hydrochloric acid
U	Unit
UV	Ultra violet
Ver	Version
xg	Relative centrifugal force (RCF) or G-Force

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Abstract

Keywords: Electronic tongue, Foodborne pathogens, Multivariate data analysis, Principal component analysis, Partial least squares model.

Background: Electronic tongue (ET) has been an essential tool in the medical field as an alternative to the traditional diagnostic method. It is based on a multi-sensor array set with characteristics of high cross-sensitivity and low selectivity. This research is carried out to investigate the possibility of using Astree II Alpha MOS ET as a fast and alternative assessment tool for early diagnosis and detection of human pathogenic bacteria by determining the limit for early detection (for colony forming unit (CFU) concentrations and incubation periods) and diagnosis of foodborne human pathogenic bacteria. Moreover, to use ET to identify unknown bacterial samples relaying on pre-stored bacterial models.

Methodology: Two bacterial strains, a gram positive and gram negative:- *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC25922), respectively, were used for this research. In which ET was verified for its ability to sense their presence in nutrient broth (NB) at the limit of detection (LOD) level for the inoculum concentrations and incubation periods using partial least square (PLS) regression. The bacteria were proliferated with original inoculum (approximately $107*10^{5}$ CFU/mL) that were diluted up to 10^{-14} and the dilutions ranged from 10^{-14} to 10^{-4} were measured using ET. Then the lowest detected concentration was identified and monitored to be used for growing the bacteria with different incubation periods (from 4 to 24 h). After that, all the measured data for both concentrations and incubation periods of each bacterial type were collected and performed in the principal component analysis (PCA) scores plot model. This was followed by projecting unknown samples of bacteria that were with specific concentration and time of incubation; to check ET's ability to recognize and categorize these unknowns.

Results: The results have approved the ability of Astree II ET in tracking bacterial growth and following the metabolic changes in the media. It was able to sensitively identify microorganisms' proliferation even with very low concentration (between the dilutions 10^{-11} and 10^{-10} for both bacteria *E.coli* and *S.aureus*). Likewise, it was able to detect *S.aureus* at a 6 h

incubation period and between 6 and 8 h for *E.coli*, which is the LOD that ET can detect these two types of bacteria. After creating the strains models, ET was able to classify each unknown sample according to their foot-printing characteristics in the media; and to determine which one was *S.aureus*, *E.coli*, and neither of them.

Conclusions: This research and results can be used for the subsequent step in considering ET as a powerful potentiometric tool for early and fast identification and classification of harmful foodborne microorganisms in their native state within a complex system, to save patients' lives. Moreover, open a wide range of applications that ET will be door as an alternative useful monitoring technique.

1. Introduction

1.1 General introduction

Microbial infections can lead to dangerous diseases, where a low count of specific pathogenic bacteria is adequate to initiate infection and cause potential damage to the human host system. Patients with dangerous bacterial diseases can be treated by accurate and early diagnosis of the causative bacterial infections, which requires combining signs and symptoms with precise diagnostic tests (Aljamali, 2021; Parry *et al.*, 2002). This is important to give suitable treatment as soon as possible and to avoid unnecessary antibiotic use. Therefore, the development of detecting strategies for different kinds of pathogens is an important aspect of health and safety. Different analytical methods such as polymerase chain reaction (PCR), colony count, enzyme-linked immunosorbent assay (ELISA), electrophoresis, biosensors, *etc*. have been employed for the detection of these pathogens (Nordin *et al*., 2016; Yu *et al*., 2016). The most popular method for bacterial detection includes culturing, colony counting, and phenotypic characteristics, which usually require 24 to 48 h to grow the pathogen and obtain a pure culture for further testing. Although some of the available diagnostic methods (such as surface recognition, PCR nucleic acid detection, enzyme-mediated and antigen testing) are quite sensitive, they are expensive, time-consuming, requires a high sophistication level

and complex sample preparation (Qin *et al*., 2011; Zhao *et al*., 2018). Hence, rapid and clear identification of the causative pathogen can be a necessary factor in the medical diagnostics sector; for saving patient lives by the implementation of appropriate therapy (Podrazka *et al*., 2018; Umesha and Manukumar, 2018).

Under these circumstances, new, advanced, ultrasensitive, and rapid methods are needed to improve the capability of detecting a few or a single pathogenic bacterial species in the target samples (such as water, food, or biological tissues) (Rohde *et al*., 2017). Bio and chemical sensor technology have become increasingly popular analytical tools for complex liquid analysis (Fernández López *et al*., 2021; Kumar *et al*., 2021; Srivastava *et al*., 2020). Electronic nose (EN) and electronic tongue (ET) are devices that try to mimic human smell and taste sensing (gas and liquid sensors, respectively) and their communication with the human brain (Abu-Khalaf, 2021; Abu-Khalaf and Masoud, 2022; Chistodoulides *et al*., 2019; Ha *et al*., 2015; Masoud *et al*., 2021; Mudalal and Abu-Khalaf, 2021; Peris and Escuder-Gilabert, 2016). Complex liquids can be analyzed using numerous promising tools such as ET systems. These systems are based on a multi-sensor array scheme characterized with pronounced cross-sensitivity and with low selectivity (Legin *et al*., 2019; Lorenz *et al*., 2009; Wang and Liu, 2019). During their process, signals obtained are processed

with multivariate data analysis (MVDA) techniques, such as principle component analysis (PCA), partial least square (PLS), discrimination function analysis (DFA), and soft independent model class analogy (SIMCA), which allow for obtaining qualitative and quantitative information on the analyzed samples (Kumar *et al*., 2018; Legin *et al*., 2019; Masoud and Abu-Khalaf, 2021; Masoud *et al*., 2021; Veloso *et al*., 2018; Wang and Liu, 2019; Wesoly and Ciosek, 2018). The usefulness of using ET in the medical analysis showed rapid bacterial detection and have been a promising alternative, rapid, reliable, and highly sensitive in shortening the detecting period as much as possible for many physicians, medical laboratories, and even patients (Al Ramahi *et al.*, 2019; Mohamed and Abdel-Mageed, 2010; Wasilewski *et al*., 2019).

1.2 Aim

This research aimed to evaluate and/or determine the limit for early detection (LOD) and diagnosis of foodborne human pathogenic bacteria using a chemical sensor such as ET and multivariate data analysis.

1.3 Objectives

The objectives of this study are:

1. To determine the limit of detection for bacteria that ET can recognize (number of colony forming unit (CFU)),

2. To evaluate the earliest time of bacterial detection using ET, and

3. To use ET to identify unknown bacterial samples relaying on a pre-stored bacterial model.

2 Literature review

2.1 Foodborne pathogens

Foods can be contaminated by different infectious pathogens, such as viruses, parasites, fungi, and bacteria. Foodborne diseases are an important cause of mortality and morbidity worldwide and have an influence on public health; more than 250 foodborne diseases have been described (Gourama, 2020; Kassahun and Wongiel, 2019; Noor, 2019). Bacterial related poisoning is one of the most common diseases, in which more than 90% of the cases each year are caused by *Staphylococcus aureus*, *Salmonella*, *Clostridium perfringens*, *Campylobacter*, *Listeria monocytogenes*, *Vibrio parahaemolyticus*, *Bacillus cereus*, and Entero-pathogenic *Escherichia coli* (Al-Mutairi, 2011; Parry *et al*., 2002; Terranova and Blake, 1978).

Among poisoning bacteria, some are more important in terms of the frequency and seriousness of the disease. Gram negative and gram positive bacteria produce toxins that adequate to initiate infection in the body and cause potential damage to the host system (Gourama, 2020; Noor, 2019). Infections can be ranged from low to severe life-threatening diseases such as upset stomach, stomach cramps, nausea, vomiting, diarrhea, fever, signs of dehydration (including little or no urination and very dry mouth and throat), while some illnesses cause long term health problems (*i.e*. chronic

arthritis, brain and nerve damage, and kidney failure) or even death (Aljamali, 2021; Ukah *et al*., 2018).

2.2 Foodborne pathogens diagnostic

Early diagnosis of bacterial infections is very crucial and can be life-saving. There has been much research related to the diagnosis of human infectious bacteria using traditional plate count and morphological characteristics, where they explained the advantage and disadvantages of using this method (Ghasemi-Varnamkhasti *et al*., 2018). Moreover, the same evaluating studies have been done relying on using other sophisticated diagnostic methods such as PCR, ELISA, nanoparticles, and microscopic staining. Likewise, in recent years the presence of bacterial infections have been confirmed using some infection markers such as interleukins, C-reactive protein, and procalcitonin; these markers, unfortunately, cannot identify the causative agent, it only can indicate if there is a bacterial infection or not (Carlson *et al*., 2018; Liu *et al*., 2019; Yu *et al*., 2016; Zhao *et al*., 2018). The currently established techniques that are used for microorganisms' identification are based on culturing the microorganisms on an appropriate medium and then morphologically determining it by their phenotypic characteristics such as the color, odor, shape, microscopic staining, selective media, and applying differential antibiotics (Ahn *et al*., 2018; Suslick *et al*., 2018). However, these

techniques are time-consuming (taking up to 72 h), expensive, need intensive laboratory preparations and consumable requirements. Moreover, it is often inadequate for differentiating phenotypically similar species or strains and gives a high possibility of false-negative and/or positive results (Kim *et al*., 2016; Law *et al*., 2015). Although some of the available sensitive diagnostic methods (*i.e.* enzyme-mediated, antigen testing, and PCR nucleic acid detection) are accurate, they require a highly sophisticated level of equipment and complex sample preparation. Moreover, they are not easy, expensive, and time-consuming (Ahari *et al*., 2017; Nordin *et al*., 2016; Qin *et al*., 2011).

According to the current situation and the need to save patients' lives; seeking new ultrasensitive and rapid methods are obligatory needed to improve the capability of detecting a few or single bacterial species in the target samples. For that, biosensor technology has become a popular analytical tool for complex liquid analysis.

2.3 Gram positive and gram negative bacteria

Gram positive and gram negative are the two major classified groups of bacteria, where their dissimilarity comes from the structure of the cell wall that gives different gram staining colors. The gram-positive bacteria have a cell wall with thick peptidoglycans layer, which retain the crystal violet dye during gram staining to have a violet color. Gram-positive

species have chemical diversity linked to the composition of the peptide crosslinks between glycan strands (repeating units of the disaccharide N-acetyl glucosamine-N-acetyl muramic acid-forming macromolecular network) and the high variability of long anionic polymers (variable structure and chemical composition like glucosyl phosphate and glycerol phosphate repeats), which are covalently attached to peptidoglycan and anchored to the head groups of the membrane lipids (Luderitz *et al*., 1982; Ruhal and Kataria, 2021; Sutcliffe and Russell, 1995). The Gram positive *Staphylococcus aureus* is a facultatively anaerobic, non-motile, and grape-like clusters bacteria. It catalase and coagulase enterotoxins called Staphylococcal enterotoxins (SEs), a group of low molecular weight proteins (about 26–30 kDa) with super antigenic activity (Moreillon *et al*., 2005; Watkins *et al*., 2019). SEs are highly resistant to denaturation conditions such as heat treatment, low pH, and proteolytic enzymes; hence retaining their activities in the digestive tract and its capacity causing a substantial impact on public health such as toxic shock syndrome and septicemia. Studies involving the incidence of new SE genes in *S.aureus* strains are growing, where strains carrying only new types of SE genes have been isolated from food poisoning cases. About 25% percent of the isolated *S.aureus* strains from food samples are considered to be enterotoxigenic strains, but this estimation varies from one food to another

and from one study to another (Dinges *et al*., 2000; Le Loir *et al*., 2003; Lowy, 1998; Ondusko and Nolt, 2018).

On the other hand, gram-negative bacteria have a cell wall with a thin layer of peptidoglycan positioned between inner and outer cell membranes. Therefore, these species do not retain the violet dye instead and appear red during the staining process. The layer of peptidoglycan in gram-negative bacteria is thin and is enclosed by an outer membrane and an asymmetric bilayer, with phospholipids in the inner leaflet and lipopolysaccharides in the outer membrane (Luderitz *et al*., 1982). The lipopolysaccharide molecules are composed of lipid A (a core oligosaccharide) and a polysaccharide O-chain. The latter is highly variable between the group species and even their strains (Pugsley, 1993; Rojas *et al*., 2018).

The gram negative *Escherichia coli* is rod shape, facultatively anaerobic, non-spore-forming, and mesophilic bacterium. It can grow in temperatures ranging from 7 to 45˚C and is considered a part of the intestine normal flora in humans and animals. *E.coli* includes various strains that vary from weak to highly pathogenic strains, which cause variable degrees of infections in both humans and animals. Pathogenic *E.coli* are categorized into five types according to their pathogenicity: enteropathogenic *E.coli*, enterotoxigenic *E.coli*, enterohemorrhagic *E.coli*,

enteroinvasive *E.coli*, and enteroaggregative *E.coli* (Kaper *et al*., 2004; Oh *et al*., 2021). The pathogenicity of this bacteria is related to the production of various virulence toxins within the host such as Shiga toxins (Stx1 and/or Stx2), which are heat-labile enterotoxins (LT) and heat-stable enterotoxins (ST) (Qin *et al*., 2011). Moreover, *E.coli* virulence properties can be affected by colonizing and adhering factors, invaded plasmids, fimbriae, and acquired genes among plasmids, phages, or other gene transfer events. Diarrhea, hemorrhagic colitis, acute inflammation, septicemia, and urinary tract infections are common symptoms of pathogenic *E.coli* (Ekici and Dumen, 2019; Meng *et al*., 2012; Nataro and Kaper, 1998).

2.4 Electronic tongue (ET)

Solutions are analyzed using a taste sensor, also called ET, which can be considered a simple analytical instrument. It is composed of four parts auto-sampler for repeatable sensor measurement (A), a chemical multi-sensor system (B), signal acquisition system (C), and multivariate (chemometrics) software and the instrument control on PC (D) (Figure 1).

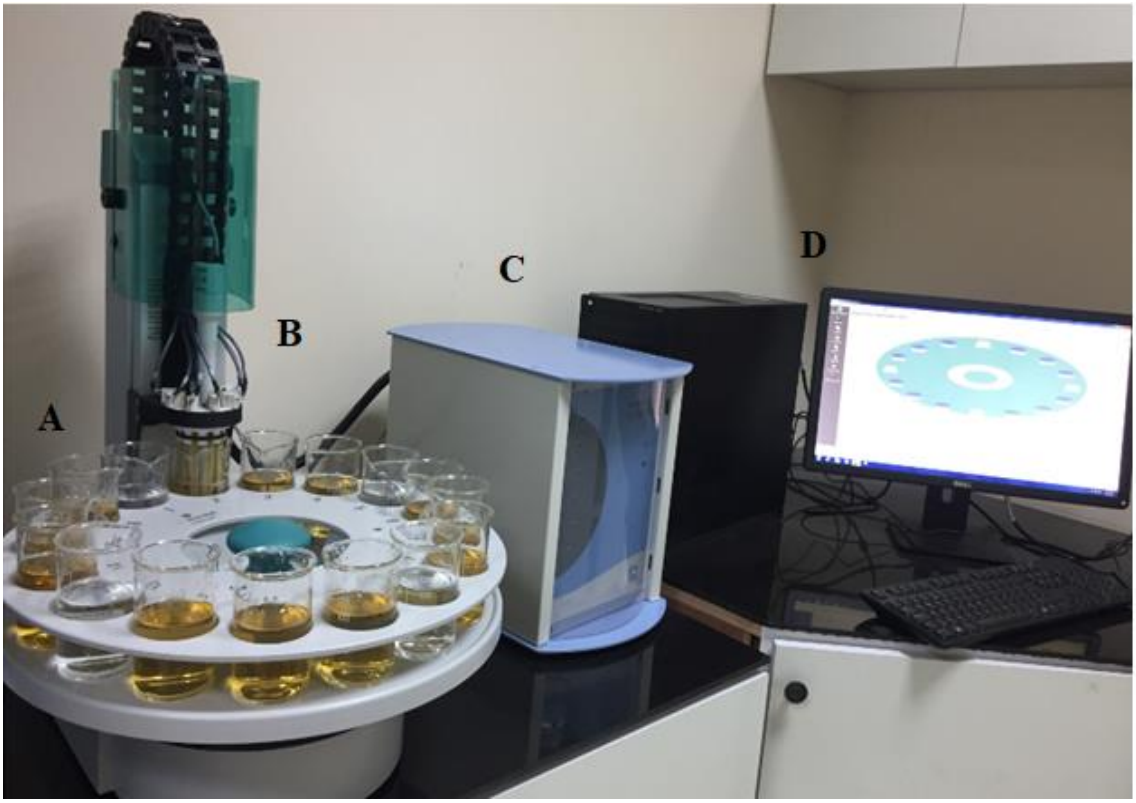

Figure 1: Typical Astree II electronic tongue package. A: auto-sampler, B: array of liquid sensors, C: electronic unit, and D: advanced chemometrics software (Alpha Soft ver. 14) (Alpha MOS, 2009).

This technology is based on transforming the information of measured substances (*i.e.* chemical composition) by a scheme of chemical multi-sensor array that transfers the sense into electrical signals that are attached with chemometrics analysis methods. In which, the intact system gives partial specificity in recognizing qualitative and even quantitative configuration of liquids. It also can provide objective low selective information with a high cross-sensitive evaluation of the previewed nonspecific complex liquids (Di Rosa *et al*., 2017; Gallardo *et al*., 2005; Jiang *et al*., 2018; Legin *et al*., 1999; Podrazka *et al*., 2018).

The necessity of using ET in this study comes from the presence of pathogenic microorganisms in food at low levels and with heterogeneous distribution; it is still capable to cause dangerous foodborne diseases in humans. Hence, this makes it hard to detect their presence within the foodstuff. Adding to that, it was able to sense the presence of different metabolites (primary and secondary) produced or consumed throughout the growth process of microorganisms, which alter the composition level within the media (Abu Rumaila, 2019; Al Ramahi *et al.*, 2019). Thus, leading to a satisfied detectable qualitative and changes reported by the system. Moreover, the chemical composition of the bacterial cell wall and/or membranes vary among different species and strains of the same class (*i.e.* different chemical groups, the activated or non-activated prone to redox reactions to electrostatic and hydrogen bond interactions), this variation could result in numerous and different cells interactions that, in long term, leads to specific sensing signals considered as finger-prints of each bacteria, which can be recognized and distinguished by sensors (Kumar *et al*., 2021; Moreno *et al*., 2006; Peris and Escuder-Gilabert, 2016; Qneibi *et al*., 2018; Skladal, 2020; Veloso *et al*., 2018; Wang and Liu, 2019).

ET system was successfully applied for microorganisms' foot-printing and determining the relationship among the tested bacterial and

fungal samples, and it was used in early identification and diagnosis of bacterial infections (Abu Rumaila, 2019; Abu-Khalaf and Abu Rumaila, 2020; Chistodoulides *et al*., 2019). Moreover, it was applied to analyze various pharmaceuticals, environmental objects, foods and drinks, fermentable microbial uses, and microorganisms' studying (Abu-Khalaf *et al*., 2015; Abu-Khalaf *et al*., 2018; Abu-Khalaf *et al*., 2018; Abu-Khalaf *et al*., 2019; Al Ramahi *et al*., 2019; Baldwin *et al*, 2011; Ceto *et al*., 2016; Gomez *et al*., 2019; Masoud *et al*., 2021; Taha and Abu-Khalaf, 2020; Wasilewski *et al*., 2019; Wesoly and Ciosek, 2018).

2.4.1 Types of ET

There are three public types of ET: volumetric, potentiometric, and taste sensor (*i.e.* lipid/ polymer membrane). They differ by the principal of the used sensors (Bougrini *et al*., 2016; de Morais *et al*., 2019; Faura *et al*., 2016; Ghissi *et al*., 2021; Kalinke *et al*., 2019). The potentiometric Astree II ET used in this research is composed of modified seven chemical sensitive field effect transistor (ChemFETs) solid electrochemical sensors. The type of Astree's sensors is ion-sensitive field - effect transistors (ISFET), which, are patently have been developed by Alpha MOS Company (Alpha MOS, 2009). The sensors set is composed of two main parts, the first is seven sensing transducers (CA, JB, HA, ZZ, BB, JE, and GA) with a chemically coated sensitive layer and the second is Ag/AgCl

reference electrode (*i.e.* silver-silver chloride). In which, the signals are obtained by the potentiometric alteration between each sensor and likened to the reference one. Where the trapping of ions on the sensitive layer of each sensor generates a potential change that can be measured by the ISFET sensors that creates an electronic signal (Figure 2).

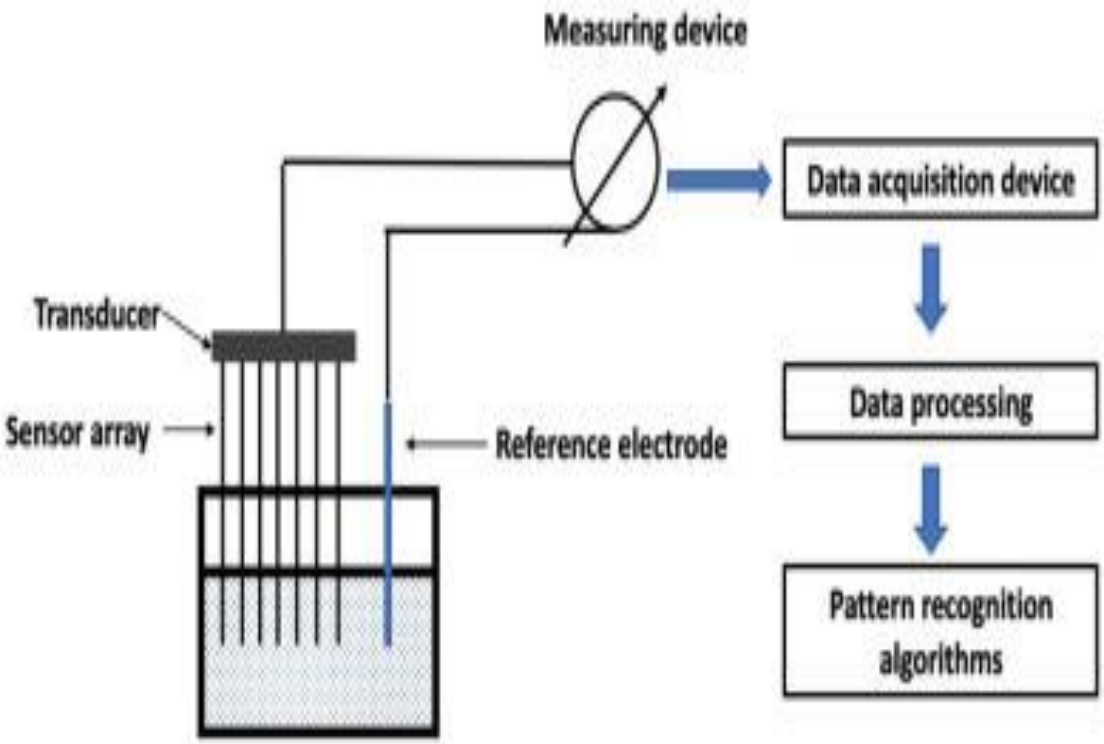

Figure 2: Schematic diagram of a potentiometric type ET, showing seven ISFET sensors immersed in a complex liquid sample, the potential difference signals are sent to a signal processor system to be analyzed using pattern recognition algorithms (Kovács *et al*., 2009).

Moreover, the organic coating of sensors was created with specificity to ensure their reproducibility, selectivity, and sensitivity of each sensor to the dissolved compounds in liquid media (Fernández López *et al*., 2021; Ha *et al*., 2015; Legin *et al*., 2019; Soderstrom *et al*., 2003). However, the main disadvantages of this type of ETs are temperature dependence and the adsorption of solution components on sensors can affect the nature of

charge transfer (Di Rosa *et al*., 2017; Ghissi *et al*., 2021). Subsequently, the outputted signals of the sensors array are then processed by the computer system and patterned with multivariate qualitative and quantitative chemometrics tools (e.g., principal component analysis (PCA); soft independent modeling of class analogy (SIMCA); linear discriminant analysis (LDA); partial least-squares regression (PLS); support vector machines (SVMs); artificial neural networks (ANNs)) and save the data for future uses (Alpha MOS, 2009; Masoud and Abu-Khalaf, 2021; Masoud *et al*., 2021; Mudalal and Abu-Khalaf, 2021; Soderstrom *et al*., 2003; Soderstrom *et al*., 2005).

2.4.2 Principal component analysis (PCA)

Acquired signals are translated to distinguished patterns using MVDA software methods. In categorizing the electro-chemical responses of sensors, some of these methods are supervised while others are not (known as exploratory data analysis method). ET Alpha software ver.14 has several methods such as principal component analysis (PCA), partial least squares (PLS), and linear discriminate analysis (LDA).

The unsupervised PCA method for example does not need any prior knowledge about the class structure of the data, where it creates grouping categories of the analyzed samples according to their close characteristics (Abu-Khalaf and Masoud, 2022; Al-Ramahi *et al*., 2019; Najjar and Abu-

Khalaf, 2021a; Najjar and Abu-Khalaf, 2021b; Qneibi *et al*., 2018). Moreover, it is widely used and often the first approach used for qualitative purposes to verify the pattern of the measured data, where it simplify and reduce the multidimensional interpreted primary variables from large dataset to lower-dimensional approximation variables (called principal components (PCs)). Scores plot is the resulted projections of points from the original data space on PCs (PC1, PC2, … PCn) and keeps most of the variance in the data by providing a set of orthogonal axes that indicates the direction of the largest variance in the data. The first principal component (PC1) books the maximum of the total variance, PC2 is the second one which orthogonally lies in the direction of the largest remaining variation, and so on inform all variance is explained by *ca.* 100% (Figure 3) (Abu-Khalaf and Masoud, 2022; Chen *et al*., 2020; Ghasemi-Varnamkhasti *et al*., 2018; Mudalal and Abu-Khalaf, 2021; Rodrigues *et al*., 2019; Winquist *et al*., 2000; Zhu *et al*., 2020).

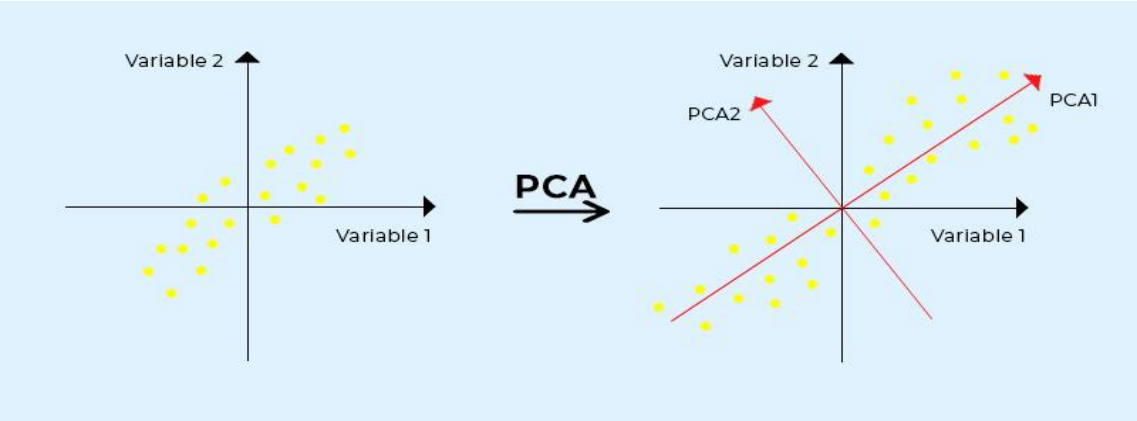

Figure 3: PCA representative chart for qualitative data analysis. It reduces multidimensional primary variables from original and large data space to lower-dimensional variables (PCA1 and PCA2) called scores plot (Scholz, 2006).

2.4.3 Partial least squares (PLS) regression and limit of detection (LOD)

PLS regression is an efficient and optimal statistical technique based on covariance, where there is multicollinearity among the variables. It works though lowering a large data within two matrices (*i.e*. X and Y) into a smaller set of uncorrelated elements and then preforms the least squares regression on them. For example, the PLS model explains the largest and multidimensional data direction in the Y matric (predicted) by trying to find the multidimensional direction in the X matric (measured or observed) (Figure 4) (Henseler, 2018; Masoud *et al*., 2021; Vinzi *et al*., 2010).

Unlike PCA, which is an unsupervised and linear method in finding hyperplanes of maximum variance among the measured data, PLS is supervised and obtains a linear regression model through projecting both predicted and observed variables into new space. It is commonly used in chemometrics and bioinformatics-related areas such as food, drug, ecology, plastic and chemical industries, and other approaches (Chen *et al*., 2018; Masoud and Abu-Khalaf, 2021; Mehmood *et al*., 2020; Najjar and Abu-Khalaf, 2021a).

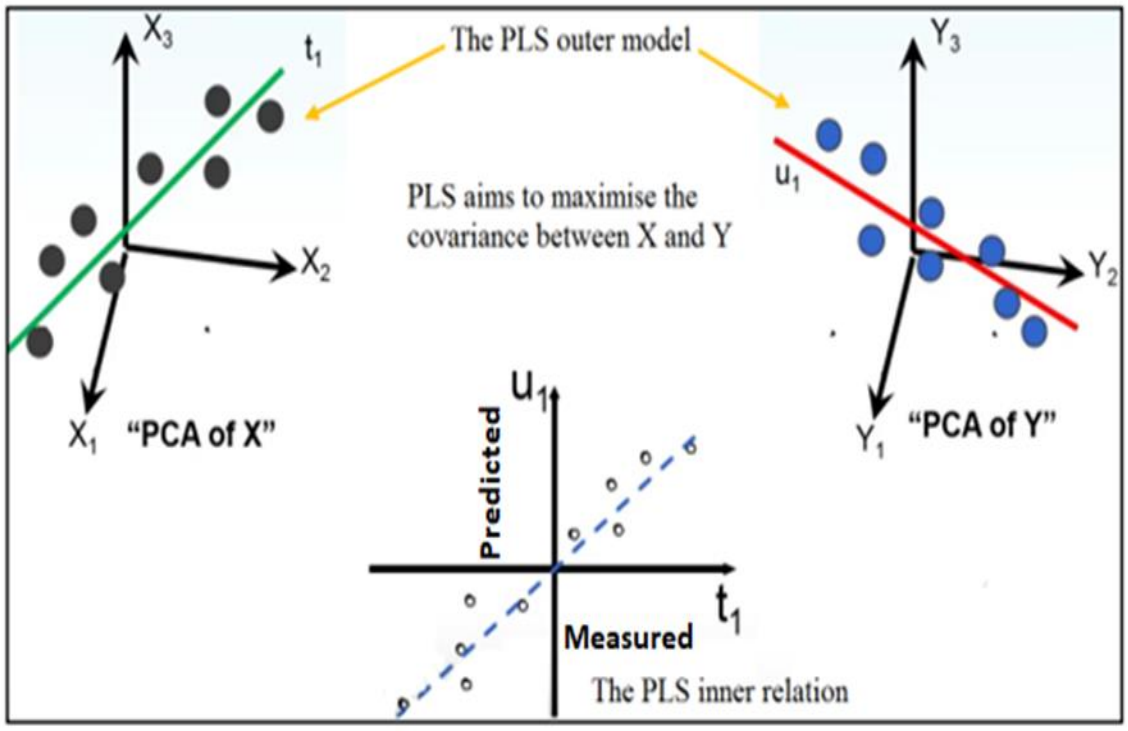

Figure 4: PLS representative chart for qualitative data analysis. It reduces multidimensional covariance variables from original and large data space to a smaller set of uncorrelated elements and then performs the least squares regression on them.

PLS is a suitable MVDA tool that can contribute to determining the limit of detection (LOD) for this study. LOD is a concept that describes the lowest concentration of a variable in a sample that can be constantly detected by a particular measurement procedure at a specified level of confidence without the necessity of being quantitated as an exact value (Abu-Khalaf and Haselmann, 2012; Chen *et al*., 2020; Masoud *et al*., 2021; Najjar and Abu-Khalaf, 2021b).

3 Material and methods

3.1 Bacterial experiment

3.1.1 Media preparation

Bacterial isolates were cultivated on nutrient agar (NA) media. The latter was prepared by dissolving a complete weight of 23 g NA powder in 1 L distilled water (DW) by heating. Then, the solution was sterilized at 121˚C and 15 psi for 15 min autoclaving program. After that, the purified solution was cooled enough to be poured in 9 cm petri dishes, this action was done under aseptic conditions on a microbiological safety cabinet (MN 120) and finally stored in a refrigerator for cultivation purposes.

Meanwhile, for ET measurements, bacterial cultivation was on nutrient broth (NB) media. The media was prepared by dissolving a complete weight of 13 g NB powder in 1 L distilled water (DW) by heating. The solution was then suspended in 250 mL Erlenmeyer flasks, each contained 100 mL of the suspension that was labeled and sealed with aluminum foil for autoclaving at 121˚C and 15 psi for 15 min and used for later bacterial growth, the overall action was also done at aseptic conditions on microbiological safety cabinet (MN 120).

3.1.2 Bacterial collecting and maintenance

Escherichia coli and *Staphylococcus aureus* were used for this study as common representative samples for both grams negative and gram positive bacterial groups, respectively. These two bacteria are also known as generic pathological causes for infectious diseases in humans. The strains were obtained from the American Type Culture Collection (ATCC) including *E.coli* (ATCC25922) and *S.aureus* (ATCC 25923).

The two bacteria were maintained with dual culturing on NA media every two weeks using a cotton swab, by spreading small bacterial inoculum of previously cultured growth over a new NA media (Figure 5), that were labeled, sealed, and incubated for 24 h at 37˚C. For liquid growth, fresh three colonies (approximately $107*10^5$ CFU/mL) of pure cultured bacteria were grown in 100 ml nutrient broth (NB) media. After that, the inculcated flasks were then incubated at 37˚C with shaking at 150 rpm for different periods and different dilution studies.

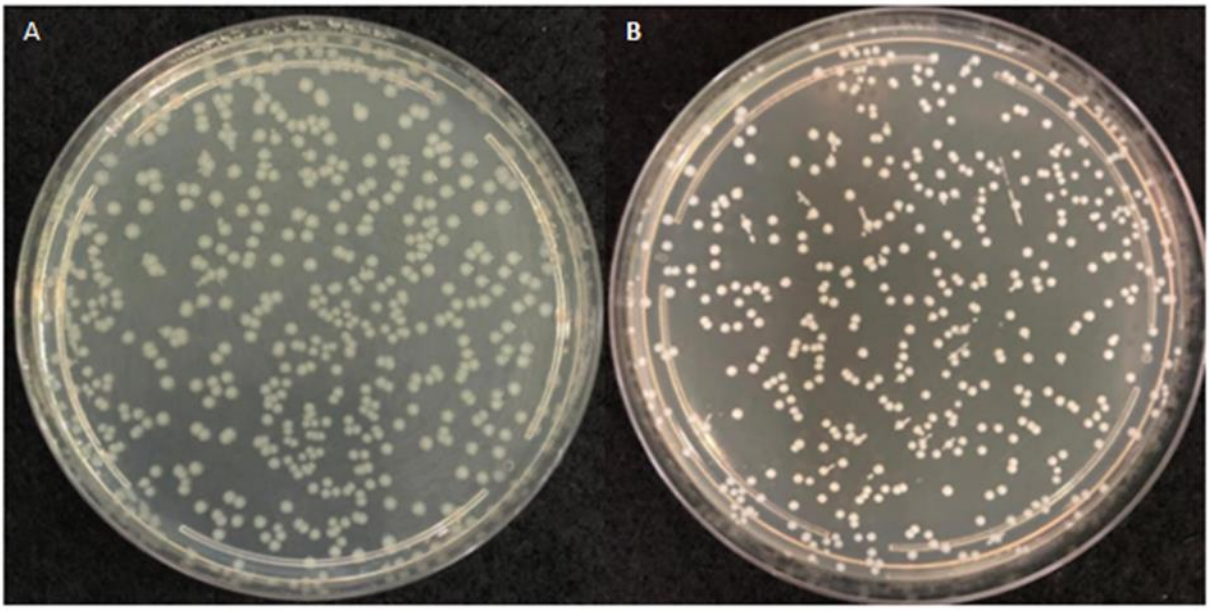

Figure 5: Full sustained *E.coli* (A) and *S.aureus* (B) over NA media after incubation at 37˚C for 24 h.

3.1.3 Bacterial colony forming unit (CFU) counting and dilution

For the original viable bacterial count, three well-isolated colonies from fresh NA culture media were suspended in 1 ml sterile NB media that were homogenized using a vortex, then 0.1 ml of stock was serially diluted in 0.9 ml NB for ten folds. After that, 0.1 ml of each dilution was plated on agar with sterile pipettes, spread with glass hockey sticks, and incubated at 37˚C. Then after 24 h of incubation, well-isolated colonies were counted and those within the average of 25-250 CFU were recorded for applying on the following equation:

CFU/mL = (no. of colonies x dilution factor) / volume of culture plate

The process was repeated three times for the average count. For the dilution test, the same dilution process was applied where 1 ml of each dilution was suspended in 99 ml of sterilized NB media in 250 ml flasks that were then incubated at 37˚C with shaking.

3.1.4 Bacterial molecular phylogenetic

3.1.4.1 Bacterial DNA isolation

TRIzol reagent manual (TRI reagent) (Cat. # T942) was applied for the bacterial DNA isolation procedure. Where 1 mL of TRI reagent was used to dissolve a small portion of freshly grown bacteria (grown in NA media) in 1.5 mL microfuge tubes. Then it was variously homogenized using a vortex, and allowed to stand for 5 min at room temperature. After that, per 1 ml added TRI reagent 200 µL of absolute cold chloroform was added to the suspension and shaken vigorously for 15 sec, and left to stand for 15 min at room temperature.

Afterward, at 12000 xg (11573 rpm) the resulted mixture was centrifuged for 10 min at 4˚C to give three phases:

- Colorless upper phase (RNA),

- Inter phase (DNA), and

- Red organic phase (protein lower phase).

At this point, for the added 1 mL of TRI reagent, a 300 µL of cold 100% ethanol was added after removing and discarding the aqueous overlying phase. Tubes were inverted a few times to be mixed and let to stand for 3 min at room temperature then centrifuged at 2000 xg (4730 rpm) for 5 min at 4˚C. The resulted supernatant was removed to be discarded or saved for later protein isolation (if needed). Now, 1 mL of cold 0.1 M Trisodium-citrate in 10% ethanol solution was used for washing the remaining DNA pellets (twice). Subsequently, tubes were allowed to stand for at 30 min with occasional mixing, centrifuged at 2000 xg (4730 rpm) for 5 min at 4˚C, and the resulting pellets were resuspended with 1.5 mL of 75% cold ethanol and allowed to stand for 20 min at room temperature. Later, tubes were centrifuged at 2000 xg (4730 rpm) for 5 min at 4˚C with discarding the resulting supernatant. In the end, under the vacuum hood pellets were dried for 10 min, dissolved in 50 µL of TE buffer, and stored at -20˚C for further uses.

3.1.4.2 Sequences amplification, electrophoresis, and data analysis

- 16S rRNA gene sequence amplification reaction

A universal 16S bacterial primer set was used for DNA templates' PCR amplification. Forward 27F (AGATTTGATCTGGCTCAG) and reverse primers 1492R (TACGGTTACCTTGTTACGACTT) were dissolved in sterilized distilled DNase free water volume to have a final concentration of 100 µM and stored at -20˚C.

Amplification mixture was done using Go taq green 2X PCR master mix with 3 mm $MgCl_2$ (Cat. # AF9PIM712 0418M712). In which, for 25 µL PCR reaction mixture it contains 12.5 µL of 2X ready mix PCR master mix (75 mM Tris-HCl, 20 mM $(NH_4)^2SO_4$, 0.625 U Thermo prime taq DNA polymerase, 0.2 mM of each dNTPs, 1.5 mM $MgCl_2$), 0.5 µL of 50 mM $MgCl_2$, 0.125 µL of 100 µM forward primer, 0.125 µL of 100 µM reverse primer, 10.75 µL of free DNase water and 1 µL of DNA template.

-The PCR amplification program

VertiTM 96 well thermal cycler (Cat. #: 4375786) (Applied Biosystems company, California, USA) was used to perform a PCR

amplification program. The program started with an initial 94˚C denaturation cycle for 3 min, followed by 35 cycles of 45 sec denaturation cycle at 94˚C, 50 sec of 51˚C annealing cycle, and 1 min extension cycle at 72˚C, and then 7 min of final extension cycle at 72˚C.

PCR procedure was duplicated for each isolate, to guarantee the reproducibility of the amplified DNA fragments. A blank sample without DNA was also run to confirm the results (as negative control).

- Gel electrophoresis

To separate total extracted bacterial DNA, a 0.8% agarose electrophoresis gel was used. Meanwhile, 2% agarose electrophoresis gel was prepared to separate 16S rRNA gene PCR products according to their molecular weight. The gel was prepared by dissolving 2 g of agarose powder completely in 100 mL of 1X TBE buffer (add 10.8 g Tris and 5.5 g Boric acid in 900 ml distilled water, then add 4 ml 0.5 M Na_2EDTA (pH 8.0), then adjust the volume to 1 L) with heating using the microwave. The mixture was cooled to 60˚C, after that, 4 µL Gel Red DNA stain 1000X (Cat. #41003) was added and stirred. The suspension was then powered and allowed to solidify in a (10 x 10) tray with 13 wells comp. After submerging

the gel in 1X TBE buffer and loading 5 µL of PCR products, the device was run for 2 h at 70 volts.

10000X Gel Red DNA stain and UV-illuminator were used to visualize DNA fragments and SynGene gene tool system (Synoptics Ltd., Cambridge C, UK) was used to document it using image acquisition and documentation. For estimating DNA fragments size, a DNA ready-to-use (RTU) ladder (Cat. # DM001-R500) of 100 bp was used as a molecular size marker. Finally, stored PCR products were sent for sequencing through Biotech company. The obtained bacterial sequences were aligned using the universal BLAST program (National Center for Biotechnology Information, Maryland, USA).

3.2 ET experiment

For bacterial broth analysis, a liquid taste analyzer Astree II ET (Alpha MOS Company, Toulouse, France) was used. That is composed of seven sensor arrays (CA, JB, HA, ZZ, BB, JE, and GA) with an Ag/AgCl reference electrode.

3.2.1 Bacterial broth sample preparation

On ET five testing rounds of bacterial samples (two for *E.coli*, two for *S.aureus,* and one for both bacteria together) were measured. For the two rounds of each, the first round was for determining the limit of detection (LOD) (limited CFU) that ET can detect after 24 h incubation period. The second round was for determining the least incubation time that ET can detect after adding the previously detected least CFU. The final round (*i.e.* the fifth) was done to test our result by applying an unknown bacterial sample of *E.coli*, *S.aureus,* and others (*S.agalactiae* and *P.aeruginosa*) that were grown at the least incubation time and CFU.

In each round, 11 bacterial samples with an NB sample (control) were tested in triplicate. The media was prepared as mentioned in section 3.1.1. For the concentration experiment, a serial dilution of an original concentration (approximately $107*10^{5}$ CFU/mL) was serially diluted up to 10^{-14} as elaborated in section 3.1.3 with proper labeling (see section 3.2.2 for labeling code), in which only the samples with dilution 10^{-14} to 10^{-4} were analyzed using ET after 24 h incubation period (Figure 6). Followed by the growth period experiment, that was done by inoculation the determined least concentration CFU of each type of bacteria (approximately $88*10^{-9}$

CFU/mL) on prepared NB under sterilized conditions that were incubated for different periods (4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 h) that were properly labeled (see section 3.2.2 for labeling code).

Figure 6: Nutrient broth (NB) media grown with bacterial sample with different inoculum CFU from 10^{-4} to 10^{-14}.

3.2.2 ET sequence preparation and auto-sampler loading

To create the sequence in ET a two parts labeling was applied, where the first part has the bacterial name (*i.e.* E.coli or Staph) the other for the concentration (*i.e.* _-04 to _-14) and/or incubation period (*i.e.* _04h to _24h).

For the first ET round, only *E.coli* samples (*i.e*. E.coli_-04, E.coli_-05, …, and E.coli_-14) with NB as a control sample (E.coli_ NB) were tested (Table 1). Which, these samples were with different concentrations, but all incubated for 24 h with shaking at 37˚C (to determine *E.coli* concentration LOD that ET can recognize).

The second round of *E.coli* was with the same concentration but with different incubation periods (E.coli_04h, E.coli_06h, …, and E.coli_24h) with NB as a control sample (E.coli_ NB) were tested (Table 1). These samples were measured after 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 h of inoculation to find out the earliest time of incubation that ET can recognize their growth.

The third one, only *S.aureus* (*i.e.* Staph_-04, Staph_-05, …, and Staph_-14) with NB as a control sample (Staph_ NB) were tested. These samples were also with different inoculated CFU but incubated for 24 h with shaking at 37˚C (to determine *S.aureus* concentration LOD that ET can recognize) (Table 1).

In the fourth round of bacterial samples, *S.aureus* was tested with different incubation periods (Staph_04h, Staph_06 h, …, and Staph _24h) with NB as a control sample (Staph_ NB) (Table 1). These samples were

measured after 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 h of inoculation with approximately the same CFU concentration (to determine S.aureus LOD for incubation period that ET can recognize).

In the fifth final round, bacterial samples of *E.coli*, *S.aureus*, and two different unknown bacteria (*S.agalactiae* and *P.aeruginosa*) with NB as a control sample were tested. In which, those samples were measured at 10^{-9} CFU concentration after 10, 12, and 14 h of inoculation to identify unknown bacterial samples relaying on pre-stored bacterial data and if it can recognize them from other types of bacteria (Table 1).

Before ET testing, bacterial growth was filtered using a white cheese cloth to obtain approximately 80 mL of each broth to be placed on ET's 16-position autosampler (Figure 7), with an automatic stirrer, after creating the sequence. Samples were separated by four water samples (cleaning) for cleaning ET sensors after each test (Figure 8).

Figure 7: The auto-sampler 16 positions with 12 tested samples and 4 water samples for cleaning the sensors.

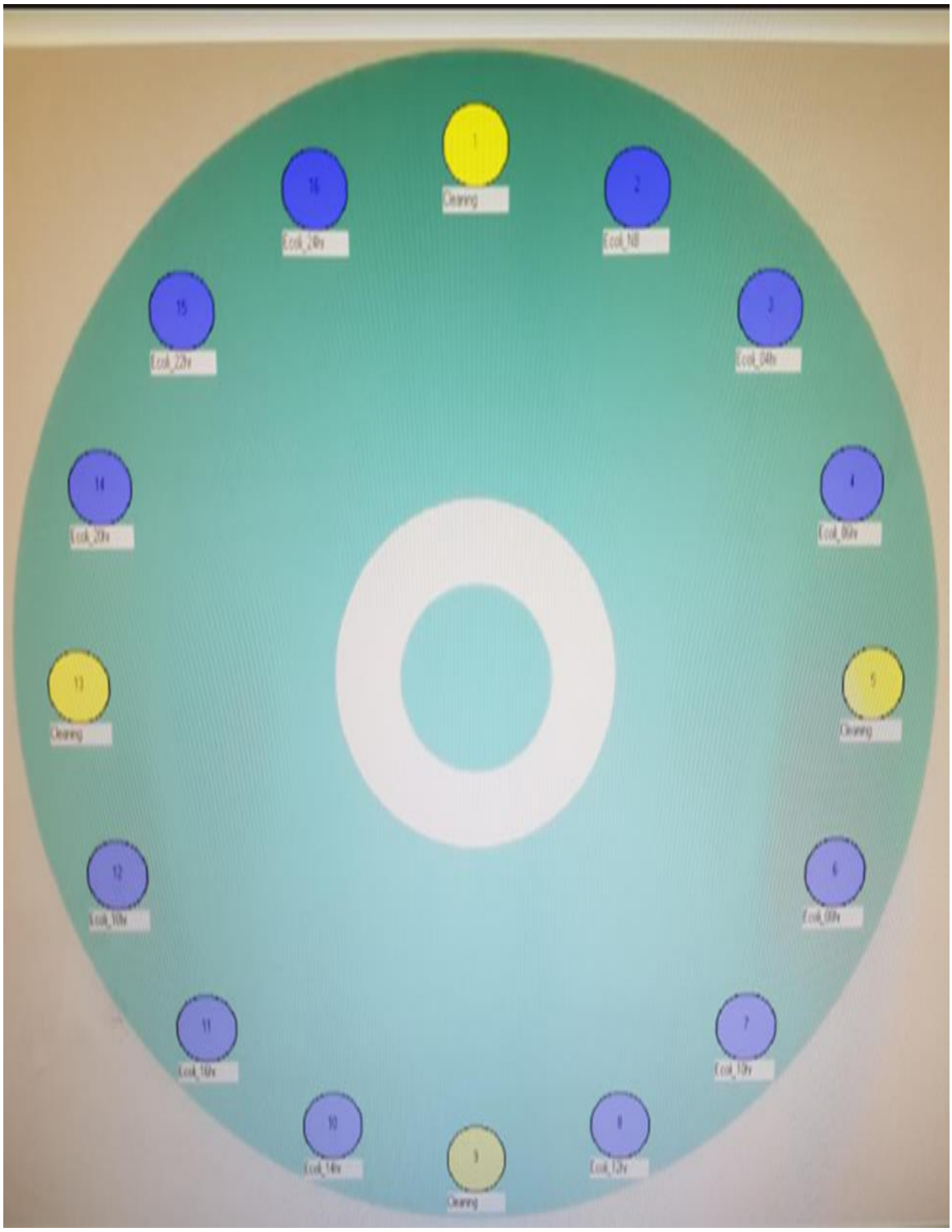

Figure 8: An example of sequence arrangement on the auto-sampler with the tested samples and cleaning water (at positions 1, 5, 9, and 13).

Table 1: The ET five experiments rounds and the labeling for each tested sample.

Round No.	Sample No.	Bacterial type	Dilution factor	The incubation period (h)	ET code	Goal of experiment
Round 1	1	*E.coli*	10^{-4}	24	E.coli_-04	***E.coli*** **concentration limit of detection test**
	2	*E.coli*	10^{-5}	24	E.coli_-05	
	3	*E.coli*	10^{-6}	24	E.coli_-06	
	4	*E.coli*	10^{-7}	24	E.coli_-07	
	5	*E.coli*	10^{-8}	24	E.coli_-08	
	6	*E.coli*	10^{-9}	24	E.coli_-09	
	7	*E.coli*	10^{-10}	24	E.coli_-10	
	8	*E.coli*	10^{-11}	24	E.coli_-11	
	9	*E.coli*	10^{-12}	24	E.coli_-12	
	10	*E.coli*	10^{-13}	24	E.coli_-13	
	11	*E.coli*	10^{-14}	24	E.coli_-14	
	12	--------	-------	24	E.coli_NB	
Round 2	1	*E.coli*	10^{-9}	4	E.coli_04h	***E.coli*** **limit of detection for incubation periods test**
	2	*E.coli*	10^{-9}	6	E.coli_06h	
	3	*E.coli*	10^{-9}	8	E.coli_08h	
	4	*E.coli*	10^{-9}	10	E.coli_10h	
	5	*E.coli*	10^{-9}	12	E.coli_12h	
	6	*E.coli*	10^{-9}	14	E.coli_14h	
	7	*E.coli*	10^{-9}	16	E.coli_16h	
	8	*E.coli*	10^{-9}	18	E.coli_18h	
	9	*E.coli*	10^{-9}	20	E.coli_20h	
	10	*E.coli*	10^{-9}	22	E.coli_22h	
	11	*E.coli*	10^{-9}	24	E.coli_24h	
	12	--------	-------	24	E.coli_NB	
Round 3	1	*S.aureus*	10^{-4}	24	Staph_-04	***S.aureus*** **concentration limit of detection test**
	2	*S.aureus*	10^{-5}	24	Staph_-05	
	3	*S.aureus*	10^{-6}	24	Staph_-06	
	4	*S.aureus*	10^{-7}	24	Staph_-07	
	5	*S.aureus*	10^{-8}	24	Staph_-08	
	6	*S.aureus*	10^{-9}	24	Staph_-09	
	7	*S.aureus*	10^{-10}	24	Staph_-10	
	8	*S.aureus*	10^{-11}	24	Staph_-11	
	9	*S.aureus*	10^{-12}	24	Staph_-12	
	10	*S.aureus*	10^{-13}	24	Staph_-13	
	11	*S.aureus*	10^{-14}	24	Staph_-14	
	12	--------	-------	24	Staph_NB	
Round 4	1	*S.aureus*	10^{-9}	4	Staph_04h	***S.aureus*** **limit of detection for incubation**
	2	*S.aureus*	10^{-9}	6	Staph_06h	
	3	*S.aureus*	10^{-9}	8	Staph_08h	
	4	*S.aureus*	10^{-9}	10	Staph_10h	

	5	*S.aureus*	10^{-9}	12	Staph_12h	**periods test**
	6	*S.aureus*	10^{-9}	14	Staph_14h	
	7	*S.aureus*	10^{-9}	16	Staph_16h	
	8	*S.aureus*	10^{-9}	18	Staph_18h	
	9	*S.aureus*	10^{-9}	20	Staph_20h	
	10	*S.aureus*	10^{-9}	22	Staph_22h	
	11	*S.aureus*	10^{-9}	24	Staph_24h	
	12	--------	-------	24	Staph_NB	
Round 5	1	*E.coli*	10^{-9}	10	UnEc_10	**Identify unknown bacterial samples relaying on pre-stored bacterial model**
	2	*E.coli*	10^{-9}	12	UnEc_12	
	3	*E.coli*	10^{-9}	14	UnEc_14	
	4	*S.aureus*	10^{-9}	10	UnSa_10	
	5	*S.aureus*	10^{-9}	12	UnSa_12	
	6	*S.aureus*	10^{-9}	14	UnSa_14	
	8	*P.aeruginosa*	10^{-9}	14	UnPs_14	
	10	*S.agalactiae*	10^{-9}	14	UnSr_14	
	11	--------	--------	12	UnNB_01	
	12	--------	--------	14	UnNB_01	

3.2.3 ET data library creation

After each measurement, the data from each sensor was collected in a folder categorized by bacterial sequence and the date of measurement for each round after creating a library of the experiment as mentioned earlier.

3.2.4 ET data analysis

The collected raw data from analyzed sensors were exported to Unscrambler X (version 10.3, Camo Software AS, Oslo, Norway), where the signals of each sensor were numerically analyzed and normalized to values be categorized using PLS and PCA.

4 Results and discussion

4.1 Bacterial experiment

4.1.1 Bacterial colony forming unit (CFU) counting and dilution

Plated counting of bacteria for the dilution process to determine the limit of detection (LOD) of CFU that ET can detect and the counting for approximately constant bacterial CFU for the earliest incubation period of identification, in which the dilution with well, separated, and countable bacterial colonies (25-250 CFU) were considered for the ET testing procedure (Figure 9).

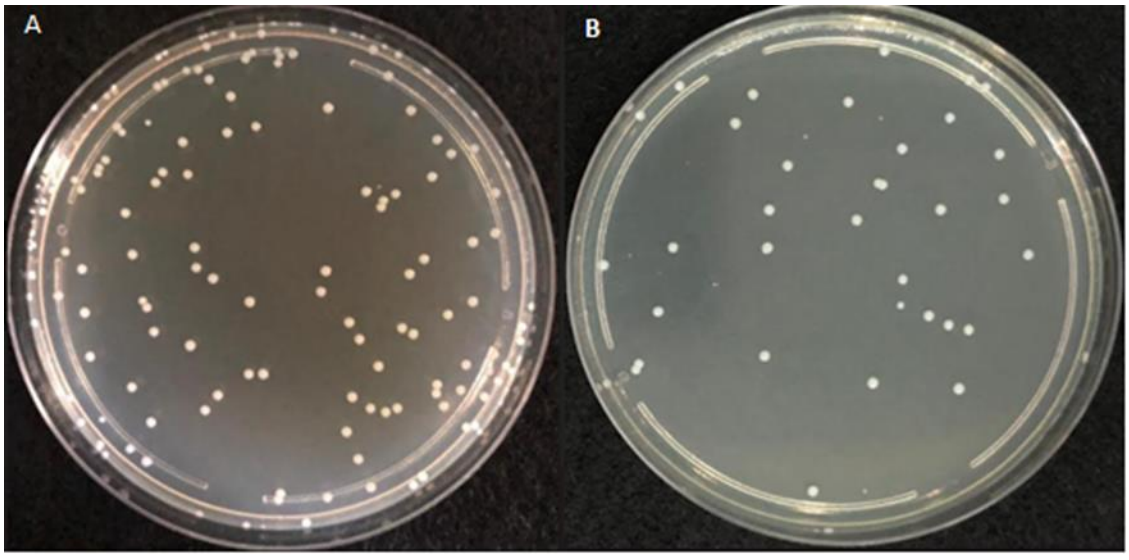

Figure 9: Type plated bacteria over NA media for counting after a serial dilution. A: plate with *E.coli*, B: plate with *S.aureus*.

4.1.2 Bacterial DNA isolation and PCR

Total DNA extraction of four bacterial samples (two for each type) using the TRI reagent method is shown in Figure 10. This was used to confirm the presence of the extracted bacterial DNA.

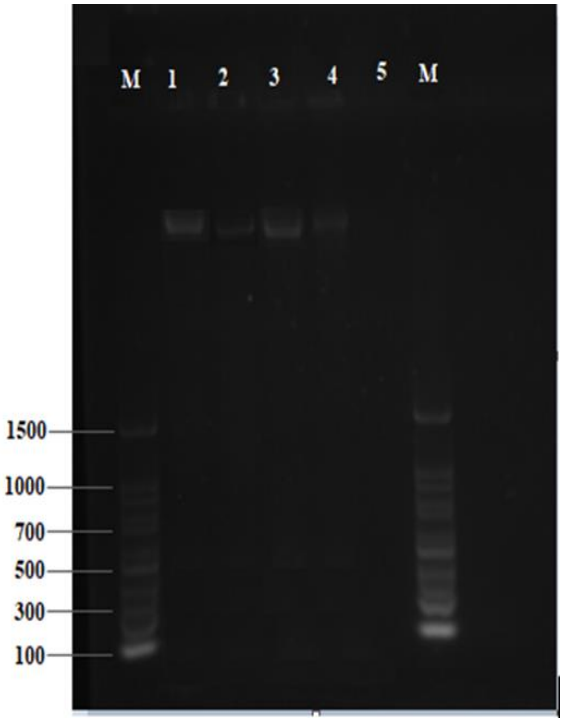

Figure 10: Gel electrophoresis documented photos of total DNA isolated from bacterial samples using TRI reagent method for genomic isolation. Where lanes from 1and 2 represent *E.coli* samples, 3 and 4 represent *S.aureus* samples, 5 is a negative control. M=100 bp ladder as a molecular size marker.

The resulted 1500 pb bands of PCR amplification for the DNA templates' using universal 16S bacterial primer set PCR amplification. Forward 27F (AGATTTGATCTGGCTCAG) and reverse primers 1492R (TACGGTTACCTTGTTACGACTT) are shown in Figure 11.

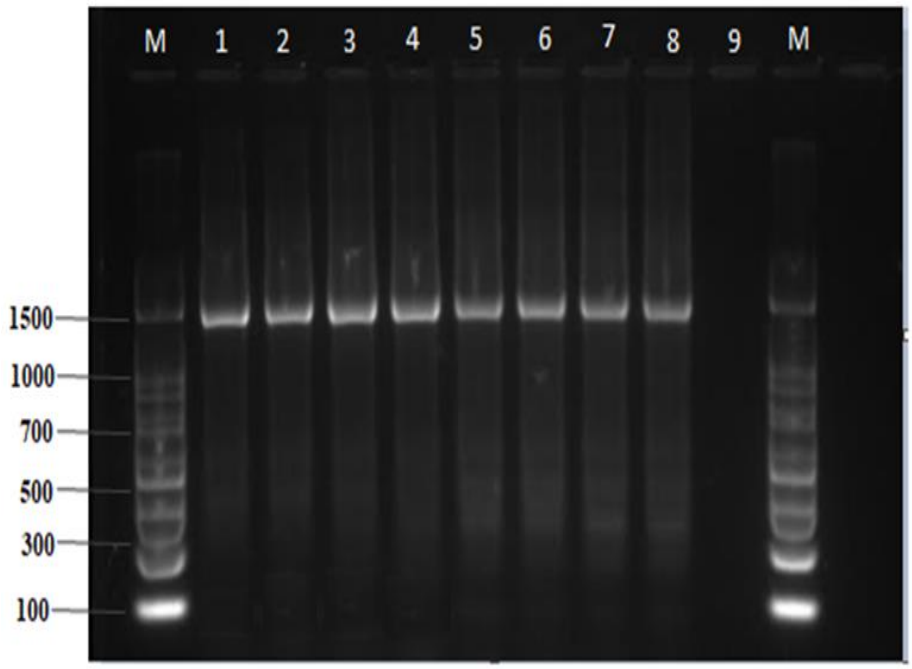

Figure 11: Gel electrophoresis documented photo for 16S rRNA amplification revealed in eight bacterial isolates using primer 27F and 1492R. 1-4 represents *E.coli* samples, 4-8 represents *S.aureus* samples, 9 is a negative control M=100 bp ladder as a molecular size marker.

4.1.3 Sequence identification

The results of BLAST alignment of the 16S rRNA gene sequences of *E.coli* and *S.aureus* bacterial samples are shown in Figure 12 and Figure 13, respectively. With a sequence homology of 99% for *E.coli* to strain NBRC 102203 and 100 % for *S.aureus* to strain ATCC 12600 were obtained. The 99% homology for *E. coli* may be due to mutations throughout the subsequent culturing or the sequencing process. It can also be attributed that *E. coli* used in this study is a different strain from strain NBRC 102203.

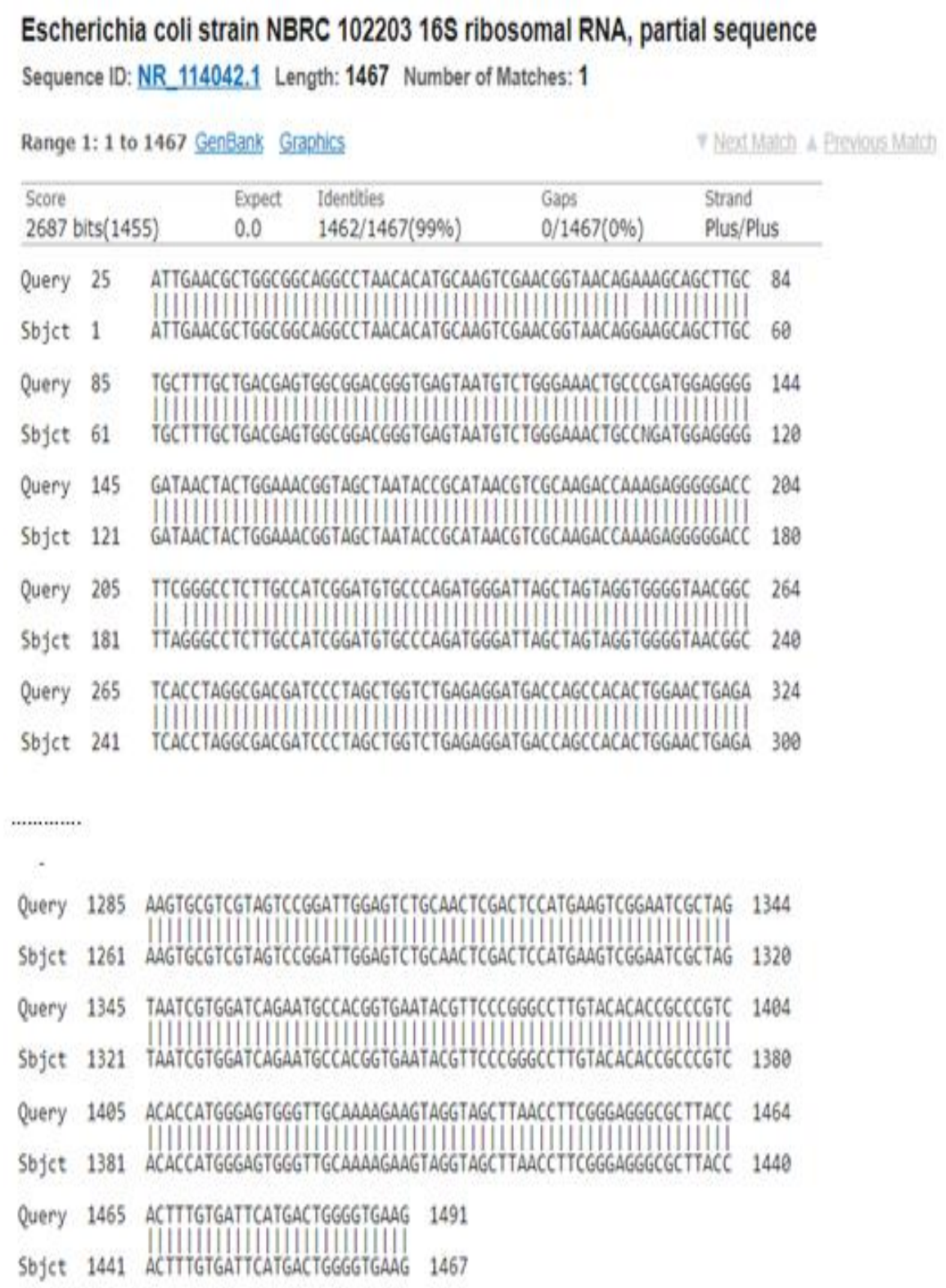

Figure 12: BLASTn alignment for *E.coli* sequenced 16S ribosomal RNA with 99% identity to *Escherichia coli* strain NBRC 102203.

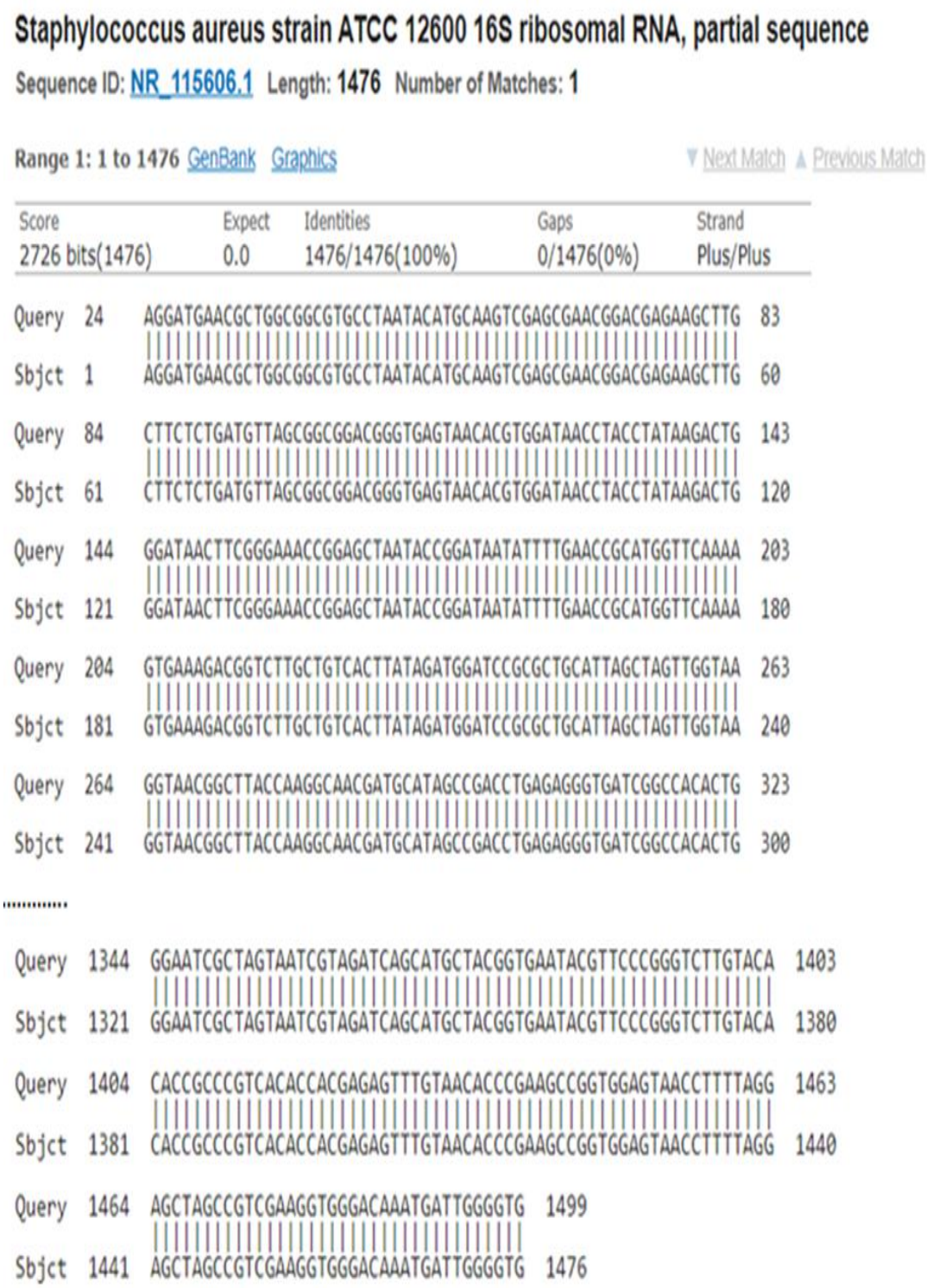

Figure 13: BLASTn alignment of *S.aureus* sequenced 16S ribosomal RNA with 100% identity to *Staphylococcus aureus* ATCC 12600.

4.2 ET data analysis

In the limit of detection (LOD) test of bacterial concentration both *E.coli* and *S.aureus* were incubated at 37˚C with shaking for 24 h but with a dilution of the original concentration ranging from 10^{-14} to 10^{-4}. The calibration curve on the PLS recognition model has identified that ET can sense the presence of bacteria, in NB media, between the dilutions 10^{-11} and 10^{-10} (the curve began to be linear) for both bacteria *E*.coli (Figure 14) and *S.aureus* (Figure 15).

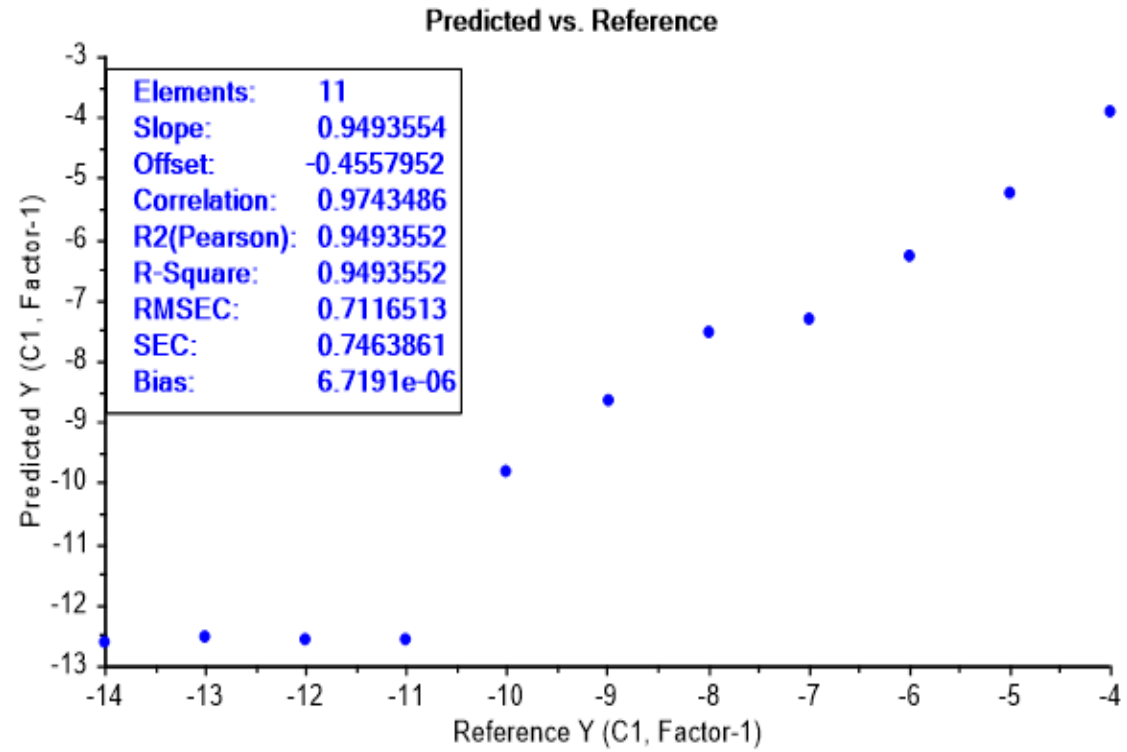

Figure 14: PLS recognition model for *S.aureus* LOD of different dilutions ranged from 10^{-14} to 10^{-4}. ET can sense the presence of bacteria, in NB media, between the dilutions 10^{-11} and 10^{-10}.

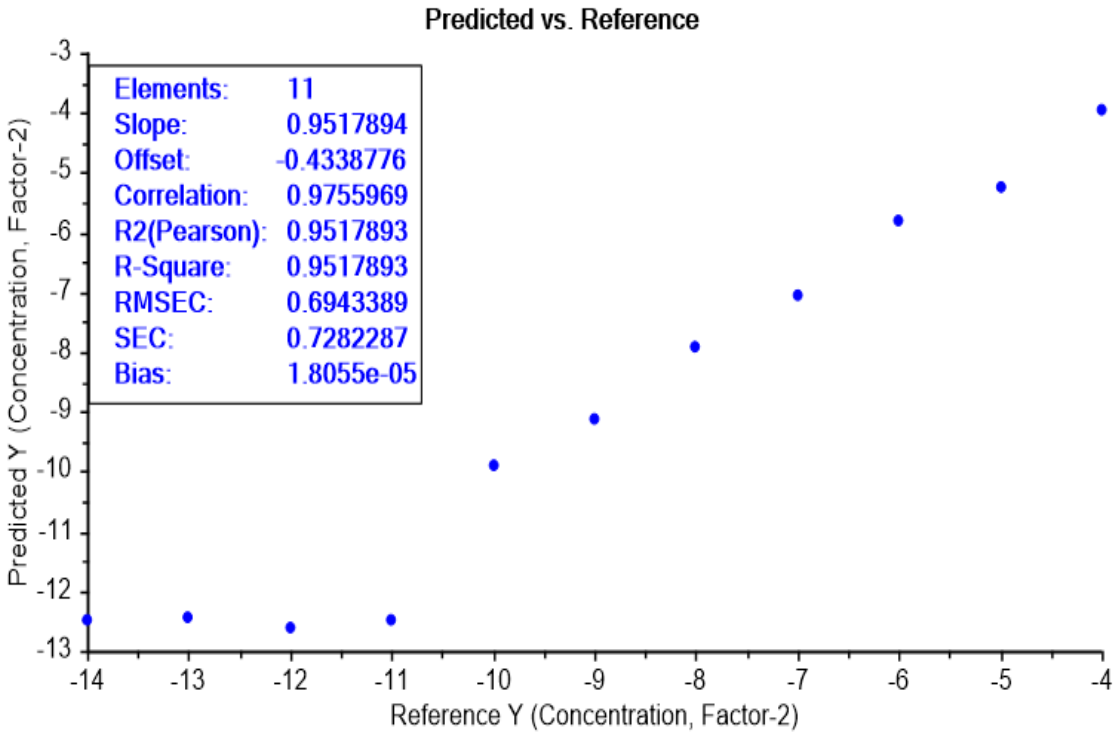

Figure 15: PLS recognition model for *E.coli* LOD of different dilutions ranged from 10^{-14} to 10^{-4}. ET can sense the presence of bacteria, in NB media, between the dilutions 10^{-11} and 10^{-10}.

Meanwhile, in the LOD test of bacterial earliest incubation period after determining the LOD concentration, both bacteria were incubated with a dilution concentration of 10^{-9} (the dilution 10^{-9} was chosen to be sure that ET will sense the growth of bacteria at 24 h of incubation (as appositive control)) but with incubation periods ranged from 4 to 24 h. The calibration curve on the PLS recognition model has identified that ET can sense the presence of *E.coli*, in NB media, between 6 and 8 h of incubation (Figure 16) and *S.aureus* after 6 h of incubation (Figure 17), the results are summarized in Table 2.

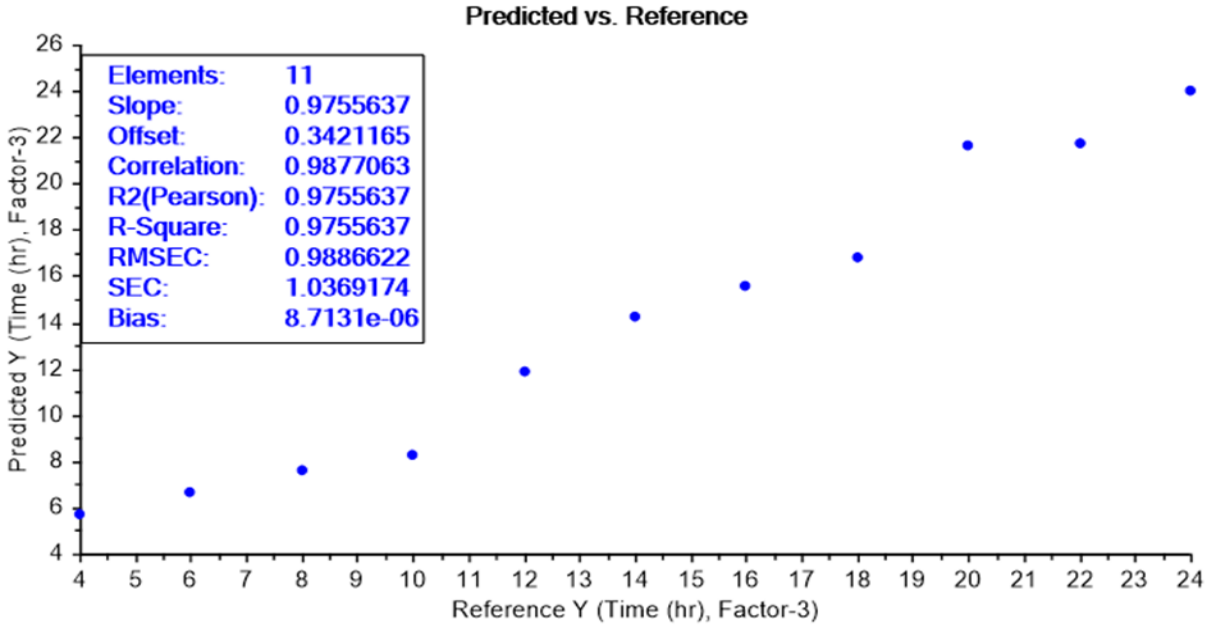

Figure 16: PLS recognition model for *E.coli* LOD of different incubation periods ranged from 4 to 24 h. ET can sense the presence of bacteria, in NB media, between incubation periods 6 and 8 h.

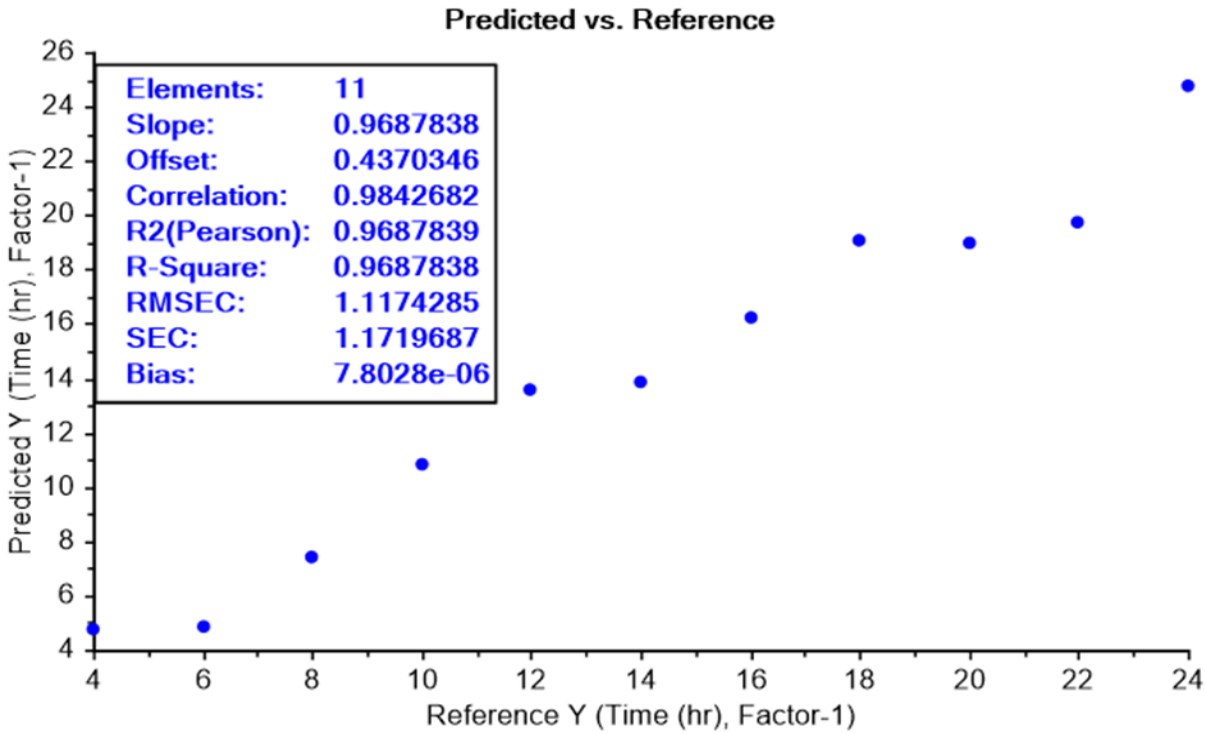

Figure 17: PLS recognition model for *S.aureus* LOD of different incubation periods ranged from 4 to 24 h. ET can sense the presence of bacteria, in NB media, at incubation period of 6 h.

Table 2: Limit of detection (LOD) results for *S.aureus* and *E.coli.*

Bacterial type	LOD of Concentration	LOD of the incubation period
S.aureus	Between 10^{-11} and 10^{-10}	After 6 h
E.coli	Between 10^{-11} and 10^{-10}	Between 6 and 8 h

ET was also tested for its ability to classify each type of bacteria if they were joined in the same PCA scores plot. The data for both recognized LOD tests (dilution greater than 10^{-10} and growth time greater than 8 h) for both bacteria were gathered in the same PCA scores plot, in which two well-separated groups were identified for *E.coli* and *S.aureus* (Figure 18).

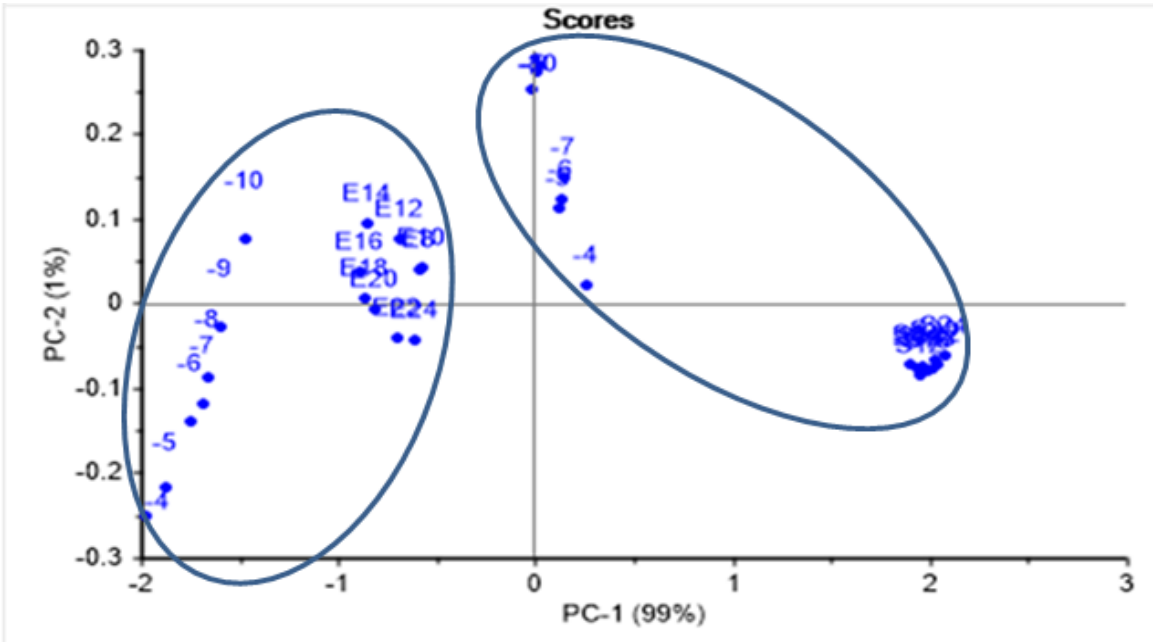

Figure 18: PCA scores plot for both bacterial data at the recognized LOD tests (dilution greater than 10^{-10} and growth time greater than 8 h). E: *E.coli*, S: *S.aureus*.

Subsequently, the resulted data with their categorization were gathered in a model for each bacterial type (a model for *E.coli* and a model

for *S.aureus*) for the projection test, where unknown samples of *E.coli* and *S.aureus* were grown with 10, 12, and 14 hr and dilution of 10^{-9}, and also another two gram positive and gram negative bacteria (*S.agalactiae* and *P.aeruginosa,* respectively), to be sure of the created model and the recognition ability of the ET.

In the *E.coli* PCA model, the projected samples of unknown *E.coli* were close enough with the created models' data. Meanwhile, the unknown gram negative *P.aeruginosa* was near the gathered samples; as the ET has recognized the differences between the two different characteristics of these two gram negative strains. Moreover, the projected gram positive samples of *S.aureus* and *S.agalactiae* on the *E.coli* model were far away; for their major different characteristics as shown in Figure 19.

Also, in the *S.aureus* PCA model, the projected samples of unknown *S.aureus* were close enough with the created model. Meanwhile, the unknown gram positive *S.agalactiae* was near (in a distance) the gathered samples; as the ET has recognized the differences between the two different characteristics of these two gram positive strains. Moreover, the projected gram negative samples of *E.coli* and *P.aeruginosa* on the *S.aureus* model were far away; for their major different characteristics as shown in Figure 20.

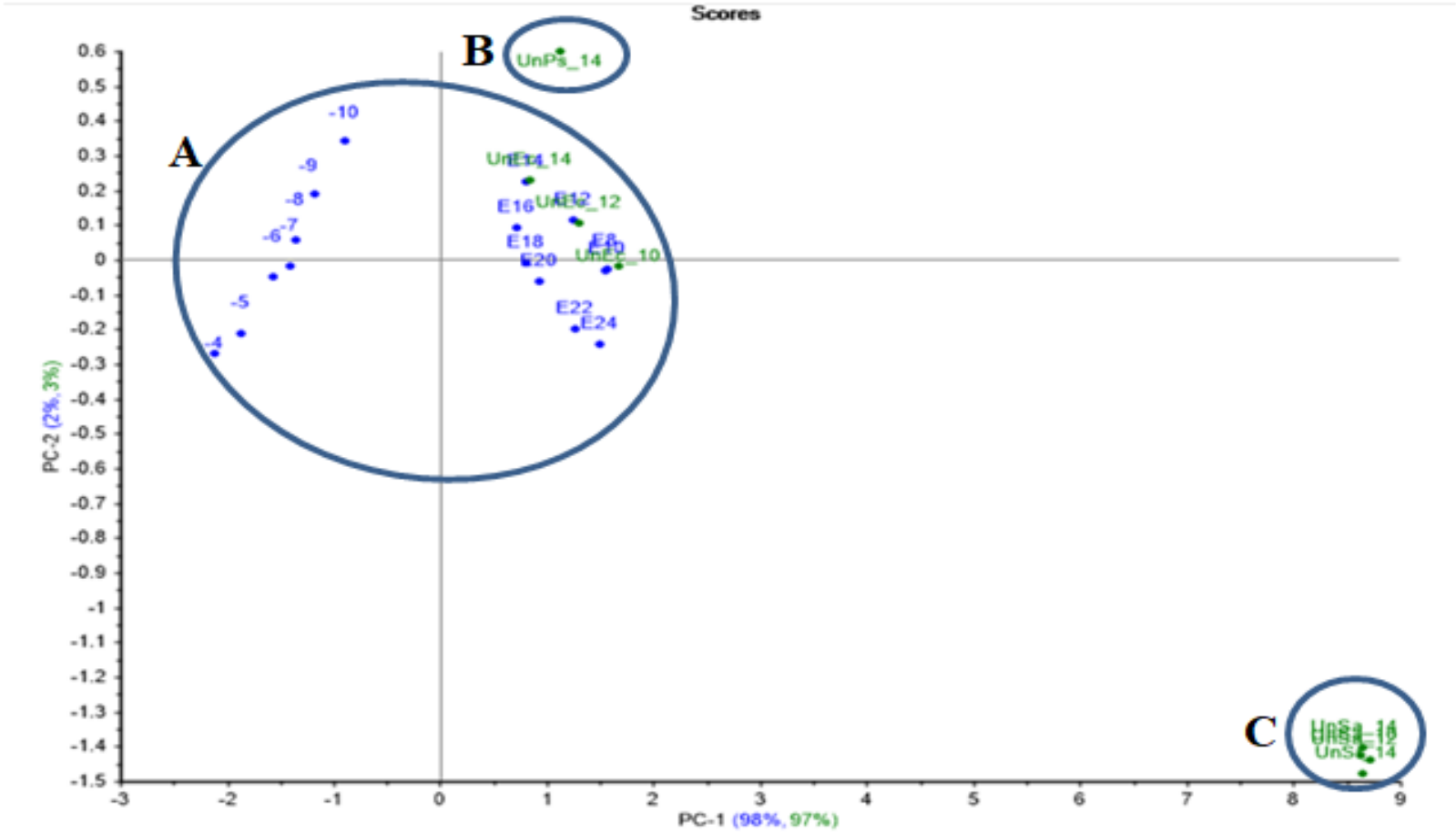

Figure 19: PCA scores plot for *E.coli* projection model with the projected unknown samples. A: a group of all gathered *E.coli* data of different concentrations and incubation periods with projected *E.colis* samples that were prepared with a dilution of 10^{-9} and incubation periods at 10, 12, and 14 h, B: projected *P.aeruginosa* that was grown at 10^{-9} dilution and for 14 h incubation period, C: projected *S.aureus* and *S.agalactiae* that were grown at 10^{-9} dilution for both and 10, 12 and 14 h incubation period (*S.aureus)* and 14 h incubation period (*S.agalactiae).*

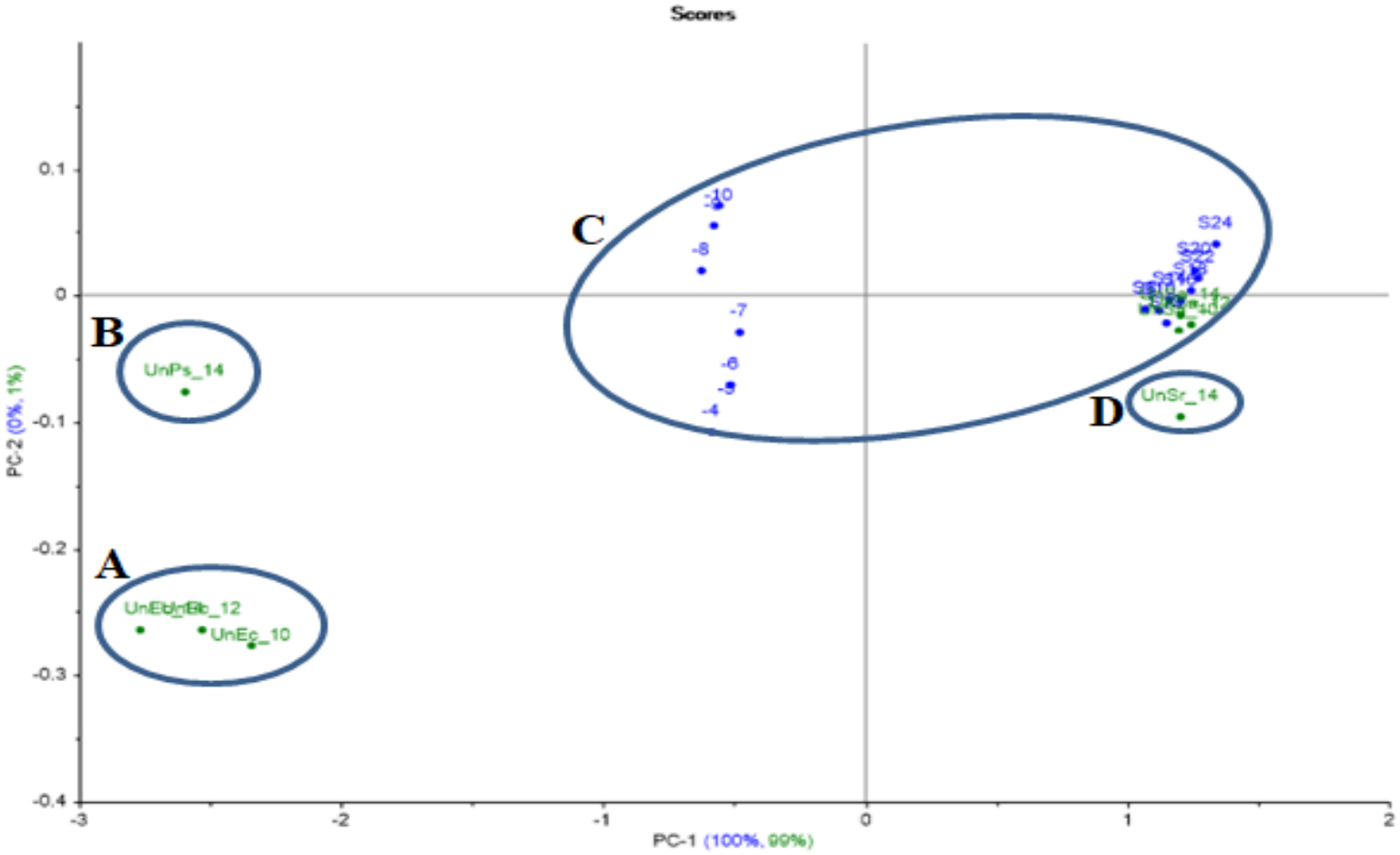

Figure 20: PCA scores plot for *S.aureus* projection model with the projected unknown samples. A: projected *E.colis* samples that were prepared with a dilution of 10^{-9} and incubation periods at 10, 12, and 14 h, B: projected *P.aeruginosa* that was grown at 10^{-9} dilution and for 14 h incubation period, C: a group of all *S.aureus* gathered data of different concentrations and incubation periods with projected *S.aureus* samples that were prepared with a dilution of 10^{-9} and incubation periods at 10, 12 and 14 h, D: projected *S.agalactiae* that was grown at 10^{-9} dilution and 14 h incubation period.

5 Conclusion

The results showed that Astree II ET was a capable technique for tracking bacterial growth and following up their metabolic changes in their environment (NB media). Additionally, it was able to create a classification model that is specific for some strains of microorganisms. Likewise, the obvious ability of ET for early detection of foodborne bacteria with an incubation period up to only 8 h and even 6 h in some strains such as *S.aureus*. Furthermore, it confirmed its sensitivity to identify microorganisms' proliferation even with a very low concentration of an original inoculum (such as a dilution factor up to 10^{-10}).

This research and results can be used for the subsequent step in considering ET as a powerful tool for early and fast identification and classification of harmful foodborne microorganisms, by creating these microorganisms' models to save patients' lives as much as possible. Moreover, in a long term, this study will open a wide door for using these sensors as an alternative fast assessment and monitoring technique in fermentable, industrial, categorizing, and other applications.

The ET importance has been cleared by its ease of use, where the foot-printing ability is coupled with distinguishing a native state

microorganism (in vitro assessment) contained in a complex system. However, combining ET with other technologies can provide a powerful combination in a wide range of applications.

As for recommendations, further studies should be carried out to monitor sensors' temperature dependence and charge transfer affected by the adsorption of solution components. Also, to enlarge the specified foot-printing databases of microorganisms that needs the first step of full work.

Abstract in Arabic (الملخص)

تقييم الحد الادنى للفحص المبكر للبكتيريا الغذائية الممرضة للانسان باستخدام اللّسان الالكتروني والتحليل المتعدد العوامل

الطالبة: آية علي أبو رميلة

المشرف الرئيس: الدكتور نواف أبو خلف

المشرف المساعد: الدكتورة وفاء مسعود

الملخص

أصبح اللّسان الإلكتروني (ET) أداة أساسية في المجال الطبي كبديل لطرق التشخيص التقليدية. وهو يعتمد على مجموعة متعددة من أجهزة الاستشعار التي تتميز بخصائص الحساسية العالية والانتقائية المنخفضة. لقد تم إجراء هذا البحث للتحقيق في إمكانية استخدام Astree II Alpha MOS ET كأداة تقييم سريعة وبديلة للتشخيص المبكر واكتشاف البكتيريا المسببة للأمراض البشرية من خلال تحديد الحد الأدنى للكشف المبكر (للتراكيز المختلفة CFU وفترات الحضانة) وتشخيص البكتيريا المسببة للأمراض البشرية. علاوة على ذلك،فقد تم استخدامه للتعرف على عينات بكتيرية غير معروفة باستخدام النماذج البكتيرية المخزنة.

لقد تم استخدام سلالتين من البكتيريا ، موجبة الجرام وسالبة الجرام لغرض هذا البحث.

(*Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC25922))

حيث تم التحقق من قدرة الجهاز على استشعار وجود البكتيرا في الوسط الغذائي (NB) عند حد الأدنى للتراكيز وفترات الحضانة باستخدام معادلات Partial least square (PLS). حيث تم تخفيف التركيز الاصلي للبكتيريا (حوالي 10^5*107) حتى 10^{-14} و باستخدام ET تم قياس التخفيفات من 10^{-14} إلى 10^{-4}. ثم تم تحديد أقل تركيز تم اكتشافه ومراقبته لاستخدامه في تجربة نمو البكتيريا بفترات حضانة مختلفة (من 4 إلى 24 ساعة حضانة). بعد ذلك تم جمع جميع البيانات المقاسة لكل نوع بكتيري من التراكيز وفترات الحضانة وحفظها في نموذج (PCA) Principle component analysis ، وبعد ذلك تم قياس عينات غير معروفة من البكتيريا ذات تركيز ووقت حضانة محدد من أجل معرفة قدرة الجهاز على التعرف عليها وتصنيفها .

أقرت النتائج قدرة Astree II ET على تتبع نمو البكتيريا والتّباع التّغيير الأيضي في الوسط الغذائي. حيث كان قادرًا على تحديد تكاثر الكائنات الحية الدقيقة بحساسية حتى مع التركيز المنخفض جدًا. وبالمثل فقد تمكن من الكشف عن بكتيريا *S.aureus* بعد فترة حضانة دامت 6 ساعات و 8 ساعات للِ *E.coli*. و بعد إنشاء نماذج السلالات فقد تمكن ال ET من تصنيف كل عينة غير معروفة وفقًا لخصائصا في الوسط الغذائي؛ وتحديد أيهما *S.aureus* أو *E.coli* أوغيرهما.

يمكننا القول أنه يمكن استخدام هذا البحث وهذه النتائج في اعتبار الجهاز كأداة قوية لتحديد وتصنيف الكائنات الدقيقة الضارة التي ممكن ان تتواجد في الغذاء في حالتها الأصلية ضمن نظام

معقد ، من أجل إنقاذ حياة المرضى. علاوة على ذلك لقد فتح مجموعة واسعة من التطبيقات التي سيكون ET تقنية بديلة وسهلة و مفيدة .

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