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Proteases production by two *Vibrio* species on residuals marine media

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Abstract A comparative study was carried out on the growth and production of alkaline proteases by two *Vibrio* species using different marine peptones from fish viscera residues. The bacteria tested, *Vibrio anguillarum* and *Vibrio splendidus*, are producers of high levels of proteolytic enzymes which act as factors of virulence in fish cultures, causing high mortality rates. The kinetic assays and subsequent comparison with the parameters obtained from the adjustment to various mathematical models, highlighted the potential interest of the media formulated, for their possible production on an industrial scale, particularly the production of proteases by *V. anguillarum* growing in rainbow trout and squid peptones.

Keywords Protease production · Marine peptones · Residuals media · Alkaline protease

Introduction

Proteases, also known as peptidyl-peptide hydrolases (EC 3.4.21-24 and 99), are enzymes which catalyze the hydrolysis of a peptide bond in a protein molecule [5], and which are classified into two groups, exopeptidase and endopeptidase or peptidases, according to the point of rupture of the protein chain [22], into four categories: metallo-, serine-, carboxy- and sulphydrilproteases, depending on the specific groups involved in the catalytic mechanisms [16] and into acid, neutral and alkaline proteases on the basis of the pH range in which their activity is optimum. From the point of view of their technological and economic interest, proteolytic enzymes are one of the most important groups of industrial enzymes, constituting 65% of worldwide industrial enzyme marketing [27, 39, 47] and around 25% of the total global enzyme production [28, 34]. Their use encompasses a great number of applications in different industrial sectors, such as detergent additives, in waste treatment processes, medical and basic research, silver recovery and the food, leather, photographic and pharmaceutical industries [17, 21, 30], being the most widely used, the alkaline proteases of microbial origin (60% of the total).

Diverse micro-organisms have been used to carry out this bioproduction: *Penicillium sp.* [1], *Serratia marcescens* [52], *Streptomyces sp.* [8], *Aspergillus clavatus* [51], *Beauveria feline* [1], *Rhizopus oryzae* [3], *Pseudomonas* [13, 45]; but from the point of view of both their historical interest and their high levels of production, the members of the genus *Bacillus* have been predominant [18, 23, 27, 29, 36, 46, 50]. Although all these microbes are of terrestrial origin, various genus of marine bacteria, such as *Aeromonas* [20] and *Vibrios* [15, 26, 32, 48], could be of great interest as potential alkaline proteases producers. In particular, the species *Vibrio anguillarum* and *Vibrio splendidus* have been typified as pathogens of *Schophthalmus maximus* [2, 43], being the proteolytic enzymes which they produce as the main factor of virulence [10, 41, 42].

One problem regarding the obtaining of enzyme production on a large scale by using these bacteria is the high cost that it presents, bearing in mind their simple formulation, and the specific marine culture that they require. A possible alternative for the reduction in production costs will come about with the preparation of a similar medium prepared with seawater and proteins or low-cost protein fractions (peptones) obtained from fish residues [53].

According to the definition of Green et al. [19], "peptones" are protein hydrolysates which are water-soluble, non-coagulable by heat and which are obtained from the hydrolysis of protein or proteins. This material contains a mixture of free amino acids, peptides and proteases. Generally, commercial peptones used in culture media for micro-organisms are mainly derived from

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casein, soya and meat. Peptones of marine origin are scarcely used at present, even though they yield good results in different applications, as is the case with the production of proteases by *Bacillus subtilis* [14], with gastrine and epidermal growth factor (EGF) by mouse fibroblasts [7], with glycerol by *Saccharomyces cerevisiae* [33], with bacteriocins and lactic acid bacteria [54, 56] or in microbial growth [9, 12].

Taking this into account, this work studied the suitability for the culture of *Vibrios* and the production of alkaline proteases, of media formulated with marine peptones from fish viscera wastes (swordfish, rainbow trout, squid and yellowfin tuna). As assessment criteria, the kinetic parameters of the cultures obtained by numerical adjustment of the results to several mathematical models, commonly used in this type of description, were compared.

Materials and methods

Preparation of marine peptones from fish viscera

Raw materials used were viscera from rainbow trout (*Oncorhynchus mykiss*), swordfish (*Xiphias gladius*), squid (*Loligo vulgaris*) and yellowfin tuna (*Thunnus albacares*), sampled immediately after industrial processing and maintained at −20°C until use. Storage did not exceed 15 days for the materials.

The viscera masses (stomach and intestine) were ground with ~10% (v/w) of distilled water, the homogenates were stabilized by steam flow (101°C/1 h) and were treated in a centrifuge decanter at 6,000 rpm for 15 min to obtain the corresponding sediments (potentially useful as substrate in biological silage) and supernatants [53–54]. The supernatant group (or marine peptones) was typified determining the levels of total nitrogen, protein and total sugars, and then stored at −20°C until the time of its use in the formulation of culture media. The basic composition of peptones is shown in Table 1.

Microbiological methods

The micro-organisms used were *Vibrio anguillarum* SSF 287 (abbreviated key Vb1) kindly provided by Dra. Ana Riaza (Stolt Sea Farm) and *Vibrio splendidus* DMC-1 (abbreviated key Vb2) kindly provided by Dr. Hazel Duncan (University of Glasgow). Stock cultures were stored at −75°C in commercial marine medium (Difco) with 25% glycerol [53]. Inocula (1% vol/vol) consisted of cellular suspensions from 24-h aged cultures on marine medium (MM), adjusted to an OD ($\lambda = 700$ nm) of 0.600.

The compositions of the media are summarized in Table 2. Each marine peptone was used at a level that replaced the Lowry protein concentration present in the commercial marine medium. With purposes comparatives, were also employees two commercial peptones (bactopeptone and tryptone), habitually used, as protein sources in the formulation of media for the growth of micro-organisms. The nutrients were dissolved in filtered seawater (NaCl: 32 g l^{-1}), where 1 g l^{-1} yeast extract (as in MM) was the only additional supplement. In all cases, the initial pH was adjusted to 7.5 and the solutions were sterilized at 121°C for 15 min. Micro-organisms were grown in 300 ml Erlenmeyer flasks with 200 ml of medium, at 22°C with 200 rpm orbital shaking. The cultures were carried out in triplicate.

At pre-established times, each culture was divided into two aliquots. The first was centrifuged at 5,000 rpm for 15 min, the sediment washed twice and resuspended in distilled water to a dilution suitable for measuring the optical density (OD) at 700 nm. The dry weight can then be estimated from a previous calibration curve. The corresponding supernatant was used for the determination of proteins and alkaline protease activity. The second aliquot was used for the quantification of viable cells by means of a plate count technique on MM, MB, MT and MP Agar media. Serial, tenfold dilutions were

Table 1 Main composition (g l^{-1}) of marine peptones (MP) from fish viscera

	Proteins (Lowry)	Total sugars	Total nitrogen
TR	61.5	3.4	12.0
SF	54.0	2.3	11.1
YT	68.3	4.1	13.1
SQ	43.3	2.4	8.2

TR rainbow trout, *SF* sword fish, *YT* yellowfin tuna, *SQ* squid

Table 2 Composition of the culture media tested (g l^{-1}, unless other units specified)

	MP[a]	MB	MT	MM
Ferric citrate	–	–	–	0.10
Sodium chloride	–	–	–	19.45
Magnesium chloride	–	–	–	5.90
Sodium sulphate	–	–	–	3.24
Calcium chloride	–	–	–	1.80
Potassium chloride	–	–	–	0.55
Sodium bicarbonate	–	–	–	0.16
Potassium bromide	–	–	–	0.08
Strontium chloride	–	–	–	34.0 mg l^{-1}
Boric acid	–	–	–	22.0 mg l^{-1}
Sodium silicate	–	–	–	4.0 mg l^{-1}
Sodium fluoride	–	–	–	2.4 mg l^{-1}
Ammonium nitrate	–	–	–	1.6 mg l^{-1}
Disodium phosphate	–	–	–	8.0 mg l^{-1}
Yeast extract	1.00	1.00	1.00	1.00
Peptone	–	–	–	5.00
Bactoneopeptone	–	5.00	–	
Tryptone	–	–	5.00	
Protein (Lowry) from MP[a]	2.60	–	–	–
Sea water	1 l	1 l	1 l	–
Distilled water	–	–	–	1 l

The corresponding solid media (MP[a], MB, MT and MM) for quantifying viable cells were supplemented with 20 g l^{-1} of agar

[a] *MP* Media prepared from marine peptones, as defined in Table 1

prepared in peptone-buffered solutions and 0.1 ml samples were plated in quadruplicate, incubated at 22°C for 48 h, and manually counted. Results were expressed in colony-forming units per ml (cfu ml^{-1}).

Analytical methods

Total nitrogen was determined by the method of Havilah et al. [24], applied to digests obtained by the classic Kjeldahl procedure. Proteins followed the method of Lowry et al. [37]. Total sugars were measured by means of the phenol-sulphuric reaction [11] according to the application of Strickland and Parsons [49] with glucose as a standard. Protease activity was estimated by the method of Kunitz [31] according to the application of Barker and Worgan [4]. The method is based on the release of tyrosine by the action of protease on casein (protease activity is expressed indirectly in terms of tyrosine concentration). Therefore, a 1-ml diluted sample was added to 1 ml of 0.5 % (w/v) casein in 0.1 M phosphate buffer, pH 6.4, and incubated at 40°C. After 10 min, the reaction was stopped by adding 3 ml of 5% (w/v) trichlorocetic acid solution, waiting for 5 min and centrifuging at 4,000 rpm for 10 min. About 2.5 ml of 0.19 M sodium carbonate in 0.1 M NaOH was added to 0.5 ml of supernatant, waiting 10 min and adding 0.25 ml 1 N Folin-Ciocalteu's phenol reagent. After 1 h of incubation at room temperature, the absorbance was measured at 750 nm against an appropriate reagent blank. One unit of protease activity (EU) was defined as the amount of enzyme that produced a colorimetric response equivalent to 1 μmole of tyrosine per minute at pH 7.5 at 37°C.

Numerical methods

Fitting procedures and parametric estimations calculated from the results were carried out by minimization 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 XP spreadsheet. Statistica 6.0 (StatSoft, Inc. 2001) was used to demonstrate the significance of the parameters estimated by the adjustment of the experimental values to the proposed mathematical models.

Results and discussion

Mathematical models

Different mathematical models have been proposed to describe the growth of micro-organisms and the production of the metabolites characteristic of each [25, 35, 40, 44]. Among these (Gompertz, Richards, Von Bertalanffy), the logistic equation, easily managed, of a pseudokinetic structure and highly generalized, is suitable for the adjustment of the sigmoid profiles encountered (Figs. 1, 2), also facilitating the calculation of parameters of biological significance, useful for the purpose of comparison. On the other hand, the Luedeking and Piret model [38] defines metabolites, according to their production, into primary and secondary; their use combined with the logistic equation being of special interest, for example, to describe bacteriocin production in lactic acid bacteria cultures [6, 55]. Thus, the postulates for definition of the models are described in the following points:

1. The differential equation describing the growth rate is:

$$r_X = \frac{dX}{dt} = \mu_m X\left(\frac{K - X}{K}\right) \tag{1}$$

from which, integrating between $X_0 \rightarrow X$ and $0 \rightarrow t$, one obtains the explicit expression which describes the temporal production of biomass (logistic):

$$X = \frac{K}{1 + e^{c - \mu_m t}}; \text{where } c = \ln\left(\frac{K}{X_0} - 1\right) \tag{2}$$

Another parameter of interest (proposed by Zwietering et al. [57]), of lower experimental variability (and therefore of lower statistical error) and of more direct application when comparing microbial growths in different culture media, is the maximum growth rate (v_m) or slope of the straight tangent to the logistic function at its point of inflection (t_i). Thus, calculating the second derivative and equalizing to zero, we find the value of the point of inflection on the abscissa axis ($t = t_i$):

$$\frac{d^2X}{dt^2} = 0; \text{to obtain } t_i = \frac{c}{\mu_m} \tag{3}$$

Being therefore:

$$v_m = \left(\frac{dX}{dt}\right)_{t_i} = \frac{K\mu_m}{4} \tag{4}$$

2. The rate of protease production r_{PR} can be described by means of the classical model of Luedeking and Piret (1959):

$$r_{PR} = \alpha r_X + \beta X \tag{5}$$

commonly expressed by dividing both terms by biomass, so:

$$\frac{r_{PR}}{X} = \alpha\frac{r_X}{X} + \beta \text{ and } \frac{r_{PR}}{X} = \alpha\mu + \beta \tag{6}$$

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

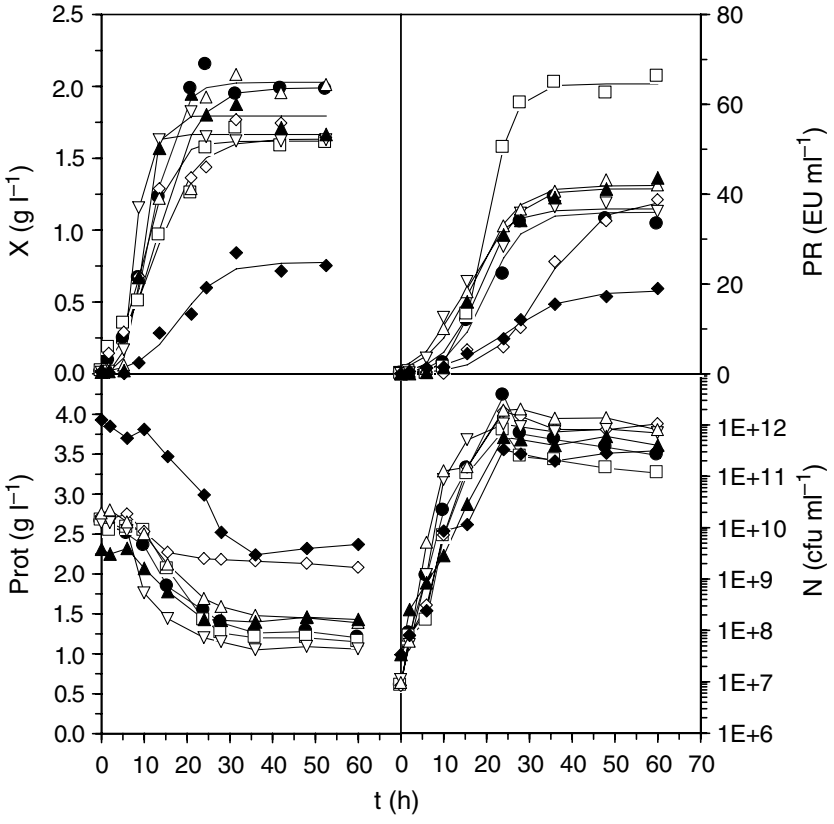

Fig. 1 Time-course of *V. anguillarum* cultures on media prepared with marine peptones as peptidic source and marine water (*TR* □, *SF* ◇, *YT* ▽, *SQ* △; Table 2), with commercial peptones (*MB* ◆, *MT* ▲) and on commercial *marine medium* ●. *X* biomass, *PR* protease activity, *Prot* proteins (Lowry), *N* colony-forming units per ml. The corresponding confidence intervals are not shown ($\alpha = 0.05$, $n = 3$), since these did not transcend in practically any case, the 10% of the experimental mean value

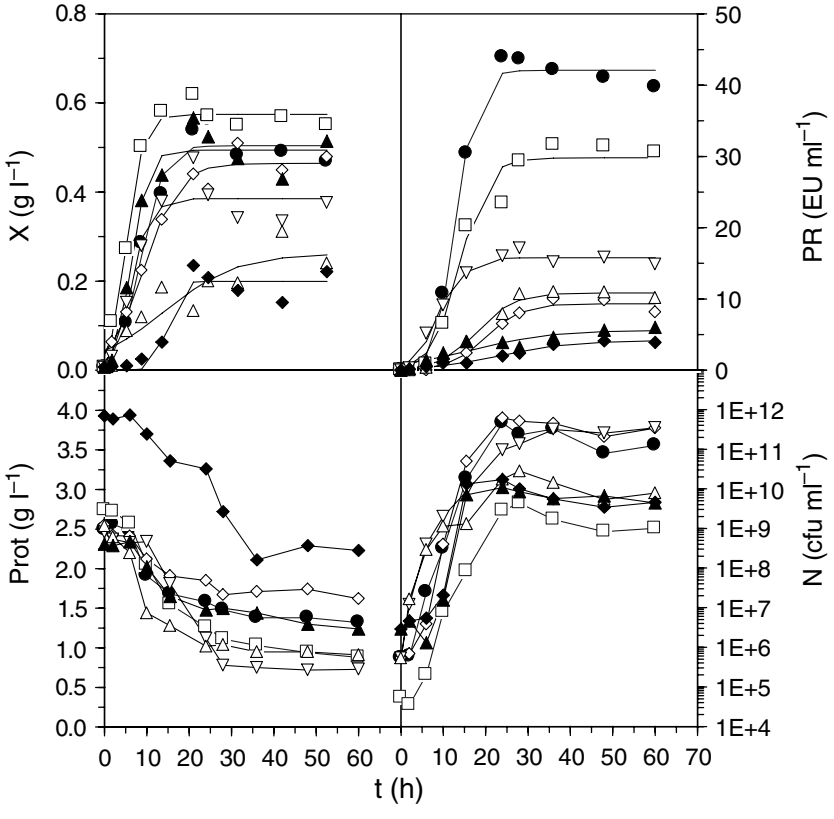

Fig. 2 Time-course of *V. splendidus* cultures on media prepared with marine peptones as peptidic source and marine water (*TR* □, *SF* ◇, *YT* ▽, *SQ* △; Table 2), with commercial peptones (*MB* ◆, *MT* ▲) and on commercial *marine medium* ●. *X* biomass, *PR* protease activity, *Prot* proteins (Lowry), *N* colony-forming units per ml. The corresponding confidence intervals are not shown ($\alpha = 0.05$, $n = 3$), since these did not transcend in practically any case, the 10% of the experimental mean value

3. The numerical integration of rate r_X provides the real biomass X_R. Substituting r_X and X_R in the Luedeking and Piret equation, the real rate of protease production, r_{PR}, can be obtained:

$$X_R = \sum_{t=0}^{t=t} r_X \tag{7}$$

$$PR_R = \sum_{t=0}^{t=t} r_{PR} = \sum_{t=0}^{t=t} (\alpha r_X + \beta X_R) \tag{8}$$

4. To calculate the maximum protease production, a similar logistical model to that expressed in Eqs. (1–2) was resorted to, where the term K was replaced (maximum biomass when $t \to \infty$) by PR_m (maximum protease when $t \to \infty$) and the parameter μ_m (specific maximum growth rate) by μ_{PR} (specific maximum protease production rate). So, the equation presents the following form:

$$PR = \frac{PR_m}{1 + e^{a - \mu_{PR} t}}; \text{ where } a = \ln\left(\frac{PR_m}{PR_0} - 1\right) \tag{9}$$

In the same way as for Eq. (2), we can define (v_{PR}):

$$v_{PR} = \left(\frac{dPR}{dt}\right)_{t_i} = \frac{PR_m \cdot \mu_{PR}}{4} \tag{10}$$

Growth and protease production on marine waste media

The experimental plan consisted of the realization of culture kinetics of the species indicated (Vb1 and Vb2), in the media with waste protein sources (MP), media with commercial peptones (bactoneopeptone:MB and tryptone:MT) and commercial marine medium (MM). The quantified variables were: biomasses (dry weight and colony forming units), protein consumption and protease production activity. Figs. 1 and 2 show the time-courses of the cultures of *V. anguillarum* and *V. splendidus*. The choice of these bacteria was motivated by their high infective capability in larval turbot cultures, causing a high mortality rate [2]. This capability is closely related to the production of high levels of extracellular proteases, which act by hydrolyzing a large proportion of the fragile intestinal walls of the fish larvae [41]. In any case, the use of these micro-organisms, in future proteases production in large-scale, would not cause a sanitary risk, since the separation processes subsequent to fermentation (clarification and ultrafiltration) and the sterilization of the decanted cell biomass would avoid this problem.

In general, all the productions led to satisfactory fits, with linear correlation coefficients between expected and observed values within the intervals specified in Tables 3 and 4, where the estimations of the parameters (significant values for $p = 0.05$; $n = 3$) and the yields are defined in the summary of symbolic notations (Table 5).

This way, keeping in mind the numeric values of the parameters, the results obtained with the species Vb1 (Fig. 1; Table 3) allow the following behaviour to be established:

1. The maximum growth, quantified as dry weight -*K*-, favoured media SQ and MM (both with very similar parametric values: 1.99 and 2.03, respectively) compared with the other media (1.79, 1.67 and 1.63 in MT, YT and TR, respectively). On the other hand, the kinetic growth profiles of viable micro-organisms -*N*- were identical in all the media assayed, with a maximum at around 20 h of culture, and a subsequent drop in bacterial viability. From a quantitative point of view, the YT, SQ and SF media maintained a final level of growth of a greater order of magnitude than the MB, TR and MM media.
2. The media with a waste protein source proved to be more efficient in the consumption of nutrients, only proteins, compared with the commercial marine medium (yield and efficiency values $Y_{X/P}$, $Y_{PR/X}$ and $Y_{PR/P}$).
3. All the marine peptones obtained from visceral fish wastes (TR, SF, YT and SQ) caused similar or higher levels of maximum protease activity (PR_m) than the formulations with commercial peptones (MB, MT) and commercial marine medium (MM). These improvements in production reached as much as 80 and 17% higher in the TR and SQ media (64.56 and 41.91 EU ml^{-1}, respectively) compared with the MM (35.94 EU ml^{-1}). If we consider the maximum protease production rate (v_{PR}), the waste TR media is also more suitable for this bioproduction than the commercial formulation.

Regarding the Vb2 cultures (Fig. 2; Table 4), the results highlighted the following characteristics:

1. Both the maximum growth and the maximum growth rate values of *V. splendidus* in the medium with trout (TR) viscera peptones were higher than those obtained in the rest of the media assayed, the worst results being in the SQ and MB medium. However, the viable count in the agarized TR medium showed restrictions on bacterial growth, of a lower order of magnitude for the inocula (identical in all cultures) and of 2.5 orders of magnitude at the end of the culture. As can be observed in Fig. 2, the colony count in the other media led to very similar results, no significant differences between them being noticeable.
2. In all cases, the yields in the consumption of proteins from the commercial medium were higher than those obtained in the waste media.
3. The highest protease production corresponded to the MM medium (42.09 EU ml^{-1}), followed by the TR medium (29.08 EU ml^{-1}), being very low in the rest of the media assayed. The production rates followed the same order of efficacy.

Table 3 Main parametric estimations (as defined in Table 5) from kinetic models (7) and (8) describing biomass and protease production by *V. anguillarum* on the specified media (Table 2)

Key	K (g l^{-1})	μ_m (h^{-1})	v_m (g l^{-1} h^{-1})	α (EU×10^{-3} g^{-1})	β (EU×10^{-3} g^{-1} h^{-1})	$Y_{X/P}$ (g X/g P)	$Y_{PR/X}$ (EU/g X)	$Y_{PR/P}$ (EU/g P)	PR^a_m (EU ml^{-1})	μ^a_{PR} (h^{-1})	v^a_{PR} (EU ml^{-1} h^{-1}
TR	1.633	0.178	0.073	115.896	4.462	1.044	41,682	43,520	64.564	0.320	5.160
SF	1.618	0.260	0.105	0	3.783	2.539	23,828	60,500	38.476	0.164	1.578
YT	1.665	0.668	0.278	52.403	2.042	1.025	22,296	22,861	36.717	0.211	1.937
SQ	1.989	0.189	0.094	95.941	1.107	1.467	20,886	30,642	41.907	0.201	2.108
MB	0.775	0.183	0.035	89.602	2.455	0.474	25,676	12,179	18.530	0.144	0.667
MT	1.793	0.519	0.232	44.729	2.968	1.875	26,424	49,545	41.182	0.223	2.293
MM	2.028	0.270	0.137	49.417	1.854	1.355	16,916	22,924	35.938	0.234	2.100

$r = 0.978$–0.996 (X); $r = 0.957$–0.999 [PR: model (8)]; $r = 0.988$–0.999 [PR: model (9)]
[a]Calculated from logistic equation for protease production [models (9) and (10)]

Table 4 Main parametric estimations (as defined in Table 5) from kinetic models (7) and (8) describing biomass and protease production by *V. splendidus* on the specified media (Table 2)

Key	K (g l^{-1})	μ_m (h^{-1})	v_m (g l^{-1} h^{-1})	α (EU×10^{-3} g^{-1})	β (EU×10^{-3} g^{-1} h^{-1})	$Y_{X/P}$ (g X/g P)	$Y_{PR/X}$ (EU/g X)	$Y_{PR/P}$ (EU/g P)	PR^a_m (EU ml^{-1})	μ^a_{PR} (h^{-1})	v^a_{PR} (EU ml^{-1} h^{-1}
TR	0.574	0.460	0.066	63.464	6.751	0.292	56,176	16,430	29.081	0.309	2.301
SF	0.464	0.218	0.025	34.502	2.637	0.509	17,230	8,763	9.331	0.242	0.564
YT	0.385	0.399	0.038	154.214	1.013	0.214	40,135	8,584	15.787	0.333	1.314
SQ	0.262	0.100	0.007	247.666	1.389	0.141	44,130	6,227	10.833	0.229	0.620
MB	0.199	0.661	0.033	62.106	2.627	0.124	18,571	2,294	4.159	0.120	0.124
MT	0.494	0.450	0.056	22.198	0.951	0.477	11,765	5,607	5.615	0.103	0.145
MM	0.504	0.335	0.042	304.202	3.948	0.395	85,931	33,932	42.085	0.417	4.392

$r = 0.988$–0.994 (X); $r = 0.935$–0.975 [PR: model (8)]; $r = 0.937$–0.997 [PR: model (9)]
[a]Calculated from logistic equation for protease production [models (9) and (10)]

Table 5 Symbolic notations used

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}
v_m	Maximum growth rate. Dimensions: g l^{-1} h^{-1}
α	Luedeking and Piret parameter (to be experimentally determined). Dimensions: EU×10^{-3} g^{-1}
β	Luedeking and Piret parameter (to be experimentally determined). Dimensions: EU×10^{-3} g^{-1} h^{-1}
r_X	Growth rate. Dimensions: g l^{-1} h^{-1}
r_{PR}	Production rate for product PR (protease). Dimensions: EU ml^{-1} h^{-1}
X_R	Rate of actual biomass production. Dimensions: g l^{-1} h^{-1}
PR_R	Rate of actual protease production. Dimensions: EU ml^{-1} h^{-1}
PR_m	Maximum protease production. Dimensions: EU ml^{-1}
μ_{PR}	Specific maximum protease production rate (protease production per unit of protease and time). Dimensions: h^{-1}
PR_0	Initial protease production. Dimensions: EU ml^{-1}
v_{PR}	Maximum protease production rate. Dimensions: EU ml^{-1} h^{-1}
$Y_{X/P}$	Biomass production / protein consumption: g biomass/g protein
$Y_{PR/X}$	Protease production / biomass production: EU/g biomass
$Y_{PR/P}$	Protease production / protein consumption: EU/g protein

EU Enzymatic (protease) units

Finally, it may be pointed out that the production of proteases by both bacteria in all of the media behaves like a mixed metabolite ($\alpha \neq 0$, and $\beta \neq 0$). Only, in the case of Vb1 in medium formulated with SF peptones, the production of proteolytic enzymes shows secondary-metabolite characteristics.

Conclusions

One of the greatest environmental problems within the coastal area of Galicia (NW Spain) is the large quantity of organic waste generated by the industries devoted to the production of foodstuffs of marine origin (canning factories, processing and prepared foodstuff plants), which is generally dumped in the sea without any kind of previous treatment. Among these, some of the most eutrophying to the environment due to their high protein content and therefore of interest for their assessment, are the viscera wastes derived from the transformation processes of fishing and aquaculture products. These wastes allowed us to obtain, in a simple way, waste protein sources (marine peptones) useful in the formulation of broths, so much solid as liquids, for the micro-organism cultures. This way, these residual media provided productions of biomass and alkaline proteases which equalled or surpassed to those obtained in the marine medium and the commercial peptones

recommended for the culture of bacteria of marine origin, as is the case of the *Vibrios* used in this work.

The highest production of protease activity by *V. anguillarum* corresponded to the media with peptones from rainbow trout (TR) and squid (SQ) as well as the medium with tryptone (MT). The cell growth in the waste marine media were slightly lower or equal to those obtained with the commercial formulation. On the other hand, in the case of *V. splendidus*, the MP media showed its greater capacity for bacterial growth, especially TR, but also its lower suitability for the production of proteolytic enzymes.

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