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Growth and bacteriocin production by *Enterococcus faecium* DPC1146 in batch and continuous culture

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Production of the bacteriocin enterocin 1146 (E1146) by *Enterococcus faecium* DPC1146 was studied in batch and continuous fermentation. Growth was strongly inhibited by lactic acid. In batch fermentations maximum E1146 activity (2.8 MBU L^{-1}) was obtained in 9 h with 20 g L^{-1} glucose. Increase in initial glucose concentration did not lead to a proportional increase in E1146 activity. A simple linear model was found to be adequate to explain the relationship between specific bacteriocin production rate and specific growth rate in batch fermentations with initial glucose concentration higher than 20 g L^{-1}. Maximum bacteriocin activity (2.9–3.2 MBU L^{-1}) was obtained in continuous fermentations at dilution rates between 0.12 and 0.17 h^{-1} and specific bacteriocin production rate increased linearly with dilution rate.

Keywords: enterocin 1146; bacteriocin; batch fermentation; continuous fermentation

Introduction

Several strains of lactic acid bacteria produce proteins or protein complexes (bacteriocins) which inhibit spoilage and pathogenic microorganisms and are therefore potentially useful as natural food preservatives [8,12]. To this end, they can be produced either *in situ* by starter bacteria or in a fermenter and added to the food. Bacteriocin production in batch and continuous culture has been studied by several authors. Bacteriocin production usually shows primary metabolite kinetics [2,7,18,20,26], starting as soon as cell growth begins and ceasing at or immediately before the end of cell growth. A decrease in bacteriocin activity may follow as a result of bacteriocin degradation by specific or non-specific proteases [2,6] or adsorption to the producer cells [18,20]. Any factor increasing cell growth increases bacteriocin production. Therefore, higher bacteriocin production is obtained in rich media [16,26] and when pH is controlled [2,7,26]. The latter has a strong influence not only on bacteriocin production but also on adsorption/degradation [10,18,20].

Although bacteriocin production is growth-associated, the relationship between growth rate and bacteriocin production rate is not straightforward. In the production of bavaricin MN by *Lactobacillus bavaricus* MN in batch and continuous culture, pH has a more important role than growth rate [7]. In continuous culture [10], nisin production is tightly regulated and influenced by cell-adhered nisin. In batch fermentations, nisin production [2] was found to be affected by carbon source regulation. Moreover, while the pre-nisin gene was expressed throughout cell growth, late increase in nisin activity was attributed to the expression of genes related to post-translational modification and secretion [2]. The importance of factors affecting post-translational modification has also been pointed out for the lantibiotics pediocin AcH [21,26] and lactocin S [11].

Enterocin 1146 (E1146) is a bacteriocin produced by *Enterococcus faecium* DPC1146 which is active against *Listeria monocytogenes* [17]. In previous works we examined the influence of media components [16,19] and pH [18] on E1146 production. E1146 production was growth-associated and strongly influenced by pH in batch fermentations: a kinetic model was tentatively proposed and optimal production of E1146 and pH 5.5 was explained by a higher bacteriocin yield per unit biomass ($Y_{E/X}$) and a lower adsorption/degradation constant (k) [18]. The objective of this work was to evaluate the effect of growth rate on enterocin 1146 production in batch and continuous culture.

Materials and methods

Microbial strains and growth media

Enterococcus faecium DPC1146 (the producer of enterocin 1146) and *Listeria innocua* BL86/26 (the indicator strain) were kindly provided by Dr T Cogan, National Dairy Products Research Centre, Fermoy, Co Cork, Eire. Both strains were maintained as frozen (−40°C) stocks in 25% glycerol. Working cultures were stored at 4°C and were obtained at monthly intervals from frozen stocks by growing *E. faecium* DPC1146 in M17 broth + 0.5% glucose at 37°C for 16 h and *L. innocua* BL86/26 in tryptone soya broth + 0.6% yeast extract at 30°C for 16 h.

The basal medium for bacteriocin production [19] had the following composition (g L^{-1}): peptone soya neutralised 6; yeast extract 5; KH_2PO_4 1; Tween 80 (Sigma Chemical Co, Chicago, IL, USA) 2; $MgSO_4 \cdot 7H_2O$ 0.25; $MnSO_4 \cdot H_2O$ 0.05; pH 4.5. Five levels of glucose ($S_0 = 5$, 10, 20, 25 and 30 g L^{-1}) were tested in different fermentations. The medium was sterilized at 121°C for 15 min and its pH was adjusted to 5.5 with NaOH immediately before inoculation. For inoculum growth 10 g L^{-1} glucose were used, KH_2PO_4 was replaced by 19 g L^{-1} sodium β-glycerophosphate

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(Sigma) and the pH was adjusted to 6.8 with HCl before sterilization.

All bacteriological media and ingredients were obtained from Oxoid (Unipath, Basingstoke, UK). Unless otherwise indicated, chemicals were obtained from BDH Ltd, Poole, UK.

Fermentation procedures

A 2.5-L (working volume) stirred tank reactor (Applikon, Schiedam, Netherlands) equipped with two 6-bladed Rushton turbines, and instrumentation for measurement and control of temperature (37°C), pH (5.5, by addition of NaOH) and agitation speed (200 rpm) was used in batch fermentations. The fermenter was filled to 2.35 L with the production medium and inoculated with 0.15 L of a 16-h culture of *E. faecium* DPC1146.

Continuous fermentation runs were carried out in a 1-L (working volume) stirred tank reactor (Applikon, Schiedam, Netherlands), equipped with instrumentation for the measurement and control of temperature (37°C), pH (5.5, by addition of NaOH) and agitation (magnetic stirrer MR2002, Heidolph Elektro GmbH & Co, Kelheim, Germany, 500 rpm). The basal medium with 20 g L^{-1} glucose (which resulted in a measured glucose content of 21.67 ± 0.27 g L^{-1}, because of the high carbohydrate content of peptone soya neutralized) was used for all fermentations. The fermenter was inoculated with 5% (v/v) of a 16-h culture and the fermentation was carried out batchwise for 8 h before continuous feeding of the substrate was started. The volume was kept constant at 0.8 L using a peristaltic pump (model 502S, Watson Marlow, Falmouth, UK). Medium flow rate was measured several times during each cycle: the coefficient of variation of the measurements ranged from 0.5 to 4.8% (average 1.4%). Sampling was started after at least five culture volumes were passed through the vessel. Thereafter, to ensure that the culture had reached a steady state, the optical density of the culture was measured at 600 nm by using a spectrophotometer (DU65 Beckman Instruments, Fullerton, CA, USA) on five samples taken from the medium outlet at 20-min intervals. If the OD values of five subsequent samples were stable and did not reveal any statistically significant trend, three other samples were taken at 30-min intervals and used for analyses.

Analytical methods

Culture samples (10 ml) were centrifuged ($3150 \times g$, 10 min) in preweighed centrifuge tubes. The pellet was washed with distilled water, recentrifuged and dried at 105°C for 24 h for cell dry weight measurement. The supernatant was filter-sterilized through HV membranes (0.45 μm pore size, Millipore, Bedford, MA, USA) and stored at –20°C until needed. Glucose and L-lactic acid concentrations were measured using enzymatic test combination kits (Boehringer Mannheim, Mannheim, Germany). E1146 activity was measured in triplicate using a photometric method [15] and expressed as MBU L^{-1} (10^6 BU L^{-1}; one Bacteriocin Unit is the amount of bacteriocin needed to obtain a 50% inhibition of the indicator strain).

Statistics

Linear and non-linear regressions were carried out using Systat 5.2.1 for Macintosh [23]. Continuous lines on graphs were obtained by linear smoothing or by Distance Weighted Least Square Smoothing (DWLS) [24], as applicable.

Results

Batch fermentations

Five batch fermentations were carried out to evaluate the effect of initial glucose concentration (S_0 = 5, 10, 20, 25 or 30 g L^{-1}) on the kinetics of growth and enterocin 1146 (E1146) production. Maximum biomass concentration (2.4 g L^{-1}) was obtained with $S_0 = 20$ g L^{-1}. At higher initial glucose concentrations growth was slower and biomass concentrations at the end of fermentation were lower (1.9 and 1.7 g L^{-1} at $S_0 = 25$ and 30 g L^{-1}, respectively). The typical time course of a fermentation at $S_0 = 20$ g L^{-1} is shown in Figure 1. E1146 production started at the beginning of cell growth, slowed down at 5 h, reached a maximum (2.8 MBU L^{-1}) at the end of cell growth. A sharp decrease in E1146 concentration followed.

E1146 production at 5–30 g L^{-1} glucose is compared in Figure 2. At S_0 = 5 and 10 g L^{-1}, maximum E1146 titre was obtained before the end of growth (not shown) and was followed by a decrease in activity which was faster at $S_0 = 5$ g L^{-1}. Bacteriocin production was slower but more prolonged at $S_0 = 20$ and 25 g L^{-1}: maximum E1146 activity was reached at the end of growth and was followed by a sharp decrease. Bacteriocin production was even slower at $S_0 = 30$ g L^{-1}, and no decrease in bacteriocin activity was observed.

The experimental data for cell dry weight, lactic acid and substrate concentration were smoothed using a generalized logistic model [3]:

$$C_i = K/[1 + \exp(a_0 + a_1 \cdot t + a_2 \cdot t^2 + a_3 \cdot t^3 + a_4 t^4)] \quad (1)$$

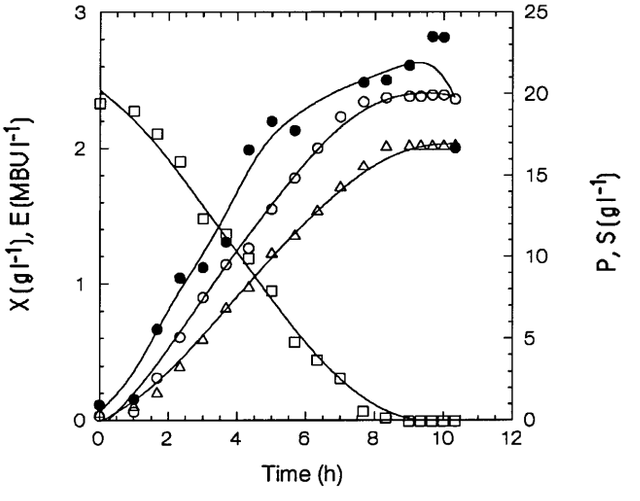

Figure 1 Kinetics of growth of *E. faecium* DPC1146 (○, X, cell dry weight, g L^{-1}), lactic acid production (△, P, g L^{-1}), glucose consumption (□, S, g L^{-1}), and enterocin 1146 production (●, E, MBU L^{-1} = 10^6 Bacteriocin Units L^{-1}) in a medium containing 20 g glucose L^{-1} during batch fermentation at 37°C and pH 5.5.

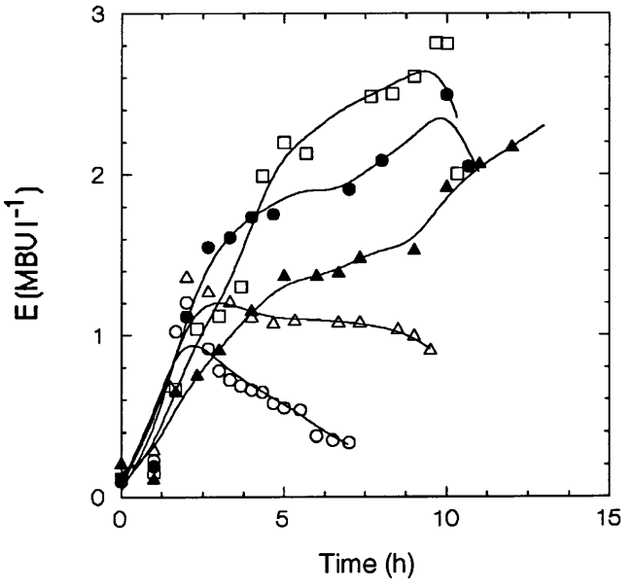

Figure 2 Kinetics of enterocin 1146 (E, MBU L^{-1} = 10^6 Bacteriocin Units L^{-1}) production by *E. faecium* DPC1146 during batch fermentation at 37°C and pH 5.5 in a medium containing 5 (○), 10 (△), 20 (□), 25 (●) or 30 (▲) g L^{-1} glucose.

where C_i is cell dry weight (X), lactic acid (P) or glucose (S) concentration, K is a constant which was arbitrarily set to 1.2 times the maximum value of C_i, a_i are parameters of the equation (estimated by non-linear regression) and t is the time in h. With this procedure the difference between calculated and experimental data was on average 1.4%. Cell growth rate (r_x), lactic acid production rate (r_p) and substrate consumption rate (r_s) were calculated by taking the first derivative of Eqn 1:

$$-dC_i/dt = -C_i \cdot (1-C_i/K) \cdot (a_1 + 2a_2 \cdot t + 3a_3 \cdot t^2 + 4a_4 \cdot t^3) \quad (2)$$

Specific rates (specific growth rate, μ; specific lactic acid production rate, π; specific glucose consumption rate, ν) were estimated by dividing the rates obtained from Eqn 2 by the smoothed values of the cell dry weight calculated with Eqn 1.

The estimated values for μ were strongly affected by lactic acid concentration. Inhibition of growth of lactic acid bacteria by lactic acid has been described as non-competitive by several authors [1,13,22]. Therefore, the relationship between specific growth rate and instantaneous glucose (S) and lactic acid (P) concentration can be described by the following equation:

$$\mu = \mu_{max} \cdot [S/(K_{S\mu}+S)] \cdot [K_{P\mu}/(K_{P\mu}+P)] \quad (3)$$

where μ_{max} is the maximum specific growth rate, $K_{S\mu}$ is the substrate saturation constant and $K_{P\mu}$ the non-competitive inhibition constant. In batch culture, where S is much higher than $K_{S\mu}$ (values of $K_{S\mu}$ close to 0.2 g L^{-1} have been calculated for enterococci [13,14]), Eqn 3 simplifies to:

$$\mu = \mu_{max} \cdot [K_{P\mu}/(K_{P\mu}+P)] \quad (4)$$

which can be rearranged as follows:

$$1/\mu = 1/\mu_{max} + [1/(K_{P\mu} \cdot \mu_{max})] \cdot P \quad (5)$$

A plot of $1/\mu$ *versus* P is shown in Figure 3. A strong deviation from linearity is observed at lactic acid concentrations higher than 8 g L^{-1}. Lower values of μ were associated to higher concentrations of P and S (which are plotted with different symbols in Figure 3). As suggested by Cachon and Diviès [1], estimates of μ_{max} (1.85 h^{-1}) and $K_{P\mu}$ (1.1 g L^{-1}) can be obtained from Eqn 5 by taking only the linear portion of Figure 3.

In batch culture, the relationship between specific growth rate and specific lactic acid production rate can be described by the Luedeking–Piret [9] type model:

$$\pi = Y_{P/X} \cdot \mu + m_P \quad (6)$$

where $Y_{P/X}$ and m_P are constants related to growth-associated and non-growth associated lactic acid production. A linear relationship was found between μ and π (not shown), and the following estimates of the constants were obtained: $Y_{P/X} = 3.8 + 0.2$ g g^{-1} and $m_P = 0.5 \pm 0.1$ h^{-1} (adj $r^2 = 0.91$).

Equation 1 cannot be used to smooth the data for bacteriocin production which follow a non-monotonous kinetics. A rough estimate of bacteriocin production rate (r_E, MBU L^{-1} h^{-1}) at time t can be obtained as follows:

$$r_E = (E_2 - E_1)/(t_2 - t_1) \quad (7)$$

where t_2 and t_1 are the sampling times immediately before and after time t and E_2 and E_1 are the corresponding bacteriocin concentrations. Maximum bacteriocin production rates were 1.0, 1.1, 0.7, 0.7 and 0.5 MBU L^{-1} h^{-1} at $S_0 = 5$, 10, 20, 25 and 30 g L^{-1} glucose, respectively. Average bacteriocin production rates (calculated with $t_1 = 0$ and t_2 corre-

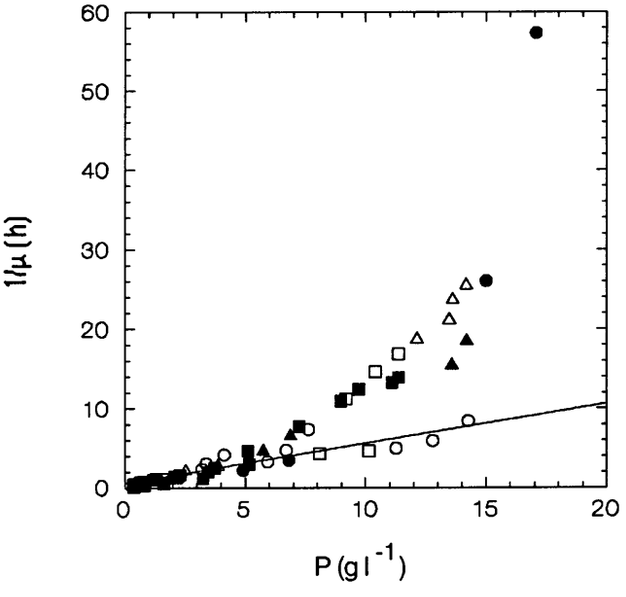

Figure 3 Relationship between lactic acid concentration (P) and the reciprocal of specific growth rate ($1/\mu$) during batch fermentation at 37°C and pH 5.5. Different plot symbols were used for different instantaneous glucose concentrations (S, g L^{-1}): $S \leq 5$ (○), $5 < S \leq 7.5$ (△), $7.5 < S \leq 10$ (□), $10 < S \leq 12.5$ (●), $12.5 < S \leq 15$ (▲), $S > 15$ (■).

sponding to the time at which E was maximum) were 0.6, 0.6, 0.4, 0.2 and 0.2 MBU L^{-1} h^{-1} at $S_0 = 5$, 10, 20, 25 and 30 g L^{-1} glucose, respectively.

Specific bacteriocin production rates (ϵ, MBU g^{-1} h^{-1}) were obtained by dividing r_E by the smoothed values of X calculated using Eqn 1. A plot of ϵ as a function of μ is shown in Figure 4. The plot markedly deviated from linearity for fermentations with $S_0 = 5$ and 10 g L^{-1} glucose. In previous works [18,20] we proposed the following model to explain the relationship between bacteriocin production rate (r_E) and cell growth rate (r_X):

$$r_E = Y_{E/X} \cdot r_X - k \cdot X \cdot E \quad (8)$$

where X (g L^{-1}) and E (MBU L^{-1}) are concentrations of biomass and E1146, respectively, $Y_{E/X}$ the bacteriocin yield per unit biomass (MBU g^{-1}) and k (g^{-1} h^{-1}) an adsorption/degradation constant. By dividing both members of Eqn 8 by X, the following relationship is obtained:

$$\epsilon = Y_{E/X} \cdot \mu - k \cdot E \quad (9)$$

If bacteriocin adsorption/degradation is assumed to be a function of cell concentration only, Eqn 9 becomes:

$$\epsilon = Y_{E/X} \cdot \mu - k \quad (10)$$

A poor fit was obtained when the parameters of either Eqn 9 or Eqn 10 were estimated by linear regression using estimated values of μ and ϵ and experimental values of E ($Y_{E/X} = 1.9 \pm 0.2$, $k = 0.17 \pm 0.07$, residual mean square, r.m.s. = 0.395, adj $r^2 = 0.76$ for Eqn 9; $Y_{E/X} = 2.1 \pm 0.2$, $k = 0.32 \pm 0.11$, r.m.s. = 0.337, adj $r^2 = 0.72$ for Eqn 10). However, if only cases with $\epsilon > 0$ were taken into account, a significantly better fit was obtained ($Y_{E/X} = 1.9 \pm 0.1$, $k = 0.09 \pm 0.04$, r.m.s. = 0.118, adjusted $r^2 = 0.90$ for Eqn 9; $Y_{E/X} = 2.0 \pm 0.2$, $k = 0.18 \pm 0.07$, r.m.s. = 0.100, adjusted $r^2 = 0.86$ for Eqn 10). In both instances, similar estimates for $Y_{E/X}$ are obtained. Moreover, since Eqn 9 does not provide a significantly better fit, the simpler model described by Eqn 10 should be preferred.

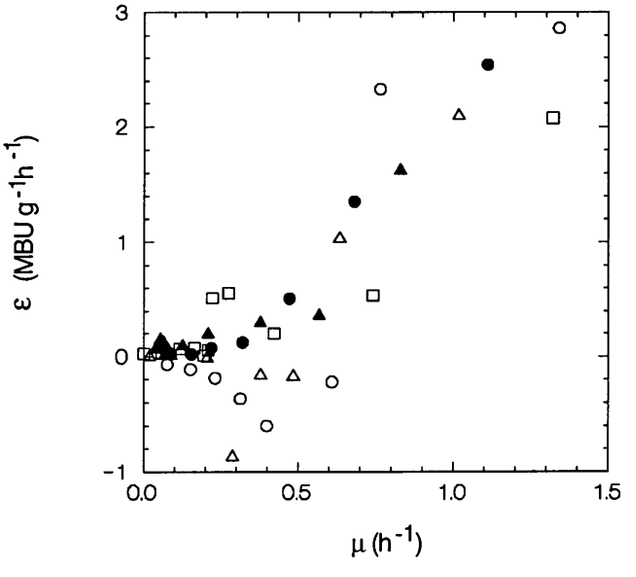

Figure 4 Relationship between specific enterocin 1146 production rate (ϵ) and specific growth rate μ during batch fermentation at 37°C and pH 5.5 in a medium containing 5 (○), 10 (△), 20 (□), 25 (●) or 30 (▲) g L^{-1} glucose.

Continuous fermentations

Steady state biomass, glucose, lactic acid and enterocin 1146 concentrations as a function of dilution rate during continuous fermentation are shown in Figure 5. Biomass concentration fell from 1.45 ± 0.1 to 0.9 ± 0.03 (mean ± standard error) as D increased from 0.10 to 0.60 h^{-1}. Residual glucose concentration was never lower than 1.9 g L^{-1} and a maximum lactic acid concentration of 18.2 g L^{-1} was obtained at the lowest dilution rate. The highest bacteriocin titre (3.2 ± 0.07 MBU L^{-1}) was observed at $D = 0.14$ h^{-1}.

In continuous fermentations specific lactic acid production rate (π, h^{-1}), specific glucose consumption rate (ν, h^{-1}) and specific E1146 production rate (ϵ, MBU g^{-1} h^{-1}) were calculated as follows:

$$\pi = P \cdot D/X \quad (11)$$

$$\nu = (S_F - S) \cdot D/X \quad (12)$$

$$\epsilon = E \cdot D/X \quad (13)$$

where D is the dilution rate (h^{-1}), and P, X, S (g L^{-1}), E (MBU L^{-1}) are the steady state concentrations of lactic acid, biomass, glucose and E1146, respectively, while S_F is the glucose concentration in the feed medium.

The relationships between π, ν, ϵ and D (=μ) are shown in Figure 6. The data were not sufficient to obtain accurate estimates of the constants of Eqns 4–6. However, by using the value of μ_{max} estimated for batch fermentations, a value of $K_{P\mu} = 1.4 \pm 0.15$ was estimated by non-linear regression using Eqn 4. Estimates of $Y_{P/X}$ (4.1 ± 0.5) and m_P

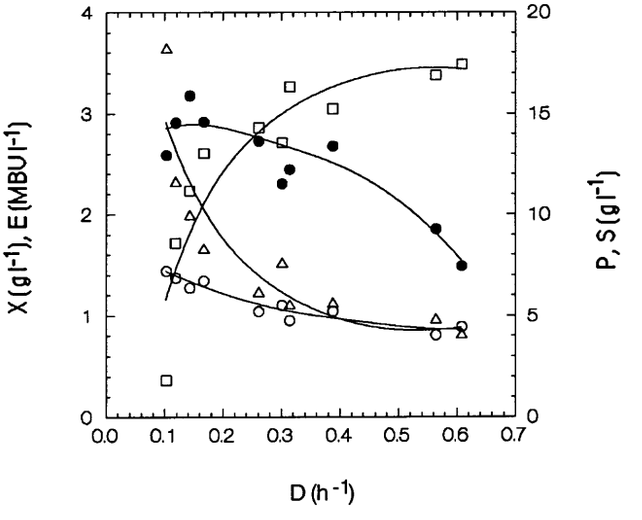

Figure 5 Steady state values of biomass (○, as cell dry weight, X, g L^{-1}), lactic acid (△, P, g L^{-1}), glucose (△, S, g L^{-1}) and enterocin 1146 (●, E, MBU L^{-1} = 10^6 Bacteriocin Units L^{-1}) as a function of dilution rate (D, h^{-1}) during continuous fermentation at 37°C and pH 5.5 with *E. faecium* DPC1146.

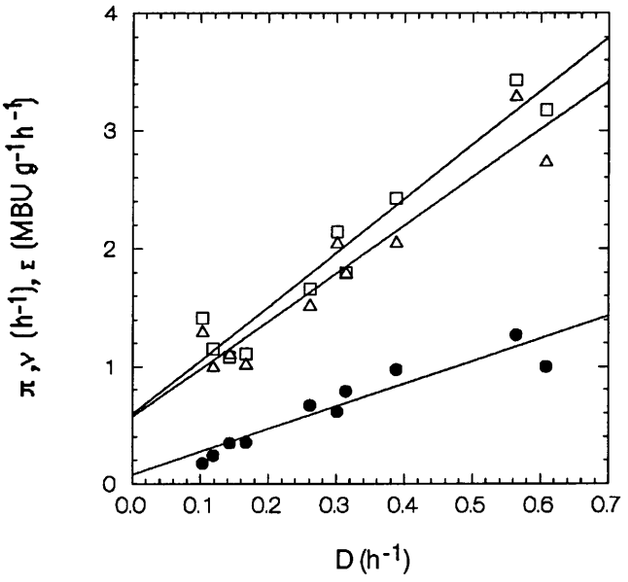

Figure 6 Specific lactic acid production rate (△, π, h^{-1}), specific glucose consumption rate (□, ν, h^{-1}) and specific enterocin 1146 production rate (●, ϵ, MBU g^{-1} h^{-1}) as a function of dilution rate (D = μ, h^{-1}) during continuous fermentation at 37°C and pH 5.5 with *E. faecium* DPC1146.

(0.57 ± 0.16) which were not significantly different from those calculated from batch fermentations were obtained by using Eqn 6. A linear relationship was found between ϵ and μ, as predicted by Eqn 10. However, although the estimated value of $Y_{E/X}$ (1.9 ± 0.2) was not significantly different from that obtained from batch fermentations, the adsorption/degradation constant k was not significantly different from 0.

Discussion

Growth and bacteriocin production were studied in batch fermentations with initial glucose concentrations between 5 and 30 g L^{-1}. Maximum cell dry weight and bacteriocin activity were obtained with S_0 = 20 g L^{-1}. Growth appeared to be inhibited by lactic acid and possibly by glucose. Non-competitive inhibition of growth by lactic acid has been reported by several authors [1,13,22] and concomitant substrate and product inhibition kinetics has been reported for *Lactobacillus delbrueckii* [4]. However, *E. faecium* DPC1146 appears to be extremely sensitive to inhibition by lactic acid (the estimated $K_{P\mu}$ was 1.1 g L^{-1}, while values quoted for lactococci [1,22] or enterococci [13,14] are higher than 9 g L^{-1}), and possibly to substrate inhibition.

In batch fermentations with glucose (5–25 g L^{-1}) bacteriocin titres reached a peak at or before the end of growth and then decreased. At higher glucose concentrations (30 g L^{-1}) no decrease in bacteriocin titre was observed, but this may be due to the relatively short time used for incubation, which was stopped at the end of growth. Maximum and average bacteriocin production rates decreased as initial glucose concentration increased. The lack of proportionality between maximum bacteriocin titre and initial glucose concentration is in agreement with the predictions of Eqn 10, because of the increasing weight of the negative term in prolonged fermentations. Equation 10 allowed the estimation of bacteriocin yield ($Y_{E/X}$) and adsorption/ degradation constant (k) and provided a reasonable fit of experimental data at specific bacteriocin production rates $\epsilon > 0$. Assuming that the estimates of the parameters are correct, ϵ should become negative when μ becomes lower than 0.09 h^{-1}: although this is in good agreement with the results of fermentations at $S_0 \geq 20$ g L^{-1}, it does not explain the early decrease in bacteriocin activity which occurred in fermentations at S_0 = 5 or 10 g L^{-1} when μ was still larger than 0.35 h^{-1}. In conclusion, Eqn 10 is not completely adequate to explain the results obtained in batch fermentations. Although difficulties in estimating the parameters of the model may be due to the procedure used to calculate the specific bacteriocin production rate, other factors in addition to those included in the model may be important in influencing bacteriocin production rate. Studies on the effect of initial carbohydrate concentration on bacteriocin production in batch fermentations are rare. In a study of nisin production in batch fermentation [2], a pattern similar to that observed in this work was found: highest nisin titre occurred at 40 g L^{-1} sucrose, while nisin yield decreased as initial substrate concentration increased. This led the authors to postulate carbon-source regulation for nisin production.

In continuous fermentations, the quantitative study of the relationship between growth and bacteriocin is simplified because bacteriocin production rate can be calculated in a more direct way. Maximum E1146 activity was obtained at dilution rates between 0.12 and 0.17 h^{-1}. In this interval E1146 activity was slightly higher than that obtained in batch fermentation. When the parameters of Eqn 10 were estimated from the data from continuous fermentations, a value of $Y_{E/X}$ which was not significantly different from that obtained in batch fermentations was obtained. Although the value of the adsorption/degradation constant k was not significantly different from 0, the experimental data for ϵ tended to 0 as μ decreased toward 0.05 h^{-1}.

It is difficult to compare our data with those on other bacteriocins or lantibiotics produced by lactic acid bacteria. Higher bacteriocin activities in continuous fermentation compared to batch fermentation were obtained for bavaricin MN [7] and for nisin [25]. During continuous fermentation of APT broth [7], bavaricin MN activity remained constant when D was varied between 0.058 and 0.205 h^{-1}. Even if a semiquantitative method was used for bacteriocin activity and biomass was measured as CFU ml^{-1}, when specific bacteriocin production rate is calculated, it increases from 0.06×10^{-6} to 0.5×10^{-6} AU CFU^{-1} h^{-1} as μ increases from 0.058 to 0.205 h^{-1} with a linear pattern, as observed from E1146.

On the other hand, in continuous nisin production under non-limiting lactose concentration [10], a strongly non-linear relationship was found between specific nisin production rate and dilution rate, with a peak in both nisin activity and specific production rate between D = 0.2 h^{-1} and D = 0.3 h^{-1} (the highest tested D was 0.4 h^{-1}). The authors explained their results on the basis of both carbon source regulation and effect of growth rate, indicating that maximum specific production rate was obtained with a specific lactose consumption rate of 3.3 h^{-1}. The occurrence

of a peak in nisin specific production rate may reflect the complexity of nisin synthesis and of its regulation [2,10].

The results of our experiments suggest some areas for further work. Taniguchi *et al* [25] showed that higher nisin productivities can be obtained in continuous fermentation with cell recycle by microfiltration compared to batch fermentation, although the average nisin concentration may vary significantly with a number of factors (glucose concentration in the feed/bleed ratio). In fact, whether or not the adsorption degradation/term is significantly different from 0, it can be calculated from mass balances for bacteriocin, biomass and substrate and from Eqn 10 that higher bacteriocin activity and productivity and lower residual glucose concentrations can be obtained at higher dilution rates if cell recycle is used in continuous fermentations. This approach would also simplify the development of integrated production, recovery and purification processes by adsorbing the bacteriocin from the permeate stream on appropriate resins, as described for epidermin [5], leading to more economic processes for bacteriocin production.

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