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Correlation analysis of *Pediococcus acidilactici* 3G3 batch fermentation parameters with bacteriocin production

Hamid Ahmadian Moghadam[1]* and Francisco B Elegado[2]

[1]College of Art and Science, Institute of Biological Science and
[2]National Institute of Molecular Biology and Biotechnology (BIOTECH)
University of the Philippines Los Baños (UPLB), College, Laguna 4031, Philippines

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Bacteriocin production is usually proportional to bacterial growth. There is lack of information with regards to time and concentration dependency of the bacteriocin production, and partial oxygen consumption in *Pediococcus acidilactici*. The aim of the study was to define the correlation among various growth factors of *P. acidilactici* 3G3 in relation to bacteriocin production within 24 h of batch fermentation process. The results confirmed a strong negative association between bacteriocin production and reduction in pH, but a strong positive association between dissolve oxygen and sugar consumption. Also, it was confirmed that a strong association existed between live cells and biomass concentration through optical density reading of the bacterial culture with bacteriocin production.

Keywords: Bacteriocin, batch fermentation, correlation analysis, growth variables

Introduction

Lactic acid bacteria are the common microbes employed as probiotics. The probiotics can reduce the severity of antibiotic associated diarrhea caused by overgrowth of potentially pathogenic organisms[1]. In fact, Pediococci exert antagonistic action against other microorganisms through the production of lactic acid and secretion of bacteriocins, known as pediocins. They have varying inhibitory properties against important food pathogens, such as, Listeria and Clostridia, and have peculiarities in terms of pH and temperature tolerance[3]. It has been elucidated that in a complex medium with yeast extract as the source of growth factors and trace elements, the yeast extract to sugar ratio defines the biomass yield and bacteriocin production. Very low values of this ratio give poor yields of bacteriocin[2]. However, lack of information exists regarding time and concentration dependency of the bacteriocin production with pH and partial oxygen consumption in *Pediococcus acidilactici*. The aim of present study was to analyze the correlation among the factors that influences growth and bacteriocin production of *P. acidilactici* 3G3 during batch fermentation.

*Author for correspondence
hamid.ahmadian@live.com

Materials and Methods

Microorganisms

Bacteriocin-producing *P. acidilactici* 3G3 and the indicator microorganism for bacteriocin assay, *Listeria innocua* 02*,* were provided by the National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines Los Baños. These were grown and maintained on De Mann, Rogosa and Sharpe (MRS) medium and Tryptic soy broth (TSB), respectively.

Batch Fermentation of *P. acidilactici*

The fermentation medium used was previously optimized TGYE medium, containing 20 g L^{-1} glucose, 20 g L^{-1} yeast extract, 15 g L^{-1} tryptone, 1.0 g L^{-1} Tween 80, 3.0 g L^{-1} triammonium citrate, 11.3 g L^{-1} sodium acetate, 3.0 g L^{-1} K_2HPO_4, 0.5 g L^{-1} $MgSO_4$ and 0.2 g L^{-1} $MnSO_4$. This medium (2 L) was placed in a 5-L bioreactor vessel (Sartorius Biostat A-Plus Bioreactor, Allentown, PA), autoclaved at 121°C for 15 min. The bacteriocin-producing strain was grown in MRS medium through daily consecutive transfers from glycerol stock to test tube (1 mL) and flask culture (50 mL), then seeded into the fermenter and incubated for 24 h at 37°C with agitation speed set at 100 rpm[4].

Analysis of Fermentation Process

P. acidilactici 3G3 grown for batch fermentation was analyzed for biomass and bacteriocin production

in duplicate runs. Samples were obtained every 2 h for 24 h with two trials. Changes in pH and partial oxygen consumption were tracked using the automatic features of the Sartorius Biostat A-Plus bioreactor. Total number of live cells as colony forming unit (CFU) was counted using a plating technique[5]. Total biomass was expressed as optical density (OD) of bacterial suspension measured as the absorbance of cell suspensions at 600 nm[6]. The "spot on lawn" method was used to assay bacteriocin production during fermentation process against *L. innocua* 02, which previously was identified as the most sensitive organism among other selected indicator microorganisms, namely, *Enterococcus faecium* 79, *E. faecalis* JCM 5803 and *P. pentosaceous* JCM 5885. An arbitrary unit (AU) of bacteriocin activity was defined as the reciprocal of highest dilution that gave clear zone of the inhibition of the indicator organism[7]. The total sugar concentration was determined according to the phenol sulfuric acid method[8].

Statistical Analysis

The "IBM SPSS Statistics 20" statistical software was used to perform the statistical analyses. Correlation analysis was performed in order to determine relationship between growth variables during the fermentation process. P-values lower than 0.05 was considered as statistically significant.

Results

The various data obtained from 24 h fermentation of *P. acidilactici* 3G3, the bacteriocin producing strain, was subjected to Pearson's correlation analysis (Table 1). The result showed a strong negative association between pH and bacteriocin production (-0.910), *i.e.*, decrease in pH increases bacteriocin activity (Fig. 1). Also, through the course of fermentation, biomass increase was negatively correlated with pH (-0.902) (Fig. 2). The results also showed a strong positive correlation between dissolve oxygen consumption and amount of total sugar consumed (0.907). Such behaviour confirmed the premise that consumption of substrate needs more oxygen, resulting to increase in bacterial population, which led to greater production of bacteriocin as primary metabolic product (Fig. 3). Although, lactic acid production is an anaerobic process, cell metabolism and bacteriocin production could be influenced by oxygen consumption. The Results showed strong positive correlation between the amount of live cell and the amount of OD (0.838). Obviously,

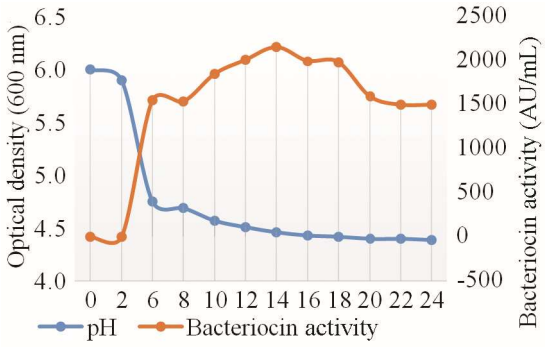

Fig. 1 — Correlation between bacteriocin activity (AU per mL) and pH.

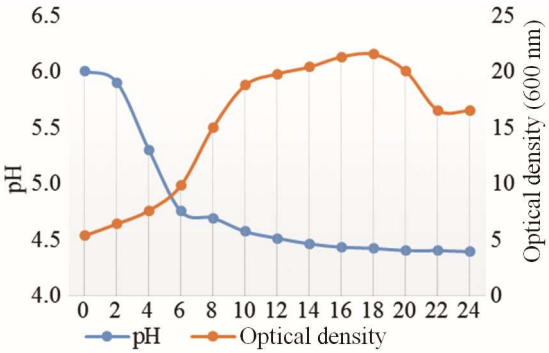

Fig. 2 — Correlation between optical density and pH.

Table 1 — Results of Pearson's correlation analysis of various data points of batch fermentation of *P. acidilactici* 3G3

	BC	TS	OD	LC	DO	PH
BC	1					
TS	-0.51 ^{NS}	1				
OD	**0.842****	-0.732**	1			
LC	0.680*	-0.616*	**0.838****	1		
DO	-0.429^{NS}	**0.907****	-0.573*	-0.409 ^{NS}	1	
PH	**-.910****	0.749**	**-0.902****	-0.659*	0.698**	1

Correlation is significant at the 0.01 level (**), Correlation is significant in 0.05 level (*), Correlation is not significant (^{NS}). Bacteriocin (BC), Total sugar (TS), Optical density (OD), Live cells (cfu/ml) (LC), Dissolve Oxygen (DO), pH (PH)

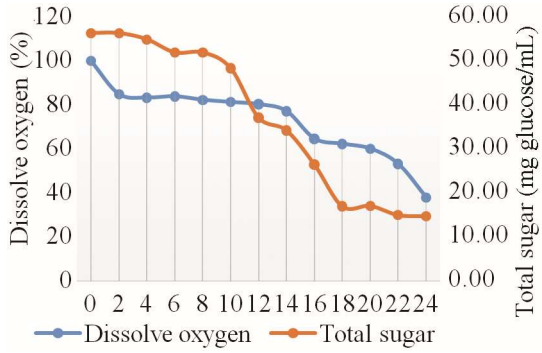

Fig. 3 — Correlation between dissolve oxygen and total sugar concentration.

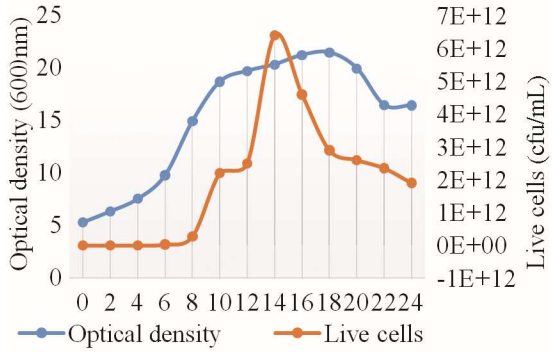

Fig. 4 — Correlation between optical density and live cells.

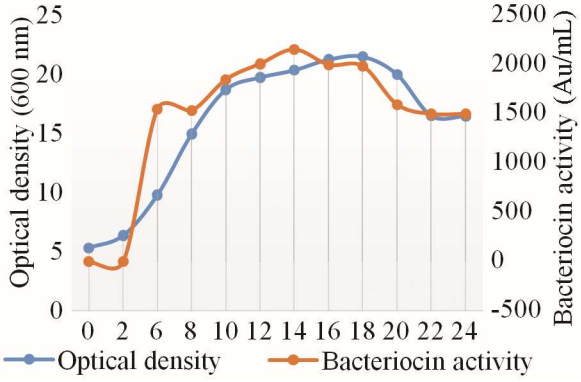

Fig. 5 — Correlation between optical density and bacteriocin activity.

increased live cells or bacterial growth is the main reason for the increase in OD of the growth medium (Fig. 4). Hence, there is strong association between OD and bacteriocin production (0.842) that confirms that bacteriocin activity was indeed proportional to biomass or microbial growth (Fig. 5). However, no significant correlation was shown between total sugar concentration and bacteriocin production (Table 1).

Discussion

The antimicrobial activity of bacteriocin is primarily through permeabilization of the membrane of susceptible microorganisms. In class IIa bacteriocin (pediocin PA-1, mesentericin Y105 & bavaricin MN) membrane pores are formed and cause the leakage of inorganic phosphate due to ionic imbalance[9,10]. Bacteriocin production is usually proportional to bacterial growth and shows primary metabolite kinetics with the rate of production paralleled to the growth rate. Bacteriocin production somewhat tapers off during the stationary phase. In general, pediocin production displays primary metabolite kinetics in parallel to the growth rate, so it shows similarity to other bacteriocins in this respect[6]. Results confirmed that there is difference in etiology of bacteriocin production but other factors that trigger bacteriocin production is the reduction in pH that was confirmed by correlation analysis. During batch fermentation, increase in OD or viable cells results to increased substrate consumption and the production of bacteriocin as another product, aside from lactic acid and other organic acids. The accumulation of lactic acid as primary product is the main reason for reduction in pH and subsequent processing or activation of bacteriocin. Oxygen consumption is also positively correlated to growth and bacteriocin production in spite of the microaerophilic nature of the bacteria. Further studies on optimization of aeration or stirring during batch fermentation of bacteriocin production by *P. acidilactici* 3G3 should be the next step to do.

Conclusion

The results confirm a strong negative association between bacteriocin production and reduction in pH and a strong positive association between dissolve oxygen and sugar consumption that led to higher biomass and bacteriocin production.

Acknowledgment

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