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Prediction of *Listeria monocytogenes* ATCC 7644 growth on fresh-cut produce treated with bacteriophage and sucrose monolaurate by using artificial neural network

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Combination of bacteriophage and sucrose monolaurate (SML) against *Listeria monocytogenes* growth on fresh-cut produce and prediction of relationship among initial bacterial load, fresh-produce type, antimicrobial concentration and residual bacteria using Artificial Neural Networks (ANNs) was investigated. Inoculated samples (tomato and carrot) containing 10^8 log cfu mL^{-1} *L. monocytogenes*, treated with bacteriophage (10^8 pfu mL^{-1}), SML (100, 250 and 400 ppm) and chlorine control (200 ppm) were stored at 4, 10 and 25 °C for 6 days. Mathematical models were developed using a linear regression and sigmoid (hyperbolic and logistic) activation functions. Data sets (120) were trained using Back propagation ANN containing one hidden layer with four hidden neurons. Phage treatment on tomato and carrot showed ($p < 0.05$) < 1 and 2 fold bacterial reductions respectively. Addition of SML at 100 and 250 ppm was ($p > 0.05$) ineffective, but showed significantly ($p < 0.05$) higher log reductions on both fresh produce at 400 ppm. Control treatment resulted in 1–2 log reductions on both fresh produce. Prediction with logistic activation function showed the highest positive correlation relationship between predicted and observed values with ~0.99 R^2-value and MSE of 0.0831. ANN offered better prediction in phage biocontrol of pathogens in fresh produce.

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1. Introduction

The demand for fresh or minimally-processed produce has notably increased in the last few decades (Abadias, Usall, Anguera, Solsona, & Viñas, 2008). Ready-to-eat (RTE) fruits and vegetables contribute to a healthy lifestyle in a more convenient way, as they contain nutritional and health-stabilising constituents (James, Ngarmsak, & Rolle, 2011). As part of advocacy by relevant global organisations, consumption of “five servings per day” of fresh produce has been advised as part of measures to reduce possible incidence of certain ailments such as cancer, diabetes, and some cardiovascular disorder (Allende, McEvoy, Luo, Artes, & Wang, 2006; FSA 2006; WHO, 2003; Warriner, Huber, Namvar, Fan, & Dunfield, 2009).

Meanwhile, safety challenges caused by pathogenic contamination of fresh produce along the food chain, resulting in disease outbreaks have been well documented (Beuchat, 2002; Olaimat & Holley, 2012). *Listeria monocytogenes* which causes listeriosis has been identified as one of such prominent bacterial contaminants in fresh produce such as tomato and carrot (Ajayeoba, Atanda, Obadina, Bankole, & Adelowo, 2015). It has a high mortality rate (20–30%) and possess the ability to survive wide-range of environmental and stress conditions (Farber & Peterkin, 1991; Gandhi & Chikindas, 2007). Effort to control this pathogen in fresh produce has led to the use of certain antimicrobial agent such as bacteriophage (Meireles, Giaouris, & Simões, 2016; Olaimat & Holley, 2012). Bacteriophages or phages are viruses that invade and kill bacteria via a lytic cycle (Simões, Simões, & Vieira, 2010). They are regarded as natural anti-microbial with an approved “GRAS” (General Recognized as Safe) status (Sulakvelidze, 2013). They co-exist with bacteria in their natural habitat over time, they are host-specific, possess effective mode of action, and they do not cause any deleterious impact on food microbiota (Bueno, García, Martínez, & Rodríguez, 2012; Guenther & Loessner, 2011; Spricigo, Bardina, Cortés, & Llagostera, 2013). These inherent attributes make them an excellent tool for the control of recalcitrant pathogen such as

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L. monocytogenes (Sillankorva, Oliveira, & Azeredo, 2012). The application of Listex P100 phage at 10^8 pfu mL^{-1} to significantly reduce *L. monocytogenes* on fresh-cut produce such as melon, pear and apple stored at 10 °C has been reported (Oliveira et al. 2014).

However, due to development of resistance mechanism reported by this pathogen, combination of phage with other antimicrobial agents have been suggested as a veritable approach to improve its efficacy (Oliveira et al. 2014; Strydom & Witthuhn, 2015; Zhao, Zhang, Hao, & Li, 2015). Phage combination with sucrose monolaurate; a surface-active antimicrobial compound could be seen as a new approach to address potential resistance by this pathogen. Previously, sucrose monolaurate (SML) at 400 ppm has been reported to be lethal to *L. monocytogenes* and *Staphylococcus aureus* broth when combined with some natural antimicrobials (Monk, Beuchat, & Hathcox, 1996). Also, SML at 100 and 250 ppm, significantly reduced *Escherichia coli* O157: H7 when combined with chlorine at 200 ppm (Xiao et al. 2011). However, results of laboratory investigation of phage combination with SML to inhibit *L. monocytogenes* on both fresh produce showed some significant reductions, but not total eradication. Hence there is need to predict its actual residual level after antimicrobial treatment.

Predictive microbiology describes the influence of environmental factors on the growth and survival of microorganism, using mathematical model (Huang, 2014; Pérez-Rodríguez & Valero, 2013). Artificial Neural Networks (ANNs) have been found to offer better modelling and predictive approach in addressing uncertainties and variations often associated with microbial growth (Jeyamkondan, Jayas, & Holley, 2001). Furthermore, application of ANN prediction on some fresh produce when treated with certain sanitizers has been reported (Keeratipibul, Phewpan, & Lursinsap, 2011; Ozturk, Tornuk, Sagdic, & Kisi, 2012).

Due to increased attention of phage biocontrol against pathogens of health importance in the fresh produce industry, the need for prediction as a risk assessment tool becomes more imminent. Therefore, the aim of this study is to predict *L. monocytogenes* population on fresh-cut tomato and carrot when treated with phage and sucrose monolaurate using artificial neural networks.

2. Materials and methods

2.1. Fresh produce preparation

Randomly picked, matured, ripe and organically grown tomato (*Lycopersicon esculentum*) and carrot (*Daucus carota* subsp. *sativus*) of approximately 1 kg was obtained from local grocery supermarket (Woolworth supermarket, Durban. South Africa). Each of the fresh produce surfaces were cleaned under running tap water disinfected with 70% ethanol and allowed to dry at room temperature before transferred into a clean plastic bowl.

2.2. Bacteria and preparation of inoculum

L. monocytogenes ATCC 7644 serovar 1/2c (Human isolate) was used in this study (Merck, South Africa). Following a modified method of Singh, Mnyandu, and Ijabadeniyi (2014), working culture was obtained from frozen stock culture kept in glycerol (−80 °C) by thawing in water bath (WB 1024, Foss tecator technology, Hoganas. Sweden) at 25 °C for 3 min and streaked on *Listeria* oxford media [LOM 75805, Sigma-Aldrich Inc. St Louis, MO 63103 USA] containing Oxford *Listeria* selective supplement (Fluka 75806 Sigma-Aldrich Inc. Buch, Switzerland) for 24 h at 37 ± 1 °C. Bacteria colonies were transferred into 50 mL fraser broth base (F6672 FB, Sigma-Aldrich Buch, Switzerland) using Fluka Fraser selective supplement (F18038 FSS, Sigma-Aldrich Buch, Switzerland). A final bacterial concentration of approximately 8 $\log_{10}$ cfu mL^{-1} was obtained using McFarland standard solutions before inoculation.

2.3. Antimicrobials

Antimicrobial compounds used in the present study includes; bacteriophage (Listex P100, Micreos food safety. Wageningen, Netherlands) stored at 4 °C in a buffered saline containing ~10^{11} plaque forming units (pfu mL^{-1}), sucrose monolaurate (SML 84110, Sigma Aldrich Pty Ltd, Aston Manor. 1630 South Africa) and chlorine-sodium hypochlorite (105614 EMPLURA, Merck Pty Ltd, Gauteng. South Africa).

2.4. Preparation of fresh-cut produce and sample inoculation

This was done by modifying the method of Xiao et al. (2011). Samples were cut thinly into average of 10 mm thickness and a 6 mm diameter wedge made into centre of each produce using a manual fruit corer (FCS 0020 Prestige Pty Ltd, Somerset West 7130, South Africa) to contain the inoculum. 100 μL of bacterial inoculum containing 10^8 cfu mL^{-1} were spot-inoculated (Chen & Zhu, 2011) into the wedges for a contact time of 30 min to attach under aeration at ambient temperature in a bio-safety cabinet (BSC-1500IIB2-X, Labotech, Midrand 1685. South Africa). The inoculated fresh-cut samples (25 g) were all placed into sterile bags (Tufflock 170 × 150 mm. Tuffy Brands Pty. Cape Town, South Africa) with perforations to avoid modified atmosphere creation prior to inoculation.

2.5. Antimicrobial treatment procedure

This was carried out by following a modified method reported by Oliveira et al. (2014). Bacteriophage concentration was diluted from ~10^{11} pfu mL^{-1} to ~10^8 pfu mL^{-1} using laboratory sterile water (pH 6.5–7.0), from which 10 ppm were inoculated into the wedges. Simultaneously, 20 mL each of SML at 100, 250 and 400 ppm prepared using sterile water (pH 6.5–7.0) at room temperature was later applied and allowed for 10 min (Ono, Miyake, & Yamashita, 2005). Chlorine dip (sodium hypochlorite at 200 ppm at 25 °C) for 3 min contact time was carried out as control. Each of the treated samples was allowed to stand for 30 min in the sterile bags under bio-safety cabinet condition before storage at 4, 10 and 25 °C for 6 days in a controlled chamber (LTIM 10 Lab design Engr Pty, Maraisburg. South Africa).

2.6. Microbiological analysis

Bacterial colony count was performed every 48 h for 6 days in all the storage temperatures (Oliveira et al. 2014). Samples from the sterile bags were mixed with 10 mL of sterile peptone water buffer (PWB HG00C134.500. Biolab Merck, Modderfontein. South Africa) and the mixture was homogenised in a Stomacher laboratory blender (Model No BA 6021; Seward Lab. London SE 19UG UK) for 120 s. Aliquots from the mixtures were serially diluted in saline peptone water (SP 1405, Conda Lab., Madrid, Spain), and 1 mL was spread-plated on a sterile petri plates containing Oxford *Listeria* agar. Inoculated plates were incubated at 37 °C for 48 h in order to obtain bacterial population as log cfu mL^{-1}.

2.7. Phage titration and pH

Phage titration was carried out by the method described by Leverentz et al. (2003). Briefly, aliquots from phage treated samples were homogenised and filtered through 0.45-μm-pore size membrane (Acrodisk; Pall Gelman, Ann Arbor, Mich). Phage titer was then determined using the soft agar overlay method using Brain

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