

OPTIMISATION OF CO-CULTURE FERMENTATION OF *Lactobacillus casei*
AND *Propionibacterium jensenii* IN RICE BRAN EXTRACT

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DEDICATION

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To my siblings;

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Rahim
Almarhum Rohman
Rokhaimie
Rokhiddin
Nornita
Siti Norniyati
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ABSTRACT

Co-culture fermentation is a fermentation process involving two defined microorganisms growing together in the same culture. A co-culture of lactic acid-producing bacteria (LAB) and propionic acid-producing bacteria (PAB) is beneficial in producing direct-fed microbial (DFM) products. The synergistic activity between LAB and PAB in co-culture fermentation can improve the survival of LAB and the growth of PAB. On this basis, the objectives of this study are two-fold. Firstly, the optimisation of co-culture fermentation involving *Lactobacillus casei* and *Propionibacterium jensenii* in the agricultural waste extract. Secondly, the development of an artificial neural network (ANN) predictive model for predicting the cell biomass concentration and the co-culture-specific growth rate. In the preliminary phase, two different substrates, namely rice bran and banana peel, were used in this study. This step was conducted to select the suitable carbon source for *L. casei* to grow and produce lactic acid for *P. jensenii* consumption. From the observation, rice bran was found more suitable as a carbon source and fermentation medium. Next, the co-culture optimisation of *L. casei* and *P. jensenii* fermentation was conducted using the one-factor-at-a-time approach. The fermentations were optimised for rice bran at concentration of 5% to 25% w/v; incubation temperature (30°C to 42°C); inoculation ratio (1:1 to 1:10 % v/v) and the initial pH (5.0 to 7.0). The optimum fermentation condition was obtained at 20% w/v rice bran concentration, incubated at 35°C with an inoculation ratio of 1:4 % v/v and initial pH of 6.5. The optimum growth (2.74 g dry cell weight/L) was recorded after 96 hours of incubation. The highest viable cell counts for *L. casei* and *P. jensenii* were 9.10 log CFU/mL and 9.42 log CFU/mL, respectively. The optimum specific growth rate, μ obtained, was 0.41 h⁻¹. The growth of *L. casei* and *P. jensenii* was compared to its monoculture fermentation, and it was found that the co-culture did not affect the growth of *L. casei* but helped maintain its survival. Moreover, *P. jensenii* gained benefits in the co-culture system, as its growth improved compared to during its monoculture. The ANN predictive model was developed using the multilayer perceptron and trained using the Levenberg-Marquardt training algorithm. Five input parameters, incubation time (h), the concentration of total reducing sugar (g/L), pH culture, incubation temperature (°C) and inoculation ratio (% v/v), were used to train the network for the prediction of cell biomass concentration (g/L) and the co-culture specific growth rate, μ (h⁻¹). The model has a low mean square error and high regression coefficient (R²) for the training and testing set, indicating the model is fit to predict the cell biomass produced and its specific growth rate during the co-culture of *L. casei* and *P. jensenii*. The structure obtained for ANN predictive model consist of five inputs, eight hidden nodes and two outputs, 5-8-2. The optimum predicted cell biomass concentration and the specific growth rate, μ , were 2.24 g dry cell weight/L and 0.51 h⁻¹, respectively. In conclusion, this work provides a strategy to produce multispecies DFM through co-culture fermentation using rice bran and presented the first predictive ANN model to predict the cell biomass concentration and the co-culture-specific growth rate of *L. casei* and *P. jensenii*.

ABSTRAK

Penapaian dwi-kultur adalah proses penapaian melibatkan dua mikroorganisma yang tumbuh bersama dalam kultur yang sama. Penapaian dwi-kultur bakteria asid laktik (LAB) dan bakteria asid propionik amat sesuai bagi tujuan penghasilan mikrob makanan (DFM). Sinergi aktiviti di antara bakteria LAB dan PAB semasa penapaian dwi-kultur mampu meningkatkan kadar jangka hayat LAB dan pertumbuhan PAB. Oleh yang demikian, kajian ini mempunyai dua objektif utama. Pertama, proses pengoptimuman penapaian dwi-kultur di antara *Lactobacillus casei* and *Propionibacterium jensenii*. Kedua, pembangunan model ramalan rangkaian saraf buatan (ANN) untuk menganggar biojisim sel dan kadar pertumbuhan tentu dwi-kultur. Dalam fasa persediaan, pemilihan dua jenis substrat iaitu dedak padi dan kulit pisang dijalankan. Pemilihan ini adalah untuk memilih substrat yang paling sesuai untuk pertumbuhan *L. casei* dan penghasilan asid laktik untuk digunakan oleh *P. jensenii*. Hasil daripada pemerhatian, didapati ekstrak dedak padi lebih sesuai digunakan sebagai substrat dan medium penapaian. Kemudian, proses pengoptimuman penapaian dwi-kultur *L. casei* dan *P. jensenii* dijalankan menggunakan kaedah satu faktor dalam satu masa. Proses pengoptimuman ini melibatkan peratus kandungan dedak padi pada kepekatan 5% hingga 20% w/v; suhu inkubasi (30°C hingga 42°C); nisbah inokulasi (1:1 hingga 1:10 % v/v) dan nilai pH awal (5.0 hingga 7.0). Pertumbuhan optimum diperoleh daripada penapaian 20% w/v kandungan dedak padi, pada suhu 35°C dengan nisbah inokulasi 1:4 % v/v dan nilai awal pH iaitu 6.5. Biojisim sel tertinggi yang diperoleh ialah 2.74 g berat sel kering/L selepas 96 jam tempoh inkubasi, dan bilangan mikrob yang hidup untuk *L. casei* ialah 9.10 log CFU/mL dan 9.42 log CFU/mL bagi *P. jensenii*. Kadar pertumbuhan tentu diperoleh ialah 0.41 j⁻¹. Pertumbuhan *L. casei* dan *P. jensenii* dibandingkan terhadap penapaian monokulturnya dan didapati bahawa penapaian dwi-kultur tidak memberikan kesan terhadap pertumbuhan *L. casei* tetapi telah membantunya kekal hidup. Selain itu, *P. jensenii* memperoleh manfaat daripada penapaian dwi-kultur kerana pertumbuhannya meningkat berbanding semasa monokultur. Model ramalan ANN yang telah dibangunkan ialah perceptron pelbagai lapisan dan menggunakan algoritma Levenberg-Marquardt. Lima parameter masukan iaitu, masa inkubasi (j), kandungan gula penurun (g/L), pH kultur, suhu inkubasi (°C) dan nisbah kepekatan inokulum (% v/v) digunakan untuk menganggar kepekatan biojisim sel dan kadar pertumbuhan tentu dwi-kultur, μ (j⁻¹). Model ini mempunyai nilai ralat min kuasa dua yang rendah dan pekali regresi (R²) yang tinggi untuk set latihan dan set ujian, menunjukkan bahawa model yang dibangunkan mampu membuat anggaran kepekatan biojisim sel dan kadar pertumbuhan tentu dwi-kultur ketika penapaian dwi-kultur *L. casei* dan *P. jensenii*. Struktur diperoleh untuk model ramalan ANN terdiri daripada 5 masukan, 8 nod tersembunyi dan 2 keluaran, 5-8-2. Kepekatan biojisim sel ramalan optimum dan kadar pertumbuhan tentu, μ masing-masing ialah 2.24 g berat sel kering/L dan 0.51 j⁻¹. Kesimpulannya, kajian ini menawarkan satu strategi penghasilan produk DFM yang kos efektif menggunakan teknik dwi-kultur dan dedak padi, serta melaporkan model ramalan ANN yang pertama untuk menganggar kepekatan biojisim sel dan kadar pertumbuhan tentu *L. casei* dan *P. jensenii* dwi-kultur.

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LIST OF ABBREVIATIONS

ANN	-	Artificial Neural Network
LAB	-	Lactic acid-producing bacteria
LC	-	<i>Lactobacillus casei</i>
MLP	-	Multilayer Perceptron
MRS	-	De Man Rogose & Sharpe
PAB	-	Propionic acid-producing bacteria
PJ	-	<i>Propionibacterium jensenii</i>
SLA	-	Sodium lactate agar
TRS	-	Total reducing sugar

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CHAPTER 1

INTRODUCTION

1.1 Study Background

In recent years, ruminant nutritionists and microbiologists have been studying new safe alternative methods to improve ruminant production efficiency. One of the approaches is manipulating the rumen microbial system to enhance the animal nutrient utilisation and performance as an alternative for an antibiotic growth promoter (Elghandour *et al.*, 2015). Safety concern about antibiotic resistance genes has arisen regarding the prolonged usage of antibiotic growth promoters. Therefore, other approaches are more desirable (Allen *et al.*, 2013), including live beneficial microorganisms or direct-fed microbial in the feeding formulation.

The direct-fed microbial term refers to the live microorganisms supplemented orally for a beneficial response in the host animal (Khan *et al.*, 2016). Direct-fed microbes (DFM) are a live microorganism supplementation to ruminants to improve animal health and nutrition (Kmet *et al.*, 1993; Seo *et al.*, 2010). DFM microbes must be safe and non-pathogenic for consumption and technologically suitable for industrial processes. The microbes must be capable of modulating the immune responses in the gut's activities, resistant to acid and bile, and producing antimicrobial substances. Accordingly, current commercial DFMs are a blending of several species to ensure efficacies and multifactorial responses (McAllister *et al.*, 2011). The production of direct-fed microbes is gaining momentum due to consumers' growing awareness of food safety and quality (Rigobelo and de Ávila, 2012). These increased studies focused on improving the DFM production process by finding new approaches such as co-culture of two different microorganisms (Jangra *et al.*, 2016), using new microorganisms such as dairy propionibacteria (Adams *et al.*, 2008) and new carbon sources from agricultural waste (Zubaidah *et al.*, 2012).

There are many types of DFM, including lactic acid-producing bacteria (LAB). LAB are more effective than other DFM types since they are environmentally friendly and can alter the environment through several mechanisms. The lactic acid produced by LAB is one of the critical factors that can change the pH of the competitor bacteria. LAB bacteria include *Streptococcus* sp., *Enterococcus* sp., *Pediococcus* sp. and *Lactobacillus* sp. The most common strain incorporated as direct-fed microbial is *Lactobacillus casei* (Naidu, 2004; Fonteles *et al.*, 2012; Huang *et al.*, 2016a; Huang *et al.*, 2016b; Lee *et al.*, 2013; Soto *et al.*, 2011, Minelli *et al.*, 2004; Millette *et al.*, 2007; Aguirre-Ezkauriatza *et al.*, 2010; Genevois *et al.*, 2016; Céspedes *et al.*, 2013) from *Lactobacillus* sp. It has been suggested that LAB co-cultured with other bacteria and yeast demonstrate positive synergistic activities in many commercial products (McAllister *et al.*, 2011). Synergistic activities such as improvement in growth and propagation of *Lactobacillus* sp. (Khan *et al.*, 2015; Ma *et al.*, 2015) and heat tolerance of *Lactobacillus* sp. against heat shock (Sánchez *et al.*, 2013a) have been reported. Until recently, the co-culture fermentation approach has not been utilised to improve the survival of *Lactobacillus* sp. in a prolonged fermentation.

Lactic utilising bacteria, such as microbes from dairy propionibacterium, usually used as a starter culture in cheese production, has been reported to demonstrate optimal properties for DFM application (Adams *et al.*, 2008; Alazzeah *et al.*, 2014; Argañaraz-Martínez *et al.*, 2013; Thierry *et al.*, 2011). The application of dairy propionibacterium as DFM is still in the early stage. However, the reports are encouraging. The study by Adams *et al.* (2008) reported that calves supplied with live *P. jensenii* improved live weight, and the treatment showed no adverse effect on the calves' health. Adams *et al.* also suggested that this might be due to the properties owned by *Propionibacterium* sp. that are not observed in *Lactobacillus* sp. *P. jensenii* was also studied for layer chicken. It was reported that the application of the microbes in the early layer hen is safe and effective in promoting the production and quality of products in layer husbandry (Luo *et al.*, 2010).

Interestingly, dairy propionibacterium is a species that prefers lactate over glucose as a carbon source and produces propionic acid as its primary product fermentation. Xie *et al.* (2019) took advantage of this preference. In his study,

Propionibacterium freudenreichii and *Lactobacillus acidophilus* was co-cultured to produce propionic acid from sugarcane molasses. *L. acidophilus* converted sugarcane molasses to lactic acid. Then, *P. freudenreichii* utilised the lactic acid for its growth and produced propionic and acetic acid. Co-culture of dairy propionibacterium also have been reported for the production of propionic acid (Ahmadi *et al.*, 2016; Farhadi *et al.*, 2013), natural folate and vitamin B12 (Hugenschmidt *et al.*, 2011; Xie *et al.*, 2019), bio-preservatives (Misirlilar *et al.*, 2012) and functional food (Ranadheera *et al.*, 2013).

The cost of carbon and nitrogen sources accounts for more than 30% of the total fermentation cost (Tufvesson *et al.*, 2013). Thus, cheap and renewable sources have caught the researcher's interest in finding a viable method to reduce the fermentation cost. Recently, agricultural waste has been among the top studied as new carbon sources in the fermentation process. Pyar *et al.*, (2014) used pineapple waste, and Zhang and Vadlani (2015) used corn stover to produce lactic acid. Banana waste was used in the production of bioethanol (Gabhane *et al.*, 2014), cellulase (Sun *et al.*, 2011), organic acid (Mufidah *et al.*, 2017; Karthikeyan and Sivakumar, 2010), and glutathione (Chen *et al.*, 2011). Another source of carbon is from the rice industry, whose the byproducts such as stalks, straws, husks, and bran remain, which could be used for microbial production (Seyoum *et al.*, 2022; Demirci *et al.*, 2017; Todhanakasem and Puanglanyai, 2012), lactic acid production (Wang *et al.*, 2015), functional food (Le *et al.*, 2019; Moon *et al.*, 2021).

Rice bran is the most nutritious part of rice by-products, rich in protein, minerals, fatty acids, dietary fibre, and antioxidants (Zubaidah *et al.*, 2012). The compositional analysis of rice bran showed that fat, protein, carbohydrate, fibre and ash content were ranged between 15.85-18.80%, 12.07-13.66%, 40.63-45.06%, 11.77-12.68%, 9.72-11.41% dry weight, respectively (Moongngarm *et al.*, 2012). The high carbohydrate composition in rice bran makes it suitable to be used as a carbon source in fermentation. Wang *et al.*, (2015) used rice bran to produce L-lactic acid production using *B. coagulans* and Zubaidah *et al.*, (2012) cultivated *Lactobacillus casei* in rice bran to promote symbiotic effect in Wistar Rats. Saman *et al.*, (2011) reported that rice bran is suitable as a carbon source for the growth of human *Lactobacillus*

plantarum while retaining the bran's functional property. Besides being cheap and easy to obtain rice bran, its nutritional properties enabled it to be used as a nitrogen source in fermentation processes. In a study by Kazeem *et al.*, (2021), rice bran was supplemented as a nitrogen source for a co-culture of *Bacillus licheniformis* and *B. paralicheniformis* to produce cellulase.

Co-culture fermentation is the fermentation of two defined microbes, an artificial association of microorganisms (Zhang and Wang, 2016; Canon *et al.*, 2020) grown together in the same culture under an aseptic environment (Bader *et al.*, 2010). Co-culture is different from a mixed culture, as mixed culture is a self-assembled community of environmental microbes in various ecosystems (Canon *et al.*, 2020). Co-culturing offered a range of improvements, such as using cheap substrates and increasing yield and product quality (Bader *et al.*, 2010). It can be summarised that co-culture is used for two purposes; to enable substrate conversion and improve process performance (Canon *et al.*, 2020).

In the last ten years, studies of co-culture fermentation have been on the rise. This approach has been utilised for the production of organic acid (Liang *et al.*, 2016), ethanol (Chen, 2011), natural folate and vitamin B12 (Hugenschmidt *et al.*, 2011), and beneficial live microorganisms such as probiotics or DFM (Ranadheera *et al.*, 2013; Ranadheera *et al.*, 2016; Wu *et al.*, 2012; Xanthopoulos *et al.*, 2012). Co-culture can be designed using the same species but different strains (multistrain), and it can also be designed using two different species of microorganisms (multispecies). Co-culturing different species have been reported to exhibit a better synergistic effect compared to a multistrain co-culture (Kumsiri *et al.*, 2018; Ma *et al.*, 2015; Wu *et al.*, 2012). It can promote the growth and survival rate of the microbes, open opportunities using cheaper carbon sources, and improve yield and product quality (Bader *et al.*, 2010). However, co-culture fermentation, due to the complexity of its dynamics, analysis and control (Kato *et al.*, 1999; Aghababaie *et al.*, 2015a; 2015b), hence, understanding and predicting the interactions and the effect between two microbes is a challenge.

The Artificial Neural Network (ANN) is a biologically inspired prediction model involving computations and mathematics. ANN is also known as neural nets, artificial neural systems, parallel distributed processing systems and connectionist systems (Mitra and Dutta, 2017). ANN is developed to deal with noisy, incomplete data and non-linear problems (Zhang *et al.*, 2020; Morris *et al.*, 1994). Previous studies have demonstrated the capabilities of ANN in estimation, optimisation and growth kinetic. For example, Vidra and Németh (2022) applied the ANN modelling approach to simulate a batch of propionic acid fermentation process from 40 *Propionibacterium freudenreichii* fermentations result. Jafari *et al.*, (2017) used neural networks to predict physicochemical and microbial properties of *Aspergillus flavus* within tomato paste containing olive leaf extract. The capabilities of ANN in optimising fermentation media were demonstrated by Velu *et al.*, (2016) for xanthan gum production. Meanwhile, Baş *et al.*, (2007) used neural networks to estimate the reaction rate of enzymatic reactions without using a kinetic model.

1.2 Problem Statement

DFM should be safe, technologically suitable, alive and in an adequate concentration of at least 10^7 CFU/mL. In addition, DFM should be able to modulate the composition and activities of the ruminant gut and improve its health and performance. For an example, lactic acid bacteria type of DFM was fed to favour the activities of lactate utilizing bacteria such as *Propionibacterium* sp. and promote the production of propionic acid, a significant precursor for glucose synthesis in ruminants (McAllister *et al.*, 2011; Nocek *et al.*, 2003; Yang *et al.*, 2004; Callaway and Martin, 1997). In order to achieve this, DFM products were produced by blending various lactic acid bacteria to ensure the effectiveness of the DFM product. Thus, the production involves separate cultivation of each microbe, separate drying and a mixing process (Jangra *et al.*, 2016), which is costly. Co-culture fermentation is a process that can reduce multiple operations while achieving a similar result, a mixture of two different species that confer health benefits to ruminants but at a lesser cost.

Lactobacillus casei is one of the most common DFM types; it is safe and technologically suitable. Lactic acid bacteria are fed to ruminants to improve the activities of lactate utilising bacteria and indirectly increase propionic acid production, an essential precursor in glucose synthesis in ruminants (Yang *et al.*, 2004; McAllister *et al.*, 2011). However, to retain the viability of the DFM to be at least 10^7 CFU/mL is a challenge, including *L. casei*. One of the methods was encapsulation. Nevertheless, the encapsulation process is an extreme environment for *L. casei* due to exposure to a high temperature and pressure that may significantly reduce activity and viability (Chávarri *et al.*, 2012). Co-culture fermentation offers a survival kit for *L. casei*. Co-culturing *L. casei* with lactate utilising bacteria will help in lactic acid removal, which helps *L. casei* to maintain its viability in a long fermentation condition. At the same time, *L. casei* will slowly be exposed and adapt to a low pH environment as lactate-utilising bacteria will produce propionic acid, and it will help in *L. casei* survival in ruminant guts. Nevertheless, there is still no study addressing the survival of *L. casei* in any *Lactobacillus* sp. co-culture fermentation reports.

Lactic acid utilising bacteria is a group that converts lactate into propionic acid. *Propionibacterium jensenii* is one of the dairy propionibacteria and belongs to lactic utilising bacteria. The applications of *P. jensenii* as live beneficial microorganisms, DFM or probiotics, have been reported by Adams *et al.*, (2008), Luo *et al.*, (2010), Huang *et al.*, (2003) and Ranadheera *et al.*, (2015). *P. jensenii* has been reported co-cultured with *Lactobacillus* sp. for the production of protective cultures (Schwenninger and Meile, 2004), probiotics powder (Ranadheera *et al.*, 2015), fermented dairy drink (Ranadheera *et al.*, 2016), probiotic ice cream (Ranadheera *et al.*, 2013) and fruit yoghurts (Ranadheera *et al.*, 2012). Until recently, a co-culture of *P. jensenii* and *Lactobacillus* sp. has not been studied for DFM production.

Utilising cheap and renewable sources will reduce the production cost of DFM. Rice bran is the most nutritious part of the rice byproducts. Rice bran has been utilised as a carbon source for producing organic acid, live beneficial microorganisms and functional food (Wang *et al.*, 2015; Zubaidah *et al.*, 2012; Saman *et al.*, 2011). It was also utilised as a nitrogen source in cellulase production (Kazeem *et al.*, 2021).

Hence, this is the evidence that rice bran is rich in nutrients for fermentation and is rich in carbon and nitrogen source for DFM production.

Although studies of co-culture *Lactobacillus* sp. with different bacteria have been reported with various synergistic effects between both microbes, almost none address the survival of *Lactobacillus* sp. itself during the co-cultivation. Here, *Propionibacterium jensenii* was chosen to be co-cultured with *Lactobacillus casei* due to the synergistic effect of its preference over lactic acid as its substrate, thus eliminating the competition over simple sugar and, as a result helping in promoting the survival of *Lactobacillus casei*. Rice bran is the nutritious part of the rice byproducts. However, reports of its usage as the primary carbon source in DFM and co-culture fermentation are scarce. Co-culture fermentation is rare due to its complex combination of different species. Regardless of the ability of ANN to deal with noisy, incomplete data, and complex and non-linear problems, only one study reported the usage of ANN as a growth characteristics model for co-culture fermentation.

1.3 Research Goal

Thus, this study highlighted three main themes: the co-culture fermentation development of *L. casei* and *P. jensenii*, the usage of rice bran as the primary carbon source and the development of a predictive model using ANN.

1.3.1 Research Objectives

The objectives of the research are :

- (a) To determine the feasibility of agricultural waste as the primary carbon source for DFM production.
- (b) To develop and optimise the co-culture fermentation of *Lactobacillus casei* and *Propionibacterium jensenii* using agricultural waste as a carbon source.

- (c) To develop an Artificial Neural Network predictive model to predict the specific growth rate and the cell biomass concentration for co-culture of *Lactobacillus casei* and *Propionibacterium jensenii*.

1.3.2 Research Scopes

In order to achieve the outlined objectives, the study was conducted as follows:

- (a) Two different types of agricultural waste were chosen, rice bran a, rice byproducts and banana peel, a banana plantain waste. The feasibility of these two agricultural wastes was determined using *L. casei*. The fermentation was conducted with only *L. casei* because *L. casei* needs the carbon source to produce lactic acid so that *P. jensenii* can utilise the lactic acid as the substrate, thus eliminating the possibility of competition for the substrate. The operating conditions were based on the work by Zubaidah *et al.*, (2012).
- (b) Objective (b) was achieved by developing and optimising the co-culture of *L. casei* and *P. jensenii* following the method by Ahmadi *et al.*, (2016). The study was conducted in batches with 300 mL working volume in a 500 mL screw-capped bottle. The fermentation media used was the extract from agriculture waste. The co-culture fermentation was optimised for carbon source concentration (% w/v), incubation temperature (°C), initial pH and inoculation ratio. The one-factor-at-a-time (OFAT) approach was used for optimisation. The co-culture was measured for cell density, cell biomass (g dry cell weight/mL), cell viability (CFU/mL), total reducing sugar (g/L), and the organic acid concentration (g/L). A mono-culture fermentation was conducted based on the optimum parameters obtained. Then, a comparative study was conducted on the performances of mono and co-culture fermentations.
- (c) An ANN model was developed for co-culture fermentation of *L. casei* and *P. jensenii*. The experimental data were first normalised in the range of 0.5 to 0.95 to speed up learning and convergence. The data were divided into train and test data at a 70:30 ratio. Levenberg Marquardt algorithm was chosen as

the training algorithm. The model was developed to predict the specific growth rate of co-culture and cell biomass of *L. casei* and *P. jensenii*. The model learned about the process from train set data and then validated using test set data. Five inputs were fed to the model to be used to predict two outputs: the specific growth rate of co-culture and cell biomass. The performance of the ANN model was evaluated via mean square error (MSE), regression analysis and error histogram.

1.4 Significance of Study

This study was conducted to develop a co-culture fermentation for DFM production. From the literatures, there has been no co-culture fermentation of *L. casei* and *P. jensenii* reported for DFM application. In addition, rice bran has been utilised as the carbon source for organic acid and probiotic production, and none has utilised rice bran in co-culture fermentation. At the same time, co-culture fermentation has a complex dynamic, analysis and control. Thus, it requires a robust model tackling the complexity and non-linear interaction in co-culture fermentation. Hence, the main contributions of this study were to provide a strategy to produce multispecies DFM through co-culture fermentation using the nutritious part of rice byproduct, rice bran, as the primary carbon source. At the same time, the first predictive ANN model was developed to predict the cell biomass concentration and the co-culture-specific growth rate of *L. casei* and *P. jensenii*.

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LIST OF PUBLICATIONS

Indexed Journal

1. **Mohamed Esivan, S. M.**, Rashid, R., Zaharudin, N. A. and Nik Mahmood, N. A. (2016) 'Growth and survival of *Lactobacillus casei* in rice bran and banana peel medium', *International Journal of Nano and Biomaterials*, 6(3/4), 151-161. doi: <https://dx.doi.org/10.1504/IJNB.2016.086107> (**Indexed by SCOPUS**)
2. **Mohamed Esivan, S. M.**, Rashid, R., Azmi, N. A., Zaharudin, N. A. and Othman, N. (2015) 'Effects of the initial rice bran concentration on the production of *Lactobacillus* as digestive bio-regulator', *Jurnal Teknologi (Sciences & Engineering)*, 74(7), 25-18. doi: <https://doi.org/10.11113/jt.v74.4691> (**Indexed by SCOPUS and ESCI-WOS**)

Indexed Conference Proceedings

1. **Mohamed Esivan, S. M.**, Rashid, R., Jati, A., Zaharudin, N. A. (2021). Growth of *Lactobacillus casei* and *Propionibacterium jensenii* in different glucose concentration and incubation temperature', In: Abdul Karim, S.A., Abd Shukur, M.F., Fai Kait, C., Soleimani, H., Sakidin, H. (eds) *Proceedings of the 6th International Conference on Fundamental and Applied Sciences*. Springer Proceedings in Complexity. Springer, Singapore. doi: https://doi.org/10.1007/978-981-16-4513-6_9 (**Indexed by SCOPUS**)

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1. **Mohamed Esivan, S. M.**, Rashid, R., Zaharudin, N. A., Mohamad, M. (2018). Fermentation variables in the cultivation of *Lactobacillus* sp. and

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2. **Mohamed Esivan, S. M.,** Rashid, R. and Zaharudin, N. A. (2018). Production of *Lactobacillus casei* as direct-fed microbial in rice bran extract: Effect of incubation time and inoculation size. *International Graduate Conference on Engineering, Science and Humanities (IGCESH 2018)*. 13-15 August 2018. Johor. 292-294.
 3. **Mohamed Esivan, S. M.,** Rashid, R. and Zaharudin, N. A. (2017). Effect of temperature and initial pH on the growth of direct-fed microbial *Lactobacillus casei* in rice bran extract. *International Postgraduate Symposium in Biotechnology 2017*. 21-22 August 2017, Johor Bahru.
 4. **Mohamed Esivan, S. M.,** Rashid, R., Zaharudin, N. A., Nik Mahmood, N. A. and Rahman, R. A. (2016). Growth *Propionibacterium jensenii* in rice bran and banana peel extract. 6th International Conference on Biotechnology for the Wellness Industry 2016 (ICBWI 2016). 16-17 August 2016, Melaka. 22-27.
 5. **Mohamed Esivan, S. M.,** Rashid, R., Zaharudin, N. A. and Nik Mahmood, N. A. (2015). Growth and survival of *Lactobacillus casei* in rice bran and banana peel medium. *International Conference on Innovation in Science and Technology (IICIST 2015)*. 20-21 April 2015, Kuala Lumpur. 67-72.