Preliminary tests on nisin and pediocin production using waste protein sources. Factorial and kinetic studies.

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Abstract

Lactic acid bacteria, the object of current interest as bacteriocin producers, are microorganisms with complex requirements for peptidic sources, making them appropriate indicators for testing the suitability of formulations based on proteinaceous wastes for use as microbiological media. Different peptones obtained from visceral and fish muscle residues promoted growth of lactic acid bacteria when applied individually or in combination. Kinetic parameters and bacteriocin production were similar and, in some cases (pediocin), far superior (>500%) to those obtained with bactopeptones and commercial media specifically recommended for lactic acid bacteria growth. Visceral residues, especially when subjected to a brief process of autohydrolysis at 20ºC, were more efficient for bacterial growth than muscle, even when muscle was treated with pepsin.

1. Introduction

Bacteriocins produced by lactic acid bacteria (LAB) are peptides degradable by intestinal proteases, with relatively specific antimicrobial activities and a number of applications in the field of food protection. In general, for LAB cultures semi-synthetic complex media are recommended, such as MRS, TGE, APT (*e.g.* Daba et al., 1993; Jensen and Hammer, 1993; De Vuyst, 1995). Although commercially available, these media are expensive, making the search for cheaper formulations for bacteriocin production attractive. Possible alternatives include residual media such as milk whey or mussel processing wastes (Goulhen et al., 1999; Guerra and Pastrana, 2002; Amiali et al. 1998; Vázquez et al., 2003).

The recommended media for LAB culture usually contain a surplus of proteins (tryptone, peptone, meat extract, yeast extract), a substantial proportion of which remain unconsumed, involving superfluous cost and hindering bacteriocin purification. If the role of the proteins is limited to the contribution of nitrogen, the problem could be resolved by using inorganic sources (Guerra and Pastrana, 2001), or by reducing the initial protein level to a value of the order of that consumed (Vázquez, 2001; Cabo, 1998). However, it has been suggested (*e.g.* Jensen and Hammer, 1993; De Vuyst, 1995) that the most important feature of these protein sources is their content of peptides that can act as inducers or precursors of bacteriocin biosynthesis, a hypothesis that explains the poor results of the aforementioned solutions.

The most common peptones – water-soluble, non-heat-coagulable protein hydrolysates, after Green et al. (1977) – in microbiological media are those derived from casein, soya, gelatine and meat. Peptones from fish are uncommon, despite their good yields in areas such as the production of proteases by *Bacillus subtilis* (Ellouz et al., 2001), production of gastrine and

epidermal growth factor (EGF) by mouse fibroblasts (Cancre et al., 1999), production of glycerol by *Saccharomyces cerevisiae* (Kurbanoglu and Kurbanoglu, 2003) and promotion of good microbial growth in other cases (de la Broise et al., 1998; Dufossé et al., 2001; Guo-Qiang and Page, 1994; Page, 1992; Green et al., 1977; Clausen et al., 1985; Gildberg et al., 1989; Vecht Lifshitz et al., 1990; Jassim et al., 1988; Dufossé et al., 1997).

The study described in this paper was an investigation of the use of protein hydrolysates obtained from residues of fishery products as peptide sources for the production of nisin and pediocin by *Lactococcus lactis* and *Pediococcus acidilactici*, respectively. The inter-changeability of different products was studied with a factorial approximation, and yields studied by kinetic analysis. In both approaches, the comparison of the results with those obtained using a commercial bactopeptone, or with MRS medium, demonstrated the efficiency of the residues for the proposed objective.

2. Methods

2.1. Preparation of fish peptones

Raw materials used were viscera from rainbow trout (*Oncorhynchus mykiss*) and squid (*Loligo vulgaris*) and muscular wastes from swordfish (*Xiphias gladius*), sampled immediately after industrial processing and maintained at –20ºC until use. Storage did not exceed 15 days for the viscera. Each sample was triturated with 10% (v/w) distilled water, followed by determination of the proteolytic activity present in the homogenate. The trout homogenates at pH 6.5 were maintained for 6 hours at 20ºC with orbital shaking at 100 rpm to promote hydrolysis with the enzymatic contents. Analogous treatment was applied to the squid homogenates, prolonging autohydrolysis until 24 hours due to the low levels of proteolytic activity. Swordfish (muscle)

homogenates, with trace proteolytic activity, were acidified to pH 2 and treated for 12 hours with 0.1% (w/w) pepsin (Merck, 700 FIP-U/g) under identical conditions of temperature and shaking. The hydrolysates were sterilized by steam flow in an autoclave at (101ºC for 1 hour), filtered with paper (Whatman Nº 2), and the filtrates obtained (fish peptones, Table 1) stored at –20ºC until use in the cultures. For trout, a peptone was also prepared by omitting the autohydrolysis step.

2.2. Microbiological methods

The microorganisms used were *Lactococcus lactis* CECT 539 (abbreviated key Lc 1.04) from the Spanish Type Culture Collection, and *Pediococcus acidilactici* NRRL B-5627 (Pc 1.02), kindly provided by the Northern Regional Research Laboratory (Peoria, Illinois, USA). The methods for pediocin and nisin extraction and quantification have been described in detail previously (Cabo et al., 1999), using *Carnobacterium piscicola* CECT 4020 as an indicator. Stock cultures were stored at –50ºC in powdered skimmed milk suspension with 25% glycerol (Cabo et al., 2001). Inocula (1% v/v) consisted of cellular suspensions from 12 (Lc 1.04) and 24 (Pc 1.02) hour-aged cultures on MRS medium, adjusted to an OD (700 nm) of 0.900. Incubations were carried out with orbital shaking at 200 rpm and 30ºC.

The compositions of media used in the kinetic studies are shown in Table 2. For comparison, a medium (D) was used in which fish peptones were substituted for a commercial bactopeptone solution with an equivalent level of protein (Lowry), as well as a commercial MRS medium. The media used in the factorial experiments are described below in Experimental Design. In all cases, initial pH was adjusted to 7.0 and solutions sterilised at 121ºC for 15 min, with cultures carried out in duplicate. In the kinetic experiments, the microorganisms were grown in 300 ml Erlenmeyer flasks with 200 ml of medium. In the factorial approaches, 300 ml Erlenmeyer flasks with 72 ml medium were used.

2.3. Analytical methods

Proteolytic activity was estimated by the method of Barker and Worgan (1981). In microbiological assays, at pre-established times each culture sample (the whole content of one flask) was divided into two aliquots. The first was centrifuged at 5,000 rpm (3220 *g*) for 15 min, and the sediment washed twice and resuspended in distilled water to the appropriate dilution for measurement of absorbance at 700 nm. The dry weight was estimated from a calibration curve. The supernatant was used for the analysis of reducing sugars (Bernfeld, 1951), proteins (Lowry et al., 1951) and glucose, lactic acid and acetic acid, the latter three compounds being measured by HPLC analysis (refractive-index detector) using an ION-300 column (Interaction Chromatography, USA) with 6mM sulphuric acid as mobile phase (flow = 0.4 ml/min) at 65ºC. The second aliquot was used for the extraction and quantification of bacteriocins, following methods described by Cabo et al. (1999) and Murado et al. (2002). All assays were carried out in duplicate.

2.4. Experimental design

Two types of experiment were performed. The first involved the comparison of Lc 1.04 and Pc 1.02 cultures in the following media:

1: MRS (8 g/l of meat extract and 10 g/l of bactopeptone as main protein sources).

2: A medium (D) formulated as for MRS, but without the meat extract.

3: A group of media (FP) based on D, but substituting increasing quantities of bactopeptone with equivalent quantities of proteins (Lowry) from fish peptones.

Total fish peptone added to FP media was obtained by combining the trout extracts (T), autohydrolysed trout (Th) and autohydrolysed squid (Qh). The proportions were adequate for

defining the three independent variables of a complete first order orthogonal design (Box et al., 1989). For Pc 1.02 it was necessary to amplify the design to a rotatable second order.

Table 3 shows the domains and criteria for codification of the variables in both designs. The model was calculated by means of the orthogonal least-squares method (see Table 4). Significance of the coefficients was verified through the Student t-test (α=0.05), and model consistency through the Fisher F test (α=0.05) applied to the following mean squares ratios: *model* / *total error*; *(model+lack of fitting)* / *model*; *total error* / *experimental error*; *lack of fitting* / *experimental error*.

The second type of experiment compared the kinetic parameters corresponding to cultures of both species in media based on D, but substituting bactopeptone for each of the fish peptones. Fitting procedures and parametric estimations calculated from the results were carried out by minimisation of the sum of quadratic differences between observed and model-predicted values, using the non linear least-squares (quasi-Newton) method provided by the macro 'Solver' of the Microsoft Excel 97 spreadsheet.

3. Results

Factorial approach

The main objective of this approach was to verify the capacity of fish peptones for promoting bacteriocin production, as well as detecting possible interactions between the different types tested (fish/cephalopods; with/without hydrolysis). Given the uncertainty concerning the capacity of waste media for meeting this objective, complementary doses of bactopeptone were

added in all cases (Table 3). This way, biomass and bacteriocin production, divided by those corresponding to medium D (only bactopeptone), provided an estimation of the capacity of the fish peptones and their combinations for bactopeptone substitution. Thus the experimental results should be interpreted accordingly. In both species, comparisons were carried out at incubation times corresponding to the late logarithmic phase, slightly before the point of maximum biomass.

With regard to *P. acidilactici*, the results of the first-order experimental plan demonstrated the insufficiency of a linear model, which thus required a second order design. Applying the significance criteria specified in Methods (see Table 4), the system was adequately described with the following empirical equations (response surface in figure 1):

$$Pd = 4.35 + 0.35\,Th + 0.27\,Qh + 0.71\,T - 0.24\,Th^2 - 0.22\,Qh^2 - 0.19\,T^2 \quad [1]$$

$$X = 3.40 + 0.42\,Th + 0.38\,Qh + 0.45\,T - 0.14\,Th\,Qh + 0.15\,Th^2 + 0.14\,Qh^2 + 0.24\,T^2 \quad [2]$$

where the responses represent:

$$Pd = \frac{\text{pediocin (40 hours) on FP media}}{\text{pediocin (40 hours) on D medium}} \;;\; X = \frac{\text{biomass (40 hours) on FP media}}{\text{biomass (40 hours) on D medium}}$$

As shown in Figure 1, the results demonstrate that both the biomass and bacteriocin production (pediocin) increased notably (~500%) as a consequence of the enrichment of the medium with fish peptones. Trout without autohydrolysis produced the greatest effect. The same figure also shows the existence of detailed differences in the responses of the biomass and pediocin, principally due to the presence of the negative interaction *Th Qh* in equation [2] which is absent in [1]. In fact, the maximum production of pediocin was found within the experimental domain, in contrast to maximum biomass production. Therefore, pediocin production cannot be increased by assigning other values to the variables considered, in contrast to biomass production.

In *L. lactis* cultures, the first order design was sufficient (calculation and significance criteria analogous to those described in Table 4; response surface in Figure 2) to obtain the empirical equation corresponding to the relative production of bacteriocin (nisin) after 16 h incubation, in analogous terms to those defined in the preceding case:

$Ns = 1.05 + 0.02\ T + 0.01\ Qh - 0.01\ T\ Qh - 0.05\ Th\ Qh$; where: [3]

$$Ns = \frac{\text{nisin (16 hours) on FP media}}{\text{nisin (16 hours) on D medium}}$$

In addition, as in the previous case, fish peptones increased nisin production, although in much more moderate proportions (~115%) due to the lowest values of the first order effects and the negative interactions detected between the squid peptones and the other two fish peptones. In addition, the effects on biomass were not sufficient to define a statistically significant relationship.

Kinetic approach

The previous results demonstrated the favourable effects of fish peptones on the production of bacteriocins when incorporated into medium D, but did not exclude the possibility that the bactopeptone represented a necessary factor for production. This possible requirement would be of little quantitative importance, since production increased with fish peptone concentration. However, it was necessary to check the efficiency of these fish peptones, assayed individually, as sole sources of organic nitrogen. In these experiments the peptone from swordfish muscle was also included, excluded from the factorial approximation due to the different nature of the starting tissue and the preparation conditions.

Figures 3 and 4 show the time courses of the cultures of both species in all the media considered, in which the production of biomass, bacteriocins, lactic and acetic acid, and consumption of proteins and glucose were determined. For clarity, the figures do not represent the values for

acetic acid in Lc 1.04, which did not exceed 0.2 g/l, the consumption of proteins in Pc 1.02, not exceeding 1.5 g/l, or biomass in medium from swordfish, whose initial turbidity made the estimations uncertain.

Growth and bacteriocin production by LAB have been described by different mathematical models (Mercier et al., 1992; Parente et al., 1994; Lejeune et al., 1998; Herranz et al., 2001; Cabo et al., 2001), among others the logistic equation. Being straightforward and generic, the logistical model is adequate for all experimental profiles in Figures 3 and 4, and allows the calculation of useful parameters for comparisons. Accordingly, the following assumptions are included:

1: The equation (logistic) describing the biomass production is:

$$X = \frac{K}{1+e^{c-\mu_m t}}; \text{ where } c = \ln\left(\frac{K}{X_0}-1\right) \qquad [4]$$

2: The rate of bacteriocin production r_P can be described by the classical model of Luedeking and Piret (1959):

$$r_P = \alpha r_X + \beta X \qquad [5]$$

commonly expressed by dividing both terms by biomass, giving:

$$\frac{r_P}{X} = \alpha \frac{r_X}{X} + \beta \quad ; \quad \text{and:} \qquad \frac{r_P}{X} = \alpha\mu + \beta \qquad [6]$$

This formulation enables microbial metabolites to be classified as primary (production rate dependent on the rate of biomass production: β=0), secondary (production rate dependent on biomass present: α=0), and mixed (production rate simultaneously dependent on growth rate and biomass present: $\alpha \neq 0$ and $\beta \neq 0$).

3: The numerical integration of rate r_X provides the real biomass X_R. From the substitution of r_X and X_R in equation [5], the actual rate of bacteriocin production, r_P, can be obtained.

$$X_R = \sum_{t=0}^{t=t} r_X \qquad [7]$$

$$BT_R = \sum_{t=0}^{t=t} r_P = \sum_{t=0}^{t=t} \left(\alpha \, r_X + \beta X_R \right) \qquad [8]$$

Figures 5 and 6 show the experimental results and corresponding fits to equations [7] and [8], which produced linear correlation coefficients in the interval 0.901-0.999 between expected and observed values in all cases. Estimations of parameters and yields defined in Table **7** are shown in Tables **5** and 6. The impossibility of quantifying the biomass in medium D impeded the application of the described model in this case.

Conclusions

1: The two approximations applied demonstrated with high agreement the utility of the peptones obtained from fish residues for the proposed objectives. The peptones from muscular waste gave poor results with materials not subjected to hydrolysis with external enzymes, but those of visceral waste promoted similar (nisin) or higher (pediocin) productions to those obtained in commercial media in all cases. In addition, the productions improved: a) in homogenates maintained for 4-8 h at 20ºC, which favoured autohydrolysis with the endogenous enzymatic contents, and b) upon combining extracts from different species. Both improvements are consistent with the known requirements of lactic acid bacteria for complex peptidic sources.

2: Fish peptones not only substituted for bactopeptone advantageously, but also for the two peptidic sources of the MRS medium, producing superior results to MRS even when reducing the concentrations of yeast extract and acetate by 50%, and omitting the Tween. In the case of pediocin, the peptones from trout viscera produced particularly notable results (increasing

medium D production by factors of 5 to 7 and MRS medium production by a factor of ~1.5). For nisin, the production of fish peptones was higher than that for medium D but not higher than that for MRS medium, despite producing most biomass. However, as illustrated in the response surface of Figure 2, the maximum was found outside the tested experimental domain. The location of the maximum is feasible by gradient methods, which could lead to further improvement. Efficiencies and yields (except $Y_{BT/X}$, higher in MRS) also improved with the fish peptones which also generally led to cultures with higher μ_m, *i.e.*, faster kinetics.

3: Analysis of the Luedeking and Piret model suggested a slight secondary metabolic component in both bacteriocins. The value of the parameter β, significantly different from zero, equalled 10% of α in the case of nisin and 4-10% for pediocin.

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Figure captions

Figure 1: Response surfaces corresponding to equations [1] (left) and [2] (right). Response values are pediocin and biomass production by *P. acidilactici* on mixed fish peptones, referred to the production in medium D (bactopeptone).

Figure 2: Response surface corresponding to equation [3]. Response values are nisin production by *L. lactis* on mixed fish peptones, referred to the production in medium D (bactopeptone).

Figure 3: Time-course of *P. acidilactici* cultures on different fish peptones considered separately (Th: △, T: ◇, Qh: ○, Sh: □) and on commercial media (MRS: ■, D: ▽). X: biomass, BT: bacteriocin, LA: lactic acid, AA: acetic acid, G: glucose.

Figure 4: Time-course of *L. lactis* cultures. Media and keys as in Figure 3. Pt: protein.

Figure 5: Fit of the kinetics represented in Figure 3 (*P. acidilactici*) to the logistical [5] (left) and Luedeking and Piret [6] models (right).

Figure 6: Fit of the kinetics represented in Figure 4 (*L. lactis*) to the logistical [5] (left) and Luedeking and Piret [6] models (right).

TABLE 1: Peptone extracts from fish wastes (see text)

	Protein (g/l)	Reducing sugars (g/l)
Th: Autohydrolyzed trout viscera	39.52	2.85
T: Trout viscera without autohydrolysis	39.89	2.55
Qh: Autohydrolyzed squid viscera	40.00	0.80
Sh: Sword fish muscle (pepsin hydrolysate)	40.60	-

TABLE 2: Compositions of media used in kinetic studies (g/l).

	FP media [1]	D medium	MRS medium
Glucose	20.00	20.00	20.00
Yeast extract	2.00	2.00	4.00
Sodium acetate	3.00	3.00	5.00
Ammonium citrate	2.00	2.00	2.00
K_2HPO_4	2.00	2.00	2.00
$MgSO_4$	0.20	0.20	0.20
$MnSO_4$	0.05	0.05	0.05
Tween 80	-	-	1.00
Meat extract	-	-	8.00
Bactopeptone	-	10.00	10.00
Fish peptone[1] protein (Lowry)	10.00	-	-

([1]): The four types of fish peptones defined in Table 1.

TABLE 3: Experimental domain and codification of independent variables in factorial experiments. For each experimental point (see matrix in Table 4), a complementary dose of bactopeptone (B=10 – (Th+Qh+T) g/l) was added.

	Natural values in g/l of protein (Lowry) present in Th, Qh and T extracts (see Table 1)		
Coded values	Th	Qh	T
-1.68 (-a)	1.10	1.10	1.10
-1	1.67	1.67	1.67
0	2.50	2.50	2.50
+1	3.33	3.33	3.33
+1.68 (-a)	3.90	3.90	3.90

Codification: $V_c=(V_n-V_0)/\Delta V_n$; Decodification: $V_n= V_0+(\Delta V_n \times V_c)$

V_n=natural value in the centre of the domain; ΔV_n= increment of V_n per unit of V_c.

Shaded area: values corresponding to the first order design.

TABLE 4: Results of experimental plan with mixed fish peptones corresponding to equation [1] and analysis of the significance of the proposed model. *Pd*: observed response, $\hat{P}d$: expected response; NS: non significant coefficient; SS: sum of squares; υ: degrees of freedom; QM: quadratic means of model (M), total error (E), experimental error (Ee) and lack of fit (LF). Independent variables according to Table 3.

Th	Qh	T	*Pd*	$\hat{P}d$	Coefficients	t	Model
-1	-1	-1	2.90	2.40			
1	-1	-1	3.42	3.10	4.39	45.76	4.35
-1	1	-1	3.18	2.94	0.35	5.44	0.35 Th
1	1	-1	3.25	3.64	0.27	4.25	0.27 Qh
-1	-1	1	3.77	3.83	0.71	11.24	0.71 T
1	-1	1	4.50	4.52	0.00	1.40	Th Qh (NS)
-1	1	1	4.62	4.37	0.00	0.58	Th T (NS)
1	1	1	4.87	5.07	0.00	1.68	QhT (NS)
-1.682	0	0	2.64	3.11	0.00	0.05	Th Qh T (NS)
1.682	0	0	4.52	4.28	-0.24	3.95	-0.24 Th^2
0	-1.682	0	2.95	3.31	-0.22	3.55	-0.22 Qh^2
0	1.682	0	4.35	4.22	-0.19	3.04	-0.19 T^2
0	0	-1.682	2.33	2.65			
0	0	1.682	5.15	5.06			
0	0	0	4.65	4.39			
0	0	0	4.54	4.39	Average value		3.940
0	0	0	4.29	4.39	Expected average value		4.390
0	0	0	4.42	4.39	Var(Ee)		0.0552
0	0	0	4.47	4.39	t(α<0.05; υ=5)		2.571
0	0	0	3.98	4.39			

	SS	υ	QM
Model (M)	11.350	6	1.891
Error (E)	1.480	13	0.114
Exp. Error (Ee)	0.276	5	0.055
Lack of fit (LF)	1.204	8	0.151
Total	12.830	19	

QM_M/QM_E= 16.6 F_{13}^{6} (α=0.05)= 2.915

$QM_{(M+LF)}/QM_M$= 0.474 F_{6}^{14} (α=0.05)= 3.956

QM_E/QM_{Ee}= 2.061 F_{5}^{13} (α=0.05)= 4.655

QM_{LF}/QM_{Ee}= 2.724 F_{5}^{8} (α=0.05)= 4.818

r^2 = 0.885

Adjusted r^2 = 0.831

TABLE 5: Main parametric estimations (as defined in Table 7) of kinetic models [5] and [6] describing biomass and bacteriocin production by *P. acidilactici* on the specified media, considered separately.

Parameter	Th	T	Qh	Sh	D	MRS
K (g/l)	1.726	1.706	1.263	-	0.278	1.169
μ_m (h^{-1})	0.186	0.198	0.267	-	0.211	0.125
X_0 (g/l)	0.075	0.079	0.033	-	0.026	0.054
r_B	0.997	0.996	0.992	-	0.951	0.999
α ($BU.10^{-3}/g$)	372.970	549.376	215.640	-	112.057	629.514
β ($BU.10^{-3}g^{-1}.h^{-1}$)	18.660	19.576	13.680	-	15.709	25.829
r_{BT}	0.997	0.983	0.948	-	0.901	0.991
$Y_{X/G}$ (g X/g G)	0.131	0.137	0.125	-	0.067	0.095
$Y_{X/P}$ (g X/g P)	1.285	1.765	2.442	-	2.154	1.095
$Y_{BT/X}$ (BU/g X)	176946.1	199606.9	142677.2	-	123478.6	243530.4
$Y_{BT/G}$ (BU/g G)	23267.7	27406.3	17887.5	10393.2	8290.9	23031.3
$Y_{BT/P}$ (BU/g P)	227307.7	352367.3	348461.5	64682.4	265307.7	266723.8
$Y_{AL/X}$ (g AL/g X)	5.695	5.052	5.102	-	14.143	7.270
$Y_{AL/G}$ (g AL/g G)	0.749	0.694	0.640	0.597	0.952	0.688
$Y_{AL/P}$ (g AL/g P)	7.315	8.918	12.462	3.718	30.462	7.962
BT_m (BU/ml) [1]	299.09	377.67	205.70	54.98	51.89	280.06

([1]): Direct experimental (not calculated) result.

TABLE 6: Main parametric estimations (as defined in Table 7) of kinetic models [5] and [6] describing biomass and bacteriocin production by *L. lactis* on the specified media, considered separately.

Parameter	Th	T	Qh	Sh	D	MRS
K (g/l)	0.994	0.887	0.670	-	0.752	0.665
μ_m (h^{-1})	0.452	0.433	0.636	-	0.856	0.740
X_0 (g/l)	0.041	0.055	0.005	-	0.011	0.013
r_B	0.998	0.995	0.999	-	0.989	0.996
α ($BU.10^{-3}/g$)	7.319	5.323	6.130	-	3.826	14.628
β ($BU.10^{-3}g^{-1}.h^{-1}$)	0.581	0.600	0.643	-	0.508	0.629
r_{BT}	0.974	0.985	0.998	-	0.971	0.975
$Y_{X/G}$ (g X/g G)	0.114	0.117	0.090	-	0.122	0.091
$Y_{X/P}$ (g X/g P)	1.269	2.556	0.835	-	6.333	6.000
$Y_{BT/X}$ (BU/g X)	5717.2	4793.5	5484.9	-	5013.2	9257.6
$Y_{BT/G}$ (BU/g G)	651.3	560.4	439.9	724.6	609.6	838.1
$Y_{BT/P}$ (BU/g P)	7256.4	12250.0	4582.3	61500.0	31750.0	55545.5
$Y_{AL/X}$ (g AL/g X)	6.697	7.424	8.045	-	6.921	10.591
$Y_{AL/G}$ (g AL/g G)	0.763	0.868	0.724	0.810	0.842	0.959
$Y_{AL/P}$ (g AL/g P)	8.500	18.972	6.722	68.750	43.833	63.545
BT_m (BU/ml) [1]	6.06	4.67	3.62	4.92	3.83	6.76

([1]): Direct experimental (not calculated) result.

TABLE 7: Symbolic notations used. BU: Bacteriocin arbitrary units.

X:	Biomass. Dimensions : $g.l^{-1}$
K:	Maximum biomass. Dimensions: $g.l^{-1}$
μ_m :	Specific maximum growth rate (biomass production per unit of biomass and time). Dimensions: h^{-1}
X_0:	Initial biomass. Dimensions: $g.l^{-1}$
α:	Luedeking and Piret parameter (to be experimentally determined). Dimensions: $BU.10^{-3}.g^{-1}$
β:	Luedeking and Piret parameter (to be experimentally determined). Dimensions: $BU.10^{-3}.g^{-1}.h^{-1}$
r_X :	Growth rate. Dimensions : $g.l^{-1}.h^{-1}$
r_p:	Production rate for product P (bacteriocin). Dimensions: $BU.ml^{-1}.h^{-1}$
X_R:	Rate of actual biomass production. Dimensions: $g.l^{-1}.h^{-1}$
BT_R:	Rate of actual bacteriocin production. Dimensions: $BU.ml^{-1}.h^{-1}$
BT_m:	Maximum bacteriocin obtained from culture. Dimensions: $BU.ml^{-1}$
$Y_{X/G}$	Biomass production / glucose consumption: g biomass / g glucose
$Y_{X/P}$	Biomass production / protein consumption: g biomass / g protein
$Y_{BT/X}$	Bacteriocin production / biomass production: BU / g biomass
$Y_{BT/G}$	Bacteriocin production / glucose consumption: BU / g glucose
$Y_{BT/P}$	Bacteriocin production / protein consumption: BU / g protein
$Y_{AL/X}$	Lactic acid production / biomass production: g lactic acid / g biomass
$Y_{AL/G}$	Lactic acid production / glucose consumption: g lactic acid / g glucose
$Y_{AL/P}$	Lactic acid production / protein consumption: g lactic acid / g protein

FIGURE 1

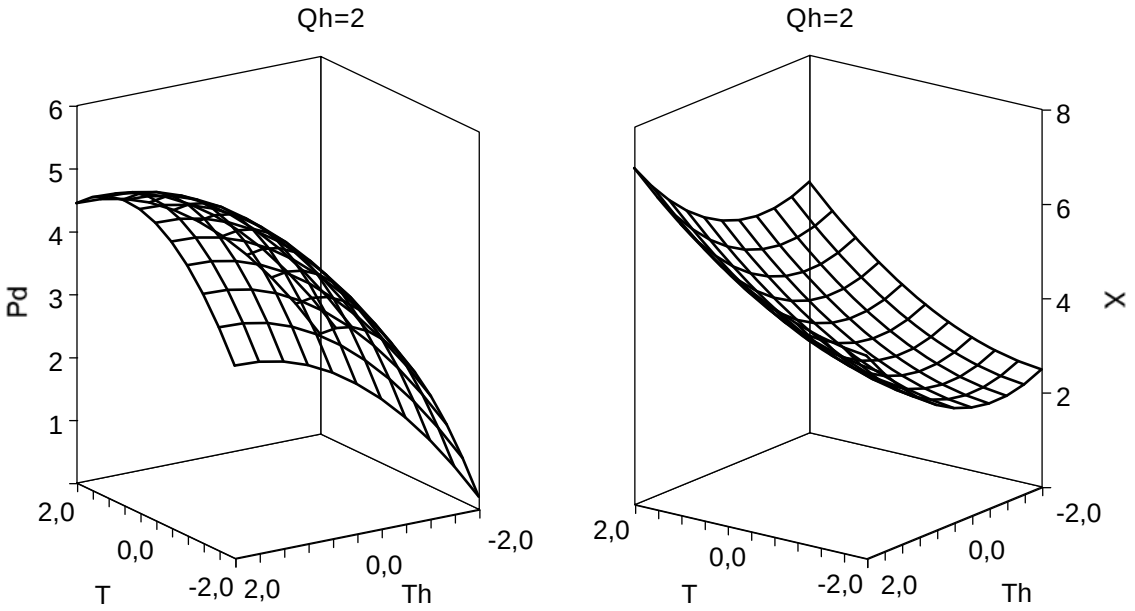


FIGURE 2

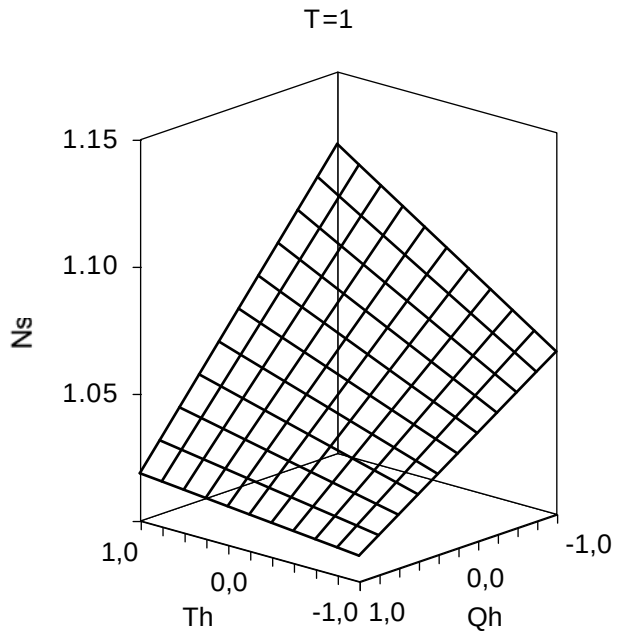


FIGURE 3

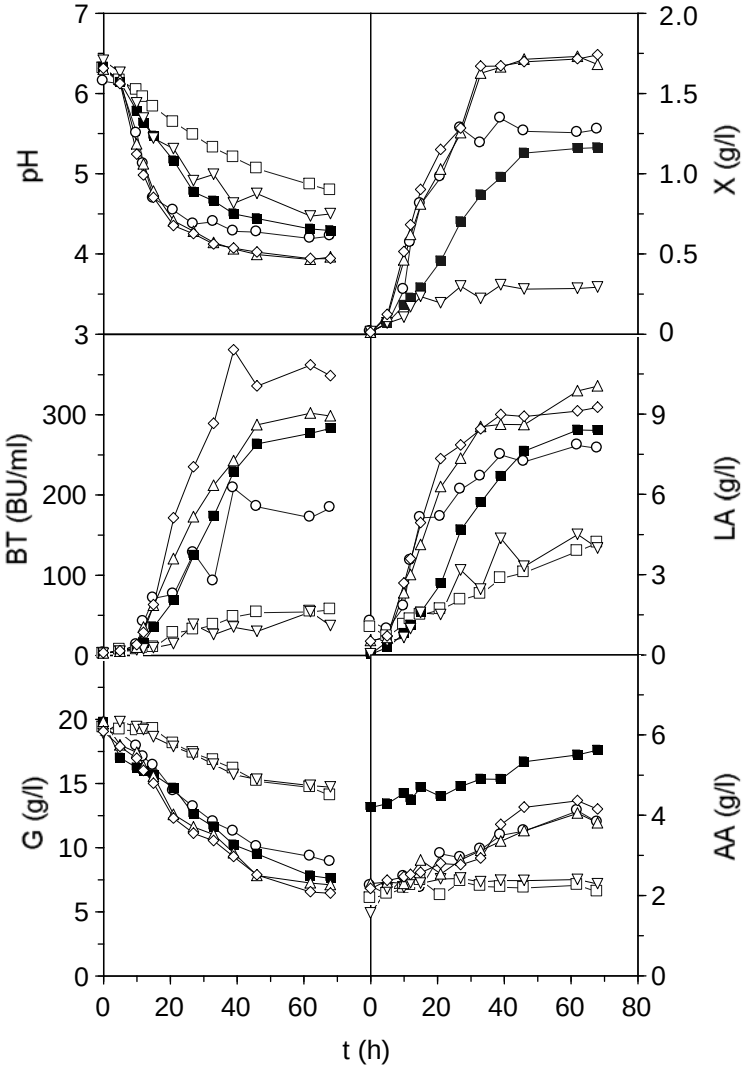


FIGURE 4

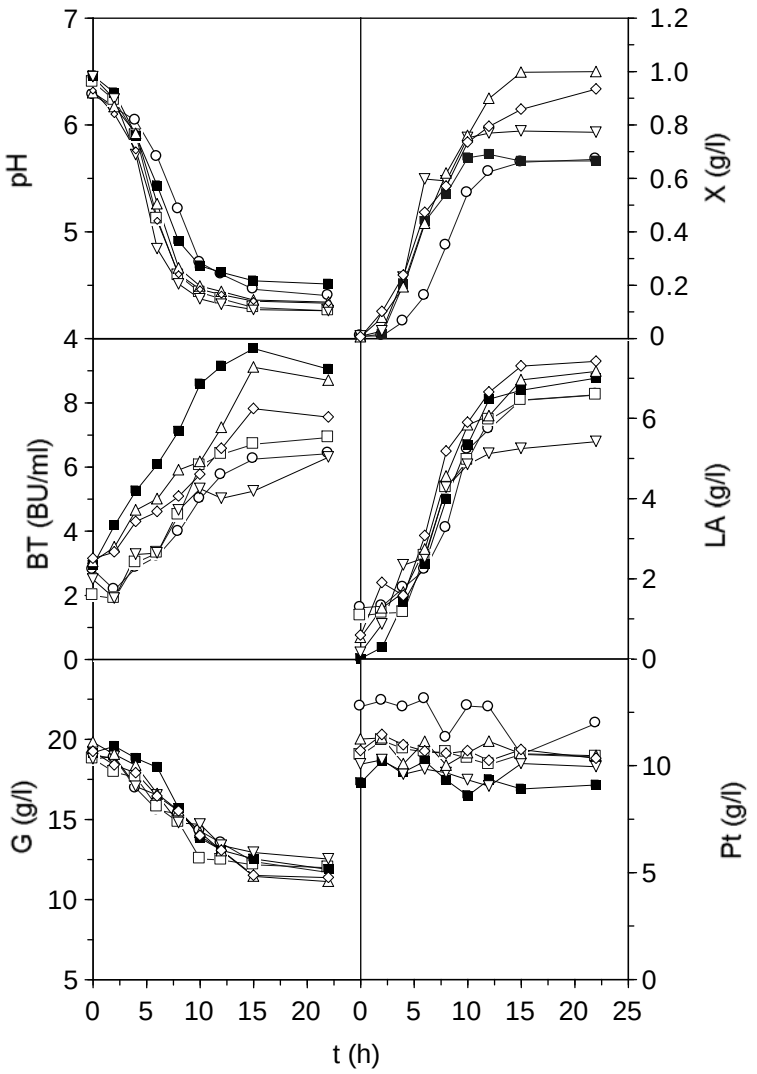
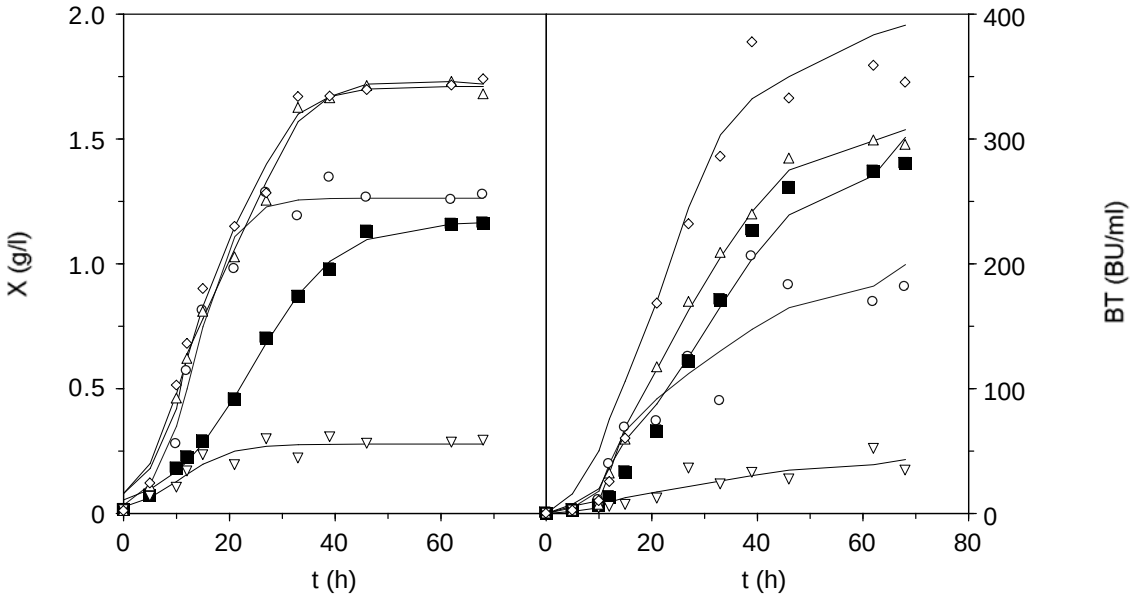


FIGURE 5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1.5
1.0
0.5
0
X (g/l)
0
20
40
60
t (h)
0
20
40
60
80
t (h)
400
300
200
100
0
BT (BU/ml)

FIGURE 6