



## Effects of carbon and nitrogen sources on bacteriocin-inhibitory activity of postbiotic metabolites produced by *Lactobacillus plantarum* I-UL4

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### ABSTRACT

**Aims:** Postbiotic metabolites are metabolic compounds produced by probiotic lactic acid bacteria. These compounds produced by *Lactobacillus* sp. have been shown to be effective substitutes for in-feed antibiotic in livestock due to their broad inhibitory activity. Therefore, the aim of this study was to determine the effects of various carbon and nitrogen sources on the bacteriocin-inhibitory activity of postbiotic metabolites produced by *Lactobacillus plantarum* I-UL4.

**Methodology and results:** The effects of various combinations of carbon and nitrogen sources on the bacteriocin-inhibitory activity (expressed as modified bacteriocin activity, MAU/mL) of postbiotic metabolites produced by *L. plantarum* I-UL4 were determined in basal media without micronutrients. The combination of glucose (20 g/L) and yeast extract (22 g/L) gave the best bacteriocin-inhibitory activity as compared to other combinations. Maximum bacteriocin-inhibitory activity of 1440 MAU/mL was achieved when 36.20 g/L of yeast extract was added as the sole nitrogen source in modified de Man, Rogosa and Sharpe (MRS) medium. The glucose concentration was further optimised to enhance the bacteriocin-inhibitory activity of the postbiotic metabolites. Lower bacteriocin-inhibitory activity was observed at 5, 10, 15 and 40 g/L in comparison to 20 g/L of glucose.

**Conclusion, significance and impact of study:** Maximum bacteriocin-inhibitory activity of postbiotic metabolites was achieved at 1440 MAU/mL when 20 g/L of glucose and 36.20 g/L of yeast extract were added as the sole carbon and nitrogen sources respectively in the modified MRS medium. Optimisation of other micronutrients present in MRS media is necessary to further enhance the bacteriocin-inhibitory activity of postbiotic metabolites produced by *L. plantarum* I-UL4.

**Keywords:** Postbiotic metabolites, *Lactobacillus plantarum*, optimisation, modified MRS media, modified bacteriocin activity.

### INTRODUCTION

Lactic acid bacteria (LAB), frequently termed as “the Lactics” are characterised as gram positive, non sporulating anaerobic but aero-tolerant bacteria with fermentative metabolism (Halasz, 2009). They are fastidious toward nutrient requirement and generally associated with carbohydrate-rich environment such as food, feed, human and animal cavities, as well as sewage and plant materials (Carr *et al.*, 2002). The use of LAB and their metabolites in the food industry is generally regarded as safe (GRAS, grade one) (Zacharoff and Lovitt, 2012). Recently, metabolites produced by probiotic

LAB were termed as postbiotic metabolites (Tsilingiri *et al.*, 2012). Postbiotic metabolites commonly produced by LAB include bacteriocins, organic acids, ethanol, diacetyl, acetaldehydes and hydrogen peroxide (Suskovc *et al.*, 2010). Several reports showed that postbiotic metabolites produced by LAB, especially *Lactobacillus* sp. exhibit inhibitory effects towards species which are closely related to the LAB and other unrelated spoilage and pathogenic bacteria (Foo *et al.*, 2003b, Thanh *et al.*, 2010, Bilkova *et al.*, 2011). This broad inhibitory property makes postbiotic metabolites a preferable alternative to antibiotics as feed supplements since abusive use of antibiotics will cause adverse effects on the environment

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due to antibiotic resistance of bacteria (Forshell and Wierup, 2006, Shazali *et al.*, 2014).

The potential use of postbiotic metabolites as substitution for in-feed antibiotics in livestock has been investigated and proven to be effective (Thu *et al.*, 2011, Choe *et al.*; 2012, Loh *et al.*, 2014). Hence, optimal production of inhibitory activity of postbiotic compounds is important due to its increasing industrial applications. Research in past decades has focused on optimisation of bacteriocin production under controlled fermentation conditions (Kaur *et al.*, 2013; Saraniya and Jeevaratnam, 2014). Bacteriocins are ribosomally-synthesized polypeptides which exhibit bactericidal or bacteriostatic actions towards specific bacteria (Sarika *et al.*, 2010).

Production of bacteriocins is dependent on various factors and is usually strain-specific. One of the factors reported to significantly enhance bacteriocin production is medium composition (Kaur *et al.*, 2013). Investigations on the effects of different carbon and nitrogen sources on production of bacteriocin showed that optimal combination of carbon and nitrogen sources can improve the bacteriocin production (Todorov and Dicks, 2005; Saraniya and Jeevaratnam, 2014). Therefore, the objectives of this study were to identify suitable carbon and nitrogen sources and to determine their concentrations for optimal bacteriocin-inhibitory activity of postbiotic metabolites produced by *Lactobacillus plantarum* I-UL4.

## MATERIALS AND METHODS

### Microorganisms and maintenance

*Lactobacillus plantarum* I-UL4 used in this study was previously isolated from a local traditional fermented food made from tapioca (Foo *et al.*, 2003a). The isolate was obtained from the Laboratory of Industrial Biotechnology, Department of Bioprocess Technology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia. *Pediococcus acidilactici* ATCC 4-46 was used as an indicator microorganism for the determination of bacteriocin-inhibitory activity. All bacteria were maintained at -20 °C as frozen stock cultures in de Mann Rogosa and Sharpe (MRS) media (Merck, Darmstadt, Germany) supplemented with 20% (v/v) glycerol.

### Preparation of inoculums

Bacterial cultures were revived twice by inoculating 1% (v/v) frozen stock cultures into 10 mL MRS broth and incubated at 30 °C under static condition. The cultures were then streaked onto the MRS agar plate and incubated for another 48 h. A single pure colony was then inoculated and incubated in 10 mL MRS broth for 48 h, followed by sub-culturing into 10 mL MRS broth for another 24 h of incubation. Active *L. plantarum* I-UL4 was then incubated for an additional 18 h at 30 °C before being used as an inoculum. The absorbance of the inoculum at 600 nm ( $OD_{600nm}$ ) was adjusted to 1.0 using

sterile 0.85% (w/v) sodium chloride (NaCl) solution prior to inoculating 1.0% (v/v) into the experimental media.

### Effects of different combinations of carbon and nitrogen sources

The effects of carbon and nitrogen sources were first evaluated in basal media containing different combinations of carbon and nitrogen sources without incorporating other micronutrients in the MRS broth. The basal media combinations and their concentrations (g/L) were: (a) glucose (20.0) only, (b) molasses (20.0) only, (c) peptone (22.0) only, (d) meat extract (22.0) only, (e) yeast extract (22.0) only, (f) glucose (20.0) and peptone (22.0), (g) glucose (20.0) and meat extract (22.0), (h) glucose (20.0) and yeast extract (22.0), (i) glucose (20.0), peptone (12.2) and meat extract (9.8), (j) glucose (20.0), meat extract (14.7) and yeast extract (7.3), (k) glucose (20.0), peptone (10.0), meat extract (8.0) and yeast extract (4.0), (l) molasses (20.0) and peptone (22.0), (m) molasses (20.0) and meat extract (22.0), (n) molasses (20.0) and yeast extract (22.0), (o) molasses (20.0), peptone (12.2) and meat extract (9.8), (p) molasses (20.0), meat extract (14.7) and yeast extract (7.3) and (q) molasses (20.0), peptone (10.0), meat extract (8.0) and yeast extract (4.0). Glucose, peptone and meat extract were purchased from Merck (Darmstadt, Germany), whereas yeast extract was purchased from Ohly GmbH (Hamburg, Germany) and molasses was purchased from PT Sdn. Bhd (Selangor, Malaysia). The experimental media were incubated at 30 °C for 36 h under static condition after inoculation with 1.0% (v/v) *L. plantarum* I-UL4. A volume of 1.0 mL culture broth was withdrawn aseptically at 24 h and centrifuged at 10,000 × g for 15 min at 4 °C. Resultant supernatants from each medium containing postbiotic metabolites were then used for the determination of bacteriocin-inhibitory activity. The experiment was performed in triplicate.

### Optimisation of selected nitrogen source

Different concentrations (g/L) of the selected nitrogen source were evaluated at 11.89, 27.84, 36.20 and 44.55 individually, while the concentration (g/L) of glucose was fixed at 20.0, sodium acetate at 5.0, diammonium hydrogen citrate at 2.0, dipotassium hydrogen phosphate at 2.0, Tween 80 at 1.0, magnesium sulphate at 0.2 and manganese sulphate at 0.04. Sodium acetate, diammonium hydrogen citrate, dipotassium hydrogen phosphate, Tween 80, magnesium sulphate and manganese sulphate were purchased from Merck (Darmstadt, Germany). The inoculated media were incubated at 30 °C for 36 h under static condition with 2 h sampling intervals. The experiment was performed in triplicate. A volume of 10 mL culture broth was withdrawn aseptically and centrifuged at 10,000 × g for 15 min at 4 °C. Collected supernatants were then analysed for bacteriocin-inhibitory activity.

### Optimisation of selected carbon source

The effects of glucose, lactose, sucrose and fructose as carbon source were evaluated individually at 20 g/L in the modified MRS media containing the selected nitrogen source at optimum concentration and the other micronutrients in the MRS broth. Lactose, sucrose and fructose were purchased from Merck (Darmstadt, Germany). Different concentrations (g/L) of the best carbon source were then evaluated individually at 0, 5, 10, 15, 20 and 40. The inoculated media were incubated at 30 °C for 36 h under static condition with 2 h sampling intervals. The experiment was performed in triplicate. A volume of 10 mL culture broth was withdrawn aseptically and centrifuged at 10,000 × *g* for 15 min at 4 °C. Supernatants containing postbiotic metabolites were then used for the determination of bacteriocin-inhibitory activity and glucose concentration.

### Determination of bacteriocin-inhibitory activity

Inhibitory activity of postbiotic metabolites was quantified as bacteriocin activity using Agar Well Diffusion Assay (Tagg and McGiven, 1971). Postbiotic metabolites were serially diluted 2 fold ( $2^0$  to  $2^{-5}$ ) using sterile 0.85% (w/v) NaCl solution prior to inoculation into respective wells at 20 µL. Diluted postbiotic metabolites were allowed to diffuse around the well for an hour. The plates were then overlaid with 3 mL of soft agar inoculated with 1 % (v/v) *P. acidilactici* 4-46 ( $OD_{600nm}$  was adjusted to 1.0) and incubated at 30 °C for 24 h. A clear inhibition zone with a diameter of more than 1 cm indicates positive bacteriocin-inhibitory activity. Bacteriocin-inhibitory activity was expressed as modified bacteriocin activity (MAU/mL) and calculated as shown below (Lim, 2003):

$$\text{Modified bacteriocin activity (MAU/mL)} = \frac{[\text{Highest dilution factor which score positive activity} / \text{Volume inoculated into wells (mL)}] \times \text{Diameter of the clear inhibition zone (cm)}}{1}$$

### Determination of microbial biomass

Five millilitres of the culture broth was filtered through a pre-dried and pre-weighed 0.45 µm cellulose acetate filter paper (Sartorius Stedim Biotech, Germany). After filtration, the filter paper was dried overnight at 105 °C and then cooled in a desiccator at room temperature. The difference between the initial and final filter paper weights is the weight of microbial biomass in the 5 mL broth. Microbial biomass was expressed as gram of dry weight per litre, as described by Li and de Orduna (2009).

### Determination of glucose concentration

Glucose concentration in postbiotic metabolites was determined using the Pico Trace Glucose Analyzer (Trace Analytics, Germany) according to the manufacturer's instructions. Ten microliters postbiotic metabolites was used for the analysis.

### Statistical analysis

The results were analysed by one-way analysis of variance (ANOVA) using the General Linear Model procedure by Statistical Analysis System (SAS, 1998). Duncan's Multiple Range Test System was used to compare the significant difference between the mean at  $p < 0.05$ . The results of statistical analysis were presented as mean ± standard error of the mean (SEM).

## RESULTS AND DISCUSSIONS

### Effects of different combinations of carbon and nitrogen sources

Based on the results presented in Table 1, no modified bacteriocin activity was detected in the postbiotic metabolites produced by *L. plantarum* I-UL4 when cultivated in basal media containing only carbon or nitrogen source. The highest modified bacteriocin activity was achieved at 300 MAU/mL when glucose and yeast extract were added at 20 g/L and 22 g/L respectively. However, no bacteriocin-inhibitory activity was detected when glucose was combined with meat extract or peptone in the media. The combination of glucose, yeast extract, peptone and meat extract did not improve the bacteriocin-inhibitory activity of the postbiotic metabolites and yet reduced the modified bacteriocin activity to 240 MAU/mL, which was 20% lower as compared to the combination of glucose and yeast extract. Similar patterns were observed when molasses was used as a carbon source, whereby 200 MAU/mL of modified bacteriocin activity was detected when molasses was combined with yeast extract and the bacteriocin-inhibitory activity was further reduced to 160 MAU/mL when molasses, yeast extract, peptone and meat extract were added into the growth medium.

The results obtained in this study inferred that multiple nitrogen sources would not enhance the bacteriocin-inhibitory activity of postbiotic metabolites produced by *L. plantarum* I-UL4. Addition of meat extract and peptone in the media gave adverse effects to the modified bacteriocin activity. Similar findings were reported by Todorov and Dicks (2005), whereby reduced bacteriocin activity was obtained for *Lactobacillus plantarum* strains when meat extract was used individually as a nitrogen source in the media or in combination with other nitrogen sources.

Yeast extract was identified as the best nitrogen source for the production of bacteriocin-inhibitory compounds by *L. plantarum* I-UL4. Indeed, the effect of yeast extract as a single organic nitrogen source on the improvement of bacteriocin activity has been reported for

*Streptococcus bovis* (Carvalho *et al.*, 2008), *Lactobacillus rhamanus* (Sarika *et al.*, 2010) and *Lactobacillus sakei* (Todorov *et al.*, 2012). It is likely that the free amino acids and growth factors present in the yeast extract (Aasen *et al.*, 2000) contributed to the stimulatory effect on the

production of bacteriocin-inhibitory compounds by *L. plantarum* I-UL4.

**Table 1:** Effect of different combinations of carbon and nitrogen sources on the bacteriocin-inhibitory activity of postbiotic metabolites produced by *Lactobacillus plantarum* I-UL4.

Media components	Concentration (g/L)	Modified bacteriocin activity (MAU/mL)
Glucose	20	0 ± 0 <sup>g</sup>
Molasses	22	0 ± 0 <sup>g</sup>
Peptone	22	0 ± 0 <sup>g</sup>
Meat extract	20	0 ± 0 <sup>g</sup>
Yeast extract	20	0 ± 0 <sup>g</sup>
Glucose + peptone	20.0+22.0	0 ± 0 <sup>g</sup>
Glucose + meat extract	20.0+22.0	0 ± 0 <sup>g</sup>
Glucose + yeast extract	20.0+22.0	300 ± 0 <sup>a</sup>
Glucose + peptone + meat extract	20.0+12.2+9.8	0 ± 0 <sup>g</sup>
Glucose + meat extract + yeast extract	20.0+14.7+7.3	100 ± 11.54 <sup>e</sup>
Glucose + peptone + meat extract + yeast extract	20.0+10.0+8.0+4.0	240 ± 11.54 <sup>b</sup>
Molasses + peptone	20.0+22.0	0 ± 0 <sup>g</sup>
Molasses + meat extract	20.0+22.0	0 ± 0 <sup>g</sup>
Molasses + yeast extract	20.0+22.0	200 ± 23.09 <sup>c</sup>
Molasses + peptone + meat extract	20.0+12.2+9.8	0 ± 0 <sup>g</sup>
Molasses + meat extract + yeast extract	20.0+14.7+7.3	80 ± 0 <sup>f</sup>
Molasses + peptone + meat extract + yeast extract	20.0+10.0+8.0+4.0	160 ± 0 <sup>d</sup>

Notes: Values are mean ± standard error of the mean (SEM), n = 3. Means in the same column not sharing a common superscript are significantly different ( $p < 0.05$ )

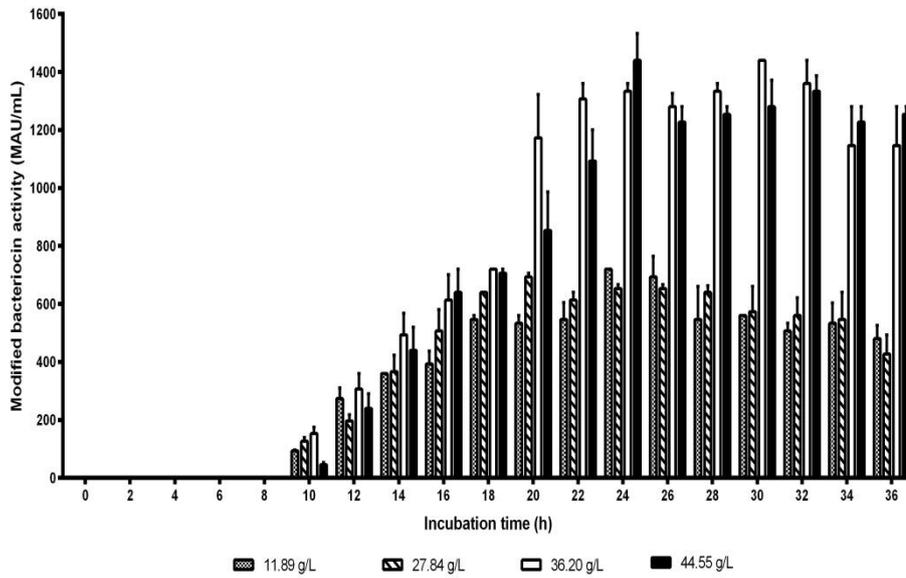
### Optimisation of yeast extract concentration

Optimisation of the yeast extract concentration was performed in the modified MRS broth to further enhance the bacteriocin-inhibitory activity of postbiotic metabolites. Total nitrogen content of the yeast extract was previously determined using Kjeltac™ 2400 (FOSS, UK) (data not shown) and the concentrations of the yeast extract used in the present study corresponds to 80 mM for 11.89 g/L (which is the same level contained in MRS broth), 200 mM for 27.84 g/L, 260 mM for 36.20 g/L and 320 mM for 44.55 g/L. Generally, bacteriocin-inhibitory activity was detected at the 10 h regardless of the concentration of yeast extracts used (Figure 1). Highest bacteriocin-inhibitory activity (1440 MAU/mL) was achieved in the media containing 36.20 g/L of yeast extract at the 30 h and 44.55 g/L of yeast extract at the 24 h of incubation. The bacteriocin-inhibitory activity achieved at 11.89 g/L and 27.84 g/L of yeast extract was in the range of 100-720 MAU/mL, which was much lower as compared to the bacteriocin-inhibitory activity achieved at 36.20 g/L and 44.55 g/L. The results obtained in this study indicated that increasing concentrations of yeast extract in the modified MRS media enhanced bacteriocin-inhibitory activity of postbiotic metabolites produced by *L. plantarum* I-UL4 significantly at  $p < 0.05$ . Similar finding was reported by Aasen *et al.* (2000), whereby production of sakacin P by *Lactobacillus sakei* was increased linearly with the

concentration of yeast extract. Likewise, higher amount of bacteriocin-like activity (640 AU/mL) was produced by *Enterococcus durans* when 2.0% (w/v) of yeast extract was added in the media as compared to the bacteriocin-like activity (320 AU/mL) achieved in the media containing 1.0% (w/v) of yeast extract (Khay *et al.*, 2013).

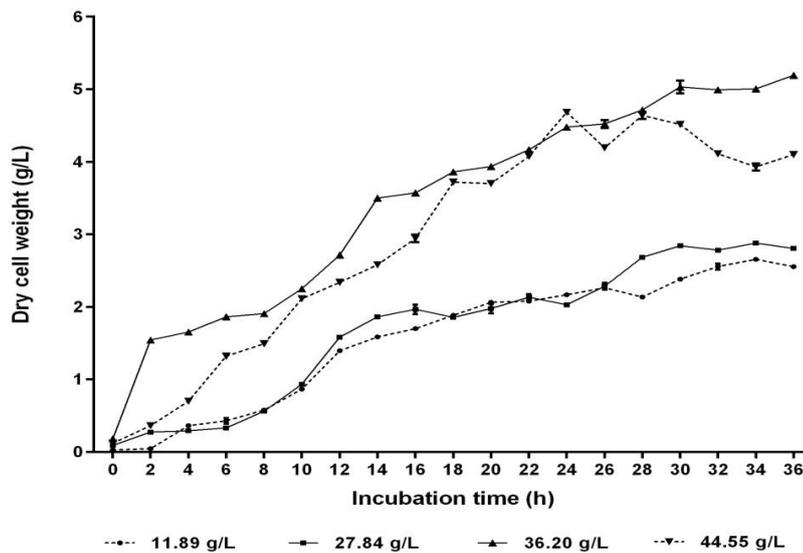
Moreover, Figure 2 shows that the growth of *L. plantarum* I-UL4 was favoured at higher concentrations of yeast extract. Shorter exponential phase (from 6 h to 12 h) was observed at 11.89 g/L and 27.84 g/L as compared to 36.20 g/L of yeast extract, whereby exponential growth phase was noted at 8 h to 18 h of incubation. Although higher nitrogen content was found in 44.55 g/L of yeast extract, biomass throughout the incubation period (except at 24 h where the maximum MAU/mL was noted) was relatively low in comparison to that in media containing 36.20 g/L yeast extract. Therefore, the highest concentration of yeast extract used may not necessarily produce maximum biomass.

This further inferred that an optimal carbon to nitrogen ratio (C/N) is essential in promoting optimal growth for *L. plantarum* I-UL4. DeGeest and De Vuyst (1999) also reported that the growth of *Streptococcus thermophilus* LY03 was influenced by C/N ratio of the growth media. By evaluating the results obtained for the growth and bacteriocin-inhibitory activity of postbiotic metabolites, 36.20 g/L of yeast extract was identified as the optimal concentration of nitrogen source for the production of bacteriocin-inhibitory compounds by *L. plantarum* I-UL4.



Values are mean ± standard error of the mean (SEM), n=3. Vertical bars represent SEM.

**Figure 1:** Effect of different yeast extract concentrations in modified MRS media on the bacteriocin-inhibitory activity of postbiotic metabolites produced by *L. plantarum* I-UL4.



Values are mean ± standard error of the mean (SEM), n=3. Vertical bars represent SEM.

**Figure 2:** Effect of different yeast extract concentrations in modified MRS media on the growth of *L. plantarum* I-UL4.

**Determination of optimum carbon source**

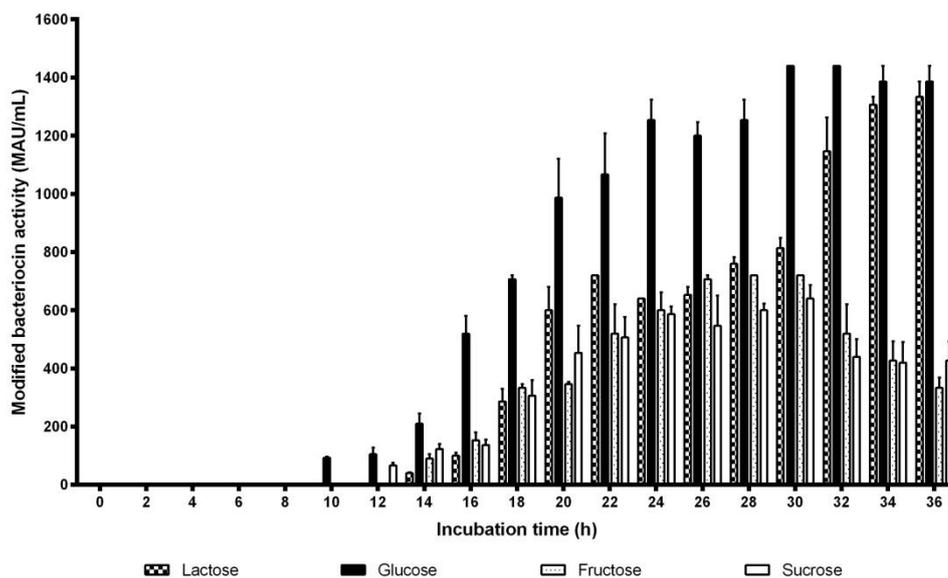
The effects of four carbon sources: lactose, glucose, sucrose and fructose on the bacteriocin-inhibitory activity of postbiotic metabolites were determined at 20 g/L in the modified MRS broth. Different initial production time of bacteriocin-inhibitory activity was detected for postbiotic

metabolites produced by *L. plantarum* I-UL4 using media containing different carbon sources. The bacteriocin-inhibitory activity of postbiotic metabolites was began to be detected at 10 h for the medium containing glucose, 12 h for medium containing sucrose and 14 h for media containing lactose and fructose respectively. Exponential production of bacteriocin-inhibitory activity of postbiotic

metabolites at 20 g/L of glucose was observed from the 12 h to 20 h and the activity was then maintained in the range of 1200-1440 MAU/mL from 22 h onwards (Figure 3). However, approximately 41.0% of increment of the modified bacteriocin activity was observed at the 32 h as compared to the 30 h when lactose was utilised as a carbon source. Before the 30 h, bacteriocin-inhibitory activity of the postbiotic metabolites was relatively low in lactose-containing medium. This is possibly due to the microorganism requiring longer time for the enzymatic degradation of the lactose disaccharide form as compared to glucose. The modified bacteriocin activity achieved in the media containing sucrose (640 MAU/mL) and fructose (720 MAU/mL) was two-fold lower as compared to that obtained in glucose and lactose medium, implying *L. plantarum* I-UL4 is capable of metabolising different carbon sources to produce different levels of bacteriocin-inhibitory activity of postbiotic

metabolites. Glucose was identified as the best carbon source attributed to the high modified bacteriocin activity (>1200 MAU/mL) was achieved from 22 h to 36 h as compared to the other three carbon sources.

The results from the effects of carbon sources on bacteriocin-inhibitory activity of the postbiotic metabolites produced by *L. plantarum* I-UL4 are also in agreement with findings from other studies, where glucose was reported to be the preferential carbon source for bacteriocin production by *Streptococcus bovicus* (Carvalho *et al.*, 2008) and *Lactobacillus sakei* (Todorov *et al.*, 2012). However, other carbon sources which support optimal bacteriocin production have also been reported for other LAB strains (Cheigh *et al.*, 2002; Drosinos *et al.*, 2005, Wang *et al.*, 2010). Moreover, Hayek and Ibrahim (2013) reported that LAB growth and their metabolic activity were influenced by different concentrations of carbon source.



Values are mean ± standard error of the mean (SEM), n=3. Vertical bars represent SEM

**Figure 3:** Effect of different carbon sources in modified MRS media containing 36.20 g/L of yeast extract on the bacteriocin-inhibitory activity of postbiotic metabolites produced by *L. plantarum* I-UL4.

### Optimisation of glucose concentration

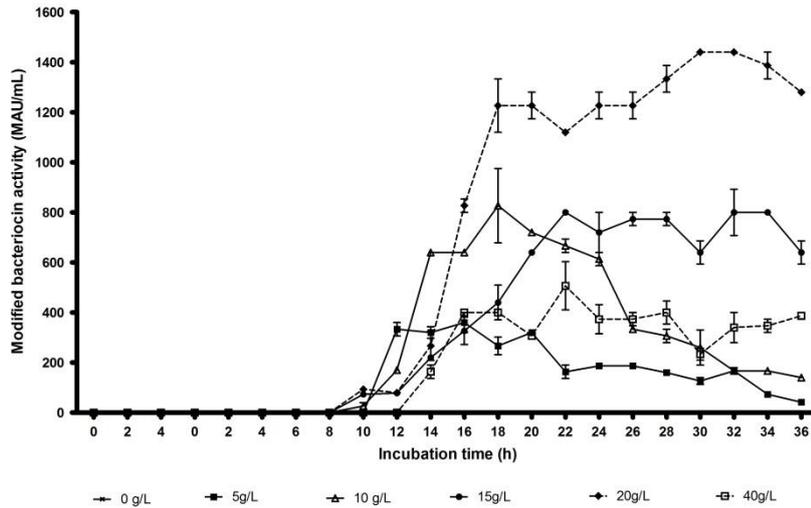
Generally, bacteriocin-inhibitory activity of the postbiotic metabolites produced by *L. plantarum* I-UL4 was detected at the 10 h in the 10 g/L, 15 g/L and 20 g/L glucose-containing media and at the 12 h for 5 g/L glucose-containing medium and 14 h for 40 g/L glucose-containing medium respectively (Figure 4). Maximum bacteriocin-inhibitory activity was achieved at 1440 MAU/mL in the medium containing 20 g/L glucose. At this concentration, intermittent production of bacteriocin-inhibitory activity was observed where substantial increment of the bacteriocin-inhibitory activity was noted between the 12 h to 18 h of incubation (from 80 MAU/mL to 1226.67

MAU/mL) and between the 26 h to 32 h (1226.67 MAU/mL to 1440 MAU/mL). Bacteriocin-inhibitory activity was relatively constant between the 20 h to 24 h. A similar profile was observed when *L. plantarum* I-UL4 was cultivated in 10 g/L of glucose although the bacteriocin-inhibitory activity was lower (826.67 MAU/mL). However, the onset time of the bacteriocin-inhibitory activity increment was observed between the 10 h to 14 h and between the 16 h to 18 h of incubations.

As for the 40 g/L of glucose, highest bacteriocin-inhibitory activity was at 506.67 MAU/mL, which was unexpectedly lower as compared to 10 g/L (826.67 MAU/mL) and 15 g/L (800 MAU/mL). The high concentration of glucose (> 20 g/L) in the media did not

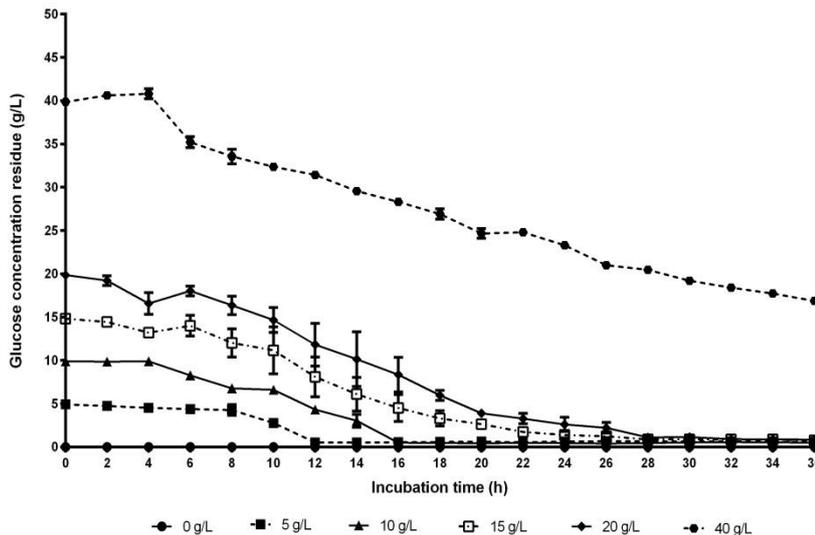
enhance the bacteriocin-inhibitory activity of postbiotic metabolites produced by *L. plantarum* I-UL4 within the 36 h of incubation. Similar observations were reported for the bacteriocin-like production by *Enterococcus durans* E204 (Khay *et al.*, 2013) and antimicrobial compound production by *Lactobacillus pentosus* SJ65 (Saraniya and Jeevaratnam, 2014). The reduction of the inhibitory activity was reported to be attributed to the

substrate inhibition when high glucose concentration was employed in the media (Khay *et al.*, 2013) as shown in the present study. Figure 5 shows that approximately 42% from the 40 g/L of glucose remained in the media at the 36 h of incubation. For the other tested glucose concentrations, the glucose residues remained in the postbiotic metabolites were generally lower than 1 g/L.



Values are mean ± standard error of the mean (SEM), n=3. Vertical bars represent SEM.

**Figure 4:** Effect of different glucose concentrations in modified MRS media on the bacteriocin-inhibitory activity of postbiotic metabolites produced by *L. plantarum* I-UL4.



Values are mean ± standard error of the mean (SEM), n=3. Vertical bars represent SEM.

**Figure 5:** The glucose concentration of postbiotic metabolites produced by *L. plantarum* I-UL4 using different glucose concentrations in modified MRS media.

## CONCLUSIONS

The optimum carbon and nitrogen sources for the bacteriocin-inhibitory activity of postbiotic metabolites produced by *L. plantarum* I-UL4 were glucose at 20 g/L and yeast extract at 36.20 g/L, whereby the maximum modified bacteriocin activity of the postbiotic metabolites was achieved at 1440 MAU/mL, which was two-fold higher as compared to activity detected in media consisting of the same amount of carbon and nitrogen content found in MRS medium. The effects of other micronutrients present in the MRS medium and their optimal concentrations should be determined to further enhance the bacteriocin-inhibitory activity of postbiotic metabolites produced by *L. plantarum* I-UL4.

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