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Effects of supplementation of succinic acid on the production and molecular weight distribution of exopolysaccharides by *Antrodia camphorata* in batch cultures

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Abstract: The effects of organic acid supplementation on both yields and molecular weight distributions of exopolysaccharide (EPS) of *Antrodia camphorata* were investigated in shaker flasks and air-lift bioreactors. In the shaker flask study, five out of six organic acid-supplemented cultures showed negative effects on cell growth, the exception being pyruvic acid-supplemented culture; lower number average molecular weights (*Mn*) of EPS were obtained in all the supplemented cultures. EPS production was enhanced by 31% due to the addition of succinic acid. Optimum product yield was obtained between 2.0 and 3.0 g dm^{-3} succinic acid; however, the specific production of EPS increased monotonically as succinic acid concentration was increased from 0 to 5 g dm^{-3}. Enhancement of EPS yield by 28% and a higher *Mn* of EPS (around 310 kDa) due to the addition of succinic acid were also demonstrated in an air-lift bioreactor. In addition, a novel fermentation process resistant to EPS degradation is proposed, based on the inhibition of β-glucanase activity by the supplementation with succinic acid.
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Keywords: *Antrodia camphorata*; exopolysaccharide; succinic acid; number average molecular weight; air-lift bioreactor

NOTATION

EPS Exopolysaccharide
P EPS concentration (mg dm^{-3})
P_{max} Maximum EPS concentration (mg dm^{-3})
Q_P Productivity for EPS (mg dm^{-3} d^{-1})
Q_X Productivity for biomass (g dm^{-3} d^{-1})
t Culture time (d)
U Unit
X Cell concentration (g dm^{-3})
X_{max} Maximum cell concentration (g dm^{-3})
$Y_{P/S}$ Product yield (g EPS g^{-1} glucose)
$Y_{P/X}$ Specific product yield (g EPS g^{-1} biomass)
$Y_{X/S}$ Cell yield (g biomass g^{-1} glucose)

μ Specific growth rate (d^{-1})

1 INTRODUCTION

Antrodia camphorata (Chinese name, niu-chang-chih or ching-chih) is a fungal parasite of the Taiwanese evergreen tree *Cinnamomum micranthum*, but is an important Chinese folk medicine for the treatment of food and drug intoxication, diarrhoea, abdominal pain, hypertension, itchy skin and liver cancer.[1] Recently, several potentially active components with therapeutic effects, such as polysaccharides, sesquiterpene lactone, steroids and triterpenoids, have been isolated and identified from the fruiting bodies of *A camphorata*.[2–5] Polysaccharides extracted from fruiting bodies and submerged culture of *A camphorata* exhibited similar biological effects in antioxidant and free radical-scavenging activities,[6] stimulating macrophage activity and anti-hepatitis B virus activity.[2] Although the biological activities of polysaccharides have been extensively investigated, relatively few reports have focused on process factors affecting yields and molecular weight distribution of polysaccharides.

Citric acid supplementation significantly influences the biosynthesis of biopolymers in both bacterial and fungal cultures. In the case of bacterial exopolysaccharide fermentation, the supplementation of citric acid in the culture medium stimulated the yield of xanthan,[7,8] and increased its pyruvic acid content.[9] In the case of fungal exopolysaccharide fermentation, the co-existence of glucose and citric acid favoured schizophyllan formation by *Schizophyllum commune*, but inhibited cell growth.[10] However, little attention

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has been focused on the effects on cell growth and exopolysaccharide formation of adding organic acids other than citric acid.

The bioactivity of exopolysaccharides is highly dependent on their physical and chemical characteristics, including molecular weight distribution,[11–13] degree of branching,[14] water solubility,[15] structure[16] and configuration.[17] It is generally considered that glucans with β-(1→3) linkages in the main chain and additional β-(1→6) branching are essential for anti-tumour activity.[14] Higher molecular weight glucans seem to be more effective in their anti-tumour activity than those of lower molecular weight.[11–13] However, the molecular weight distributions and compositions of polysaccharides were greatly influenced by medium composition,[9,18,19] cell morphology[18] and operational conditions including pH, aeration rate and harvest time.[10,18–20] In general, the operational conditions, such as culture pH and aeration, are likely to be in control throughout the fermentation; however, the harvest time might vary batch by batch due to the nature of the fermentation and the secondary metabolite kinetics of exopolysaccharide formation. In addition, the molecular weights of polysaccharides will decrease gradually due to degradation by extracellular glucanases upon the depletion of other carbon sources.[21] Therefore, if the quality of polysaccharides is closely related to their molecular weights, as most reports indicate, then it is essential to develop a robust fermentation process with high quality exopolysaccharides, which is less sensitive toward the variation of harvest time.

The main objective of this research was to investigate the effects on the yields and molecular distribution of exopolysaccharide of *A camphorata* of adding different organic acids to the culture medium. In addition, a robust fermentation process with higher yield and quality of exopolysaccharides is suggested according to the findings of this study.

2 MATERIALS AND METHODS

2.1 Microorganism and culture conditions

Antrodia camphorata BCRC 35 396 was obtained from the Bioresource Collection and Research Center (Hsinchu, Taiwan) and transferred monthly to fresh nutrient agar medium. The inoculum (3.5 cm^3 of culture) was prepared by collecting the three-week-old cells grown on agar plate using sterilized water, then transferred into 250 cm^3 Erlenmeyer flasks containing 100 cm^3 medium. The basic medium for this study contained the following components ($g\,dm^{-3}$): glucose 30, peptone 5, malt extract 3, yeast extract 3, $KH_2PO_4.H_2O$ 1, $MgSO_4.7H_2O$ 1, vitamin B1 1. The effects of organic acid supplementation on the molecular weight and production of exopolysaccharides were examined using in turn six different pure organic acids (glutamic, citric, malic, succinic, oxalic and pyruvic) at a concentration of 2 $g\,dm^{-3}$ to the flask culture medium ($n = 3$). The culture pH of the shaker flasks was then adjusted to 5.0 before sterilization. Carbohydrate was autoclaved separately for 20 min at 121 °C and added to the medium under aseptic conditions. The effect of organic acid titre was studied for a series of six different succinic acid concentrations: ie 0, 1, 2, 3, 4 and 5 $g\,dm^{-3}$, in shaker flasks ($n = 3$). These shaker flasks were incubated for 14 days at 28 °C and 150 rpm.

The effect of succinic acid supplementation on molecular weight and production of exopolysaccharide was also investigated at two succinic acid concentrations, 0 and 3 $g\,dm^{-3}$ in a 3 dm^3 air-lift bioreactor with 2.2 dm^3 working volume. A similar medium with 10 instead of 30 $g\,dm^{-3}$ glucose was used in this study. The 200 cm^3 inoculum was prepared by flask culture at 28 °C and 150 rpm for 5 days. The batch culture was controlled at temperature 28 °C, pH 5.0 and aeration 0.1 vvm for 10 days.

2.2 Analytical methods

Cell mass was determined by the dry weight method; cell mycelium obtained by filtration of broth samples through pre-weighed filter discs (Whatman Ltd, Maidstone, UK) was dried in a vacuum oven at 60 °C until the weight was constant. The filtrate was collected and stored at −20 °C for the measurement of residual glucose, residual succinic acid and exopolysaccharide. Residual glucose content was assayed by the dinitrosalicylic acid (DNS) method.[22] The exopolysaccharide was pretreated by membrane filtration (MWCO 8 kDa) before being analysed by a phenol–sulfuric acid assay.[23] The molecular weights of exopolysaccharides were determined by gel permeation chromatography (GPC) using a Waters (Milford, MA, USA) 600E system equipped with a Shodex OHPak SB-804HQ column and a model 410 refractive index detector. All chromatographic data were processed by Millennium (Milford, MA, USA) software. Polyethylene glycol (PEG) standards (Polymer Laboratories, Church Stretton, UK) with narrow polydispersity and with molecular weights ranging from 1.9 to 1260 kDa were used to construct a calibration curve. All the exopolysaccharide samples were pretreated by membrane filtration (MWCO 8 kDa) before injection. The flow rate of the mobile phase (deionized water) was 0.6 $cm^3\,min^{-1}$. The content of residual succinic acid was determined by HPLC using a Rezex organic acid (ROA) column (Phenomenex, Torrance, CA, USA) at 30 °C using water as mobile phase at 0.6 $cm^3\,min^{-1}$. The presence of β-(1→3)-D-glucans in polysaccharides was confirmed by a fluorescence method.[24] β-Glucanase activity was assayed by incubating 1 cm^3 of 5 $mg\,cm^{-3}$ laminarin in 50 mM potassium acetate buffer, pH 5.0, with 1 cm^3 of enzyme solution appropriately diluted in the same buffer.[25] After 30 min of incubation at 50 °C, the reaction was stopped by heating at 100 °C for 10 min. Then the reducing sugars contents were determined using the DNS test, with glucose as standard. Enzyme

and substrate blanks were included. One unit of β-glucanase activity was defined as the amount of enzyme that catalyses the release of reducing sugar groups equivalent to 1 mmol of glucose per min under standard assay conditions.

3 RESULTS AND DISCUSSION

3.1 Effects of organic acid supplementation on cell growth, EPS production and β-glucanase activity in shaker flask cultures

The results of adding $2\,g\,dm^{-3}$ of six organic acids on cell growth and EPS production by *A camphorata* in shaker flask fermentations are listed in Table 1. All the organic acids except pyruvic acid inhibited the cell growth, as compared with that of the flask without organic acid supplementation (control). Although cell growth was slightly inhibited by succinic acid and oxalic acid, cell yield around $0.32\,g\,g^{-1}$ was achieved. Among all the organic acids tested, succinic acid showed the greatest enhancement of EPS production, by 31% as compared with the control. Accordingly, the maximum product yield ($Y_{P/S}$) and the specific product yield ($Y_{P/X}$) were obtained as $5.46\,mg\,g^{-1}$ and $1.76\,mg\,g^{-1}$, respectively, in the succinic acid-supplemented culture.

The presence of β-glucanase activity in cultures of *A camphorata* was first demonstrated in this study. The β-glucanase activity of all organic acid-supplemented flask cultures was inhibited as compared with that of the control culture, as indicated in Table 1. Only low β-glucanase activity, with a value of $0.04\,U\,cm^{-3}$, was detected in the malic acid-supplemented culture. No β-glucanase activity was detected in the cultures supplemented with other organic acids. Thus, addition of organic acids might be used to control the expression of β-glucanase in submerged cultures.

In order to take the effect of organic acid titre into account, different concentrations of succinic acid, from 0 to $5\,g\,dm^{-3}$, in shaker flasks were investigated, and the results are described, in the following sections.

3.2 Effects of different succinic acid concentrations on cell growth, EPS production and β-glucanase activity in shaker flask cultures

As shown in Table 2, the biomass decreased monotonically from 6.14 to $4.01\,g\,dm^{-3}$ as the succinic acid concentration increased from 0 to $5\,g\,dm^{-3}$. However, an optimal cell yield ($Y_{X/S}$), $0.28\,g\,g^{-1}$, was obtained at 3 g succinic acid dm^{-3}. The production of EPS increased monotonically from 25.0 to $37.2\,mg\,dm^{-3}$ as the supplement of succinic acid increased. The stimulation by succinic acid of EPS production was more significant, as indicated by the enhancement of the specific product yield ($Y_{P/X}$) from 4.08 to $9.27\,mg\,g^{-1}$. In other words, although cell growth was moderately inhibited, EPS production could be greatly enhanced by the addition of succinic acid. The enhancement of EPS production might be reasonably suspected as a result of the consumption of succinic

Table 1. Results of batch cultures supplemented with $2\,g\,dm^{-3}$ different organic acids in shaker flasks for 2 weeks

Organic acid	X[b] ($g\,dm^{-3}$)	P[c] ($mg\,dm^{-3}$)	$Y_{P/X}$[d] ($mg\,g^{-1}$)	$Y_{X/S}$[e] ($g\,g^{-1}$)	$Y_{P/S}$[f] ($mg\,g^{-1}$)	β-glucanase[g] activity ($U\,cm^{-3}$)
Control[a]	6.19 ± 0.31	24.0 ± 1.8	3.88	0.25	0.96	0.5
Glutamic	4.50 ± 0.38	14.3 ± 2.1	3.17	0.18	0.57	ND[h]
Citric	4.64 ± 0.22	17.4 ± 3.2	3.75	0.24	0.89	ND
Malic	5.04 ± 0.37	24.9 ± 2.2	4.93	0.20	0.99	0.04
Succinic	5.77 ± 0.12	31.5 ± 1.4	5.46	0.32	1.76	ND
Oxalic	5.04 ± 0.35	27.5 ± 1.1	5.46	0.31	1.68	ND
Pyruvic	6.28 ± 0.13	25.2 ± 2.9	4.02	0.25	1.01	ND

[a] Control: No additional organic acids were supplemented, [b] X: biomass concentration ($g\,dm^{-3}$), [c] P: EPS product concentration ($mg\,dm^{-3}$), [d] $Y_{P/X}$: product yield ($mg\,g^{-1}$), [e] $Y_{X/S}$: cell yield ($g\,g^{-1}$), [f] $Y_{P/S}$: product yield ($mg\,g^{-1}$), [g] One unit of β-glucanase activity is defined as the amount of enzyme that catalyses the release of reducing sugar groups equivalent to 1 mmol glucose per min under standard assay conditions, [h] ND: not detected.

Table 2. Results of batch cultures supplemented with different concentrations of succinic acid from 0 to $5\,g\,dm^{-3}$ in the shaker flasks for 2 weeks

Initial succinic acid concentration ($g\,dm^{-3}$)	X[a] ($g\,dm^{-3}$)	P[b] ($mg\,dm^{-3}$)	$Y_{P/X}$[c] ($mg\,g^{-1}$)	$Y_{X/S}$[d] ($g\,g^{-1}$)	$Y_{P/S}$[e] ($mg\,g^{-1}$)	RS[f] ($g\,dm^{-3}$)	ES[g] ($g\,dm^{-3}$)	β-glucanase activity[h] ($U\,cm^{-3}$)
0	6.14 ± 0.20	25.0 ± 2.3	4.08	0.25	1.00	0	0	0.5
1	5.60 ± 0.18	30.5 ± 1.8	5.44	0.27	1.50	0.39	0.61	0.3
2	5.39 ± 0.15	33.6 ± 0.6	6.23	0.28	1.77	1.29	0.71	0.05
3	4.87 ± 0.31	34.4 ± 1.2	7.06	0.28	1.98	2.25	0.75	ND[i]
4	4.33 ± 0.22	35.7 ± 0.5	8.24	0.26	2.13	3.19	0.81	ND
5	4.01 ± 0.12	37.2 ± 1.0	9.27	0.25	2.33	4.15	0.85	ND

[a] X: biomass concentration ($g\,dm^{-3}$), [b] P: EPS product concentration ($mg\,dm^{-3}$), [c] $Y_{P/X}$: specific product yield (mg EPS g^{-1} biomass), [d] $Y_{X/S}$: cell yield (g biomass g^{-1} glucose), [e] $Y_{P/S}$: product yield (mg EPS g^{-1} glucose), [f] RS: residual succinic acid concentration, [g] ES: exhausted succinic acid concentration, [h] One unit of β-glucanase activity is defined as the amount of enzyme that catalyses the release of reducing sugar groups equivalent to 1 mmol glucose per min under standard assay conditions, [i] ND: not detected.

acid. However, succinic acid was not completely consumed whatever the level of supplementation and the amount of succinic acid consumption increased moderately from 0.61 to 0.85 g dm^{-3} as the added succinic acid increased from 0 to 5 g dm^{-3}. Thus, co-existence of glucose and succinic acid might be responsible for both cell growth inhibition and EPS stimulation. The contribution of succinic acid to cell growth was limited and the major carbon source for cell growth and product formation was glucose. Similar observation that citric acid was not the main energy source for cell growth has been reported elsewhere in citric acid-supplemented cultures.[7–10]

The β-glucanase activity decreased monotonically from 0.5 to 0.05 U cm^{-3} as the concentration of succinic acid increased from 0 to 2 g dm^{-3}, as indicated in Table 2. No β-glucanase activity was detected when the concentration of succinic acid was greater than 3 g dm^{-3}.

3.3 Effects of succinic acid supplementation on cell growth, EPS production and β-glucanase activity in an air-lift bioreactor

The fermentation time-course data on cell, EPS and glucose concentrations of two batch cultures in air-lift bioreactors supplemented with 0 and 3 g dm^{-3} succinic acid, are shown in Fig 1(A and B respectively) and key parameters are listed in Table 3. Cell growth exhibited a distinct exponential phase followed by the stationary phase, and EPS had the characteristics of a secondary metabolite, occurring throughout the culture and continuing even though glucose was exhausted. Similar EPS production kinetics of *A camphorata* was also observed in a stirred tank bioreactor.[20]

Cell growth in the culture supplemented with 3 g succinic acid dm^{-3} was slightly inhibited as compared with that of the culture without succinic acid. As a result, the maximum cell density (X_{max}), declined from 3.05 to 2.85 g dm^{-3}, the specific growth rate (μ) decreased from 0.43 to 0.35 d^{-1}, and fermentation time was extended from 9 to 10 days.

EPS production (P_{max}) was enhanced by 28%, from 34.8 to 44.5 mg dm^{-3}, when 3.0 g dm^{-3} succinic acid was added. As a result, the product yield ($Y_{P/S}$) increased from 3.55 to 4.54 mg g^{-1}, the specific product yield ($Y_{P/X}$) from 11.4 to 15.6 mg g^{-1}, and the productivity (Q_P) from 3.87 to 4.45 mg dm^{-3} d^{-1}.

The concentration of succinic acid declined gradually with culture time from 3.0 to 2.0 g dm^{-3}. No

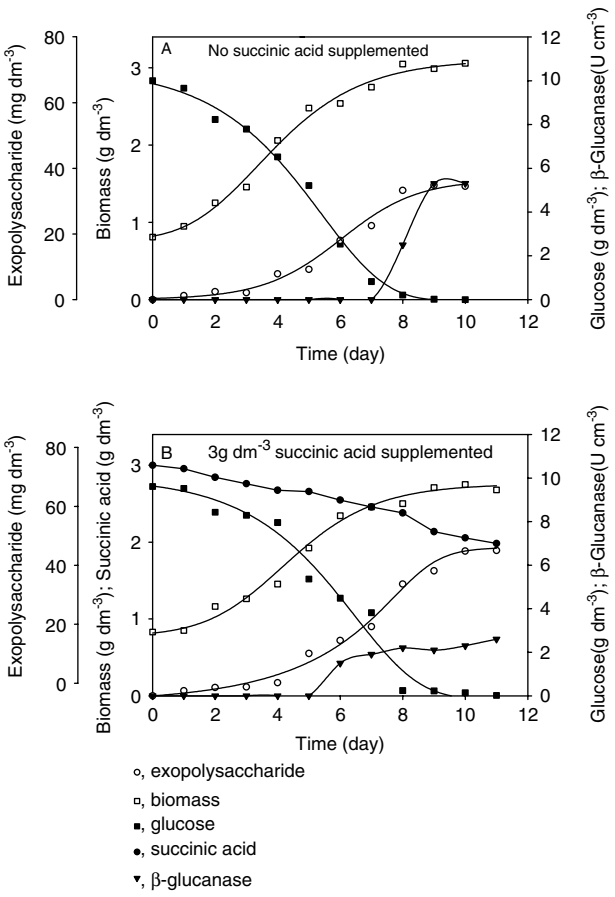

Figure 1. Batch exopolysaccharide fermentation supplemented with succinic acid at (A) 0 and (B) 3 g dm^{-3} in air-lift bioreactors.

further cell growth was observed even though succinic acid was still present at the end of the fermentation. Thus, co-metabolism of glucose and succinic acid plays an important role in the EPS fermentation. Also, the major energy source used for cell growth and product formation was glucose as indicated in the shaker flask culture in this study.

Although the EPS production of cells was subject to the medium composition and the vessels with the result that $Y_{P/X}$ of the culture without succinic acid was greatly improved from 3.88 in the shaker flask to 11.4 mg g^{-1} in the air-lift bioreactor, the effects including the inhibition of cell growth and the stimulation of EPS formation due to the supplement of succinic acid remained the same.

The β-glucanase activity of two batch cultures supplemented with 0 and 3 g dm^{-3} succinic acid in

Table 3. Results of batch cultures supplemented with 0 and 3 g dm^{-3} succinic acid respectively, in an air-lift bioreactor

Initial succinic acid concentration (g dm^{-3})	μ[a] (d^{-1})	Q_X[b] (g dm^{-3} d^{-1})	Q_P[c] (mg dm^{-3} d^{-1})	X_{max}[d] (g dm^{-3})	P_{max}[e] (mg dm^{-3})	$Y_{P/X}$[f] (mg g^{-1})	$Y_{X/S}$[g] (g g^{-1})	$Y_{P/S}$[h] (mg g^{-1})	t[i] (d)
0	0.43	0.34	3.87	3.05	34.8	11.4	0.31	3.55	9
3	0.35	0.29	4.45	2.85	44.5	15.6	0.29	4.54	10

[a] μ: specific growth rate (d^{-1}), [b] Q_X: biomass productivity (g dm^{-3} d^{-1}), [c] Q_P: EPS productivity (mg dm^{-3} d^{-1}), [d] X_{max}: maximum cell concentration (g dm^{-3}), [e] P_{max}: maximum EPS concentration (mg dm^{-3}), [f] $Y_{P/X}$: specific product yield (g EPS g^{-1} biomass), [g] $Y_{X/S}$: cell yield (g biomass g^{-1} glucose), [h] $Y_{P/S}$: product yield (g EPS g^{-1} glucose), [i] t: culture time (d).

air-lift bioreactors reached maximal values of 5.3 and 2.1 U cm^{-3}, respectively, in the stationary phase, and remained unchanged until the end of fermentation, as shown in Fig 1. This implies that the cells would start to utilize extracellular polysaccharides as the energy source by secreting EPS-degrading glucanase when glucose was exhausted in the broth. Inhibition of β-glucanase activity was also observed on the addition of succinic acid in the air-lift bioreactor.

3.4 Effects of different organic acids on the molecular weight of EPS in shaker flask cultures

The effects of different organic acid supplements on the number average molecular weight (*Mn*) of EPS and their different molecular distributions are shown in Fig 2(A and B, respectively). According to the distribution pattern of EPS of *A camphorata* on the GPC chromatograph, the EPS could be divided into three fractions: high-molecular-weight (HMW; greater than 300 kDa), medium-molecular-weight (MMW; 300–50 kDa), and low-molecular-weight fractions (LMW; less than 50 kDa).

In general, lower values of *Mn* as compared with that of the control were obtained as a result of the organic acid supplementation in the shaker flask cultures, as shown in Fig 2(A). *Mn* of the control (without organic acid supplementation) was 430 kDa, which decreased to 300 kDa in the succinic acid-supplemented culture and to 180 kDa in the pyruvic acid-supplemented culture.

HMW was the major fraction of exopolysaccharides of *A camphorata* in most of the shaker flask cultures, as shown in Fig 2(B). The proportion of HMW ranged from 58% in the control to 37% in the pyruvic acid-supplemented culture. Among the organic acids tested, higher amounts of HMW fractions (about 51%) were obtained in both the succinic acid- and oxalic acid-supplemented cultures. MMW was the next major fraction, and higher amounts of MMW fractions were observed in both the citric acid- and the pyruvic acid-supplemented cultures. The average proportion of LMW was about 10% in the shaker flask cultures. Thus, the molecular weight distribution of EPS produced was highly dependent on the composition of the medium. Since fermentation time and reactor type might play important roles in affecting molecular weight distribution of EPS, the effects of organic acid-supplemented cultures on molecular weight fractions were revealed in the study using an air-lift bioreactor.

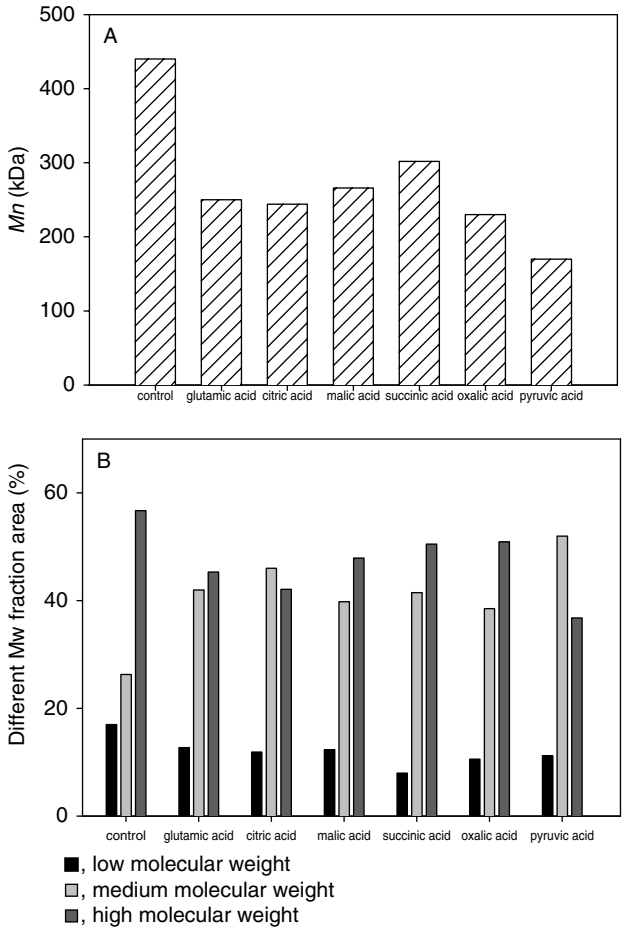

Figure 2. Effects of different organic acids supplementation on (A) the number average molecular weight (*Mn*) and (B) molecular weight (Mw) fraction area (%) in shaker flask cultures for 2 weeks.

3.5 Effect of succinic acid supplementation on molecular weight of EPS in an air-lift bioreactor

The time course data of the *Mn* and molecular weight distribution of EPS from two batch cultures supplemented with 0 and 3 g succinic acid dm^{-3} in an air-lift bioreactor are shown in Fig 3(A and B, respectively). The *Mn* of EPS of *A camphorata* in the batch culture without succinic acid supplementation decreased monotonically from 300 to 210 kDa with fermentation time, as shown in Fig 3(A). This observation was consistent with those of pullulan fermentation.[18,26] The decrease of *Mn* with the culture time resulted mainly from the decrease of HMW fraction and the increase of LMW fraction, as indicated in Fig 3(A), which could be explained by the degrading action of β-glucanase toward exopolysaccharides in the culture.[20]

However, the *Mn* of EPS from culture broth supplemented with 3 g succinic acid dm^{-3} increased slightly from 270 to 310 kDa with fermentation time, as shown in Fig 3(B). The moderate increase of *Mn* with culture time resulted mainly from the increase of HMW fraction, as indicated in Fig 3(B). This could be explained by the fact that the presence of succinic acid in the culture broth inhibited the β-glucanase activity by 2.5-fold, with a value at 2.1 U cm^{-3}.

Higher *Mn* was obtained in the succinic acid-supplemented culture as compared with that of the control in an air-lift bioreactor. This observation is not consistent with that of the shaker flask cultures. The slight increase in the HMW fraction of EPS at the end of fermentation has not yet been reported in the literature. It may be speculated that this was due to the conversion of succinic acid into EPS by *A camphorata*; however, further studies using

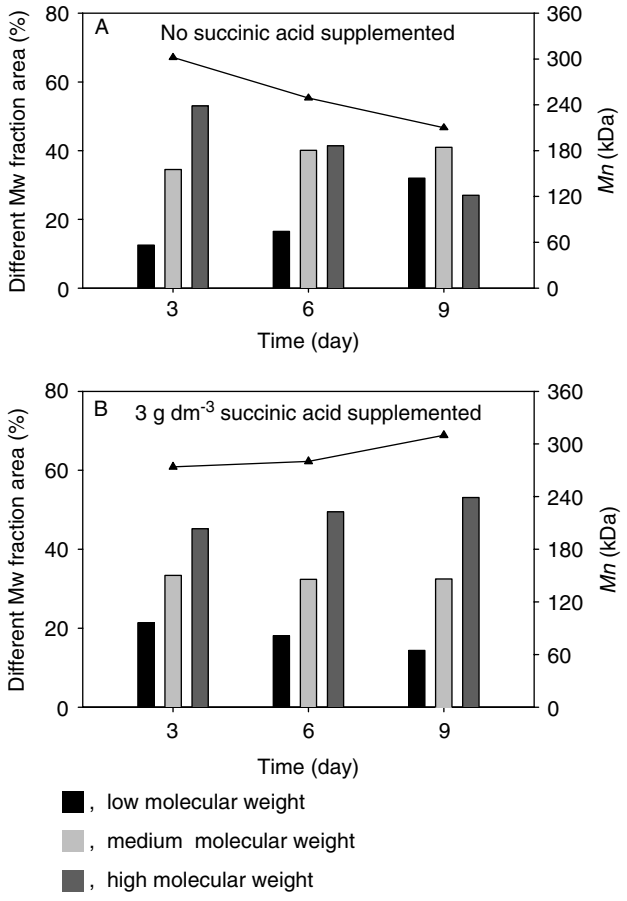

Figure 3. Effect of culture time on the number average molecular weight (*Mn*) and molecular weight (Mw) fraction area (%) of exopolysaccharides from batch cultures supplemented with succinic acid at (A) 0 and (B) 3 g dm^{-3} in an air-lift bioreactor. (▲) Number average molecular weight (*Mn*) of exopolysaccharide.

radioisotopically-labelled substrates are needed to elaborate the viewpoint.

3.6 Effect of reaction vessel type on molecular weight of EPS

Microorganisms performing differently in different reactor configurations is a commonly encountered phenomenon. In spite of the effects of succinic acid supplementation, the *Mn* of the control in the shaker flask cultures was higher than that in the air-lift bioreactor. The main fraction of the EPS in the shaker flask was HMW; however, it was MMW in the EPS in the air-lift bioreactor. This might be due to the large difference in the operational conditions such as oxygen transfer rate and mixing performance between the shaker flask and the bioreactor. In general, higher oxygen transfer and better mixing performance in the bioreactor would promote the biosynthesis of glucanases and enhance their enzymatic action. As demonstrated in this study, the β-glucanase activity in the shaker flask culture was only 9.4% of that of the air-lift bioreactor in the succinic acid-free cultures.

In succinic acid-supplemented cultures, there was little difference in terms of the molecular weight distribution of EPS from either the shaker flasks or the bioreactor. As a result, the value of the *Mn* of EPS from two different reaction vessels was similar, around 300 kDa. Besides, the enhancement of the molecular weight of EPS by succinic acid supplementation was partially explained by the lack of EPS degradation. Since the quality of polysaccharides is related to their molecular weight, and polysaccharide degradation is commonly encountered in aerated cultures upon the depletion of other carbon sources, a robust fermentation process with better quality of EPS, which is less sensitive toward the variation of harvest time, could be achieved by organic acid supplementation, as demonstrated in this study.

4 CONCLUSION

In conclusion, a significant improvement of both production and quality of EPS of *A camphorata* was demonstrated by succinic acid supplementation of cultures in the air-lift bioreactor. As a result, a novel fermentation process resistant to EPS degradation was proposed and demonstrated, using succinic acid supplementation in this study.

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