A new marine medium. Use of different fish peptones and comparative study of the growth of selected species of marine bacteria.

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ABSTRACT

Various readily obtainable 'marine' media from fishery residues were evaluated against a common commercial medium for their effectiveness for promoting growth of different bacteria. The micro-organisms investigated (genera *Pseudomonas, Vibrio, Roseobacter*) are of interest to aquaculture either for their pathogenic or probiotic character. Comparisons between the media were centered on the most important kinetic parameters of the corresponding cultures, *i.e.* maximum biomass and maximum specific growth rate, calculated by applying two widely accepted mathematical models (logistic and Gompertz equations) to data measured both in terms of dry weights and cell numbers. The parametric estimations allowed a classification of the results that demostrated the effectiveness of all the media derived from fishery residues to meeting the proposed objectives. Growths were generally superior (up to 10 times in terms of cell numbers) to those from the common commercial medium, with the best results obtained from tuna viscera.

INTRODUCTION

Numerous microorganisms require sources of organic nitrogen, which are commonly incorporated into the commercial culture media in the form of protein hydrolysates, or peptones, of variable degrees of hydrolysis [1-3], including bactopeptone, tryptone, casitone, meat extract or yeast extract. The composition of common commercial media for culturing marine bacteria is essentially seawater with peptone (5 $g.l^{-1}$) and yeast extract (1 $g.l^{-1}$), lacking the carbohydrates usually included in "terrestrial" media. Despite their essentially simple preparation, these media are expensive. It would thus be reasonable to assume that a similar medium prepared with seawater and a peptone easily obtainable from fish residues would significantly reduce culture costs. This advantage would be particularly pertinent when medium- or large-scale production is required, for example in the production of probiotics destined for aquaculture.

Commercial peptones for microbiological use are mainly obtained from casein, soya, gelatine and meat. Although much less common, fish peptones have given good results in various specific applications [1, 3-12]. In coastal areas such as Galicia (NW Spain), numerous industrial enclaves are dedicated to processing fishery or aquaculture products. Therefore, an ample availability of waste (viscera, heads and other leftovers), at present discarded, would constitute a suitable source of fish peptones for bacterial cultures.

The aquaculture industry is presently interested on two taxonomically heterogeneous subgroups of microorganisms isolated from marine environments. These groups can be pragmatically defined by their effects on the species cultivated: (a) microorganisms with pathogenic effects, a group in which *Vibrio anguillarum* and *Vibrio splendidus* are two frequent causative agents of vibriosis, one of the major bacterial diseases affecting fish, bivalves and crustaceans with great economic consequences [13-15], and (b) microorganisms with probiotics effects. Aside from diverse terrestrial lactobacteria, this latter group contains species (e.g. *Pseudomonas fluorescens*, or marine snow bacteria as *Roseobacter sp*.), that raise interest because they are producers of communication signals involved in quorum sensing effects in gram-negative bacteria [16].

In this work, diverse peptones obtained from fish viscera using simple procedures were studied to asses their suitability for preparing low price marine media. For comparison, two commercial media were assayed: marine medium (MM), widely used for the culture of marine bacteria, and MRS medium, habitual for the culture of lactic acid bacteria (a microbial group often involved in probiotic effects and specially demanding of complex organic nitrogen sources). As test micro-organisms, six species were used from the two groups (a) and (b) defined in the preceding paragraph. As validation criteria, the kinetic growth parameters in the different media were used, obtained by numerical adjustment of the corresponding time-courses to two mathematical models widely applied to this type of description.

MATERIALS AND METHODS

Preparation of marine peptones

Yellowfin tuna (*Thunnus albacares*) and squid (*Loligo vulgaris*), immediately frozen on board after catch, were provided by fish processing companies Alimentos Arousa and Clavo Congelados, respectively (Galicia, Spain). Swordfish (*Xiphias gladius*) was obtained from fish market of port of Vigo (Galicia, Spain). Rainbow trout (*Oncorhynchus mykiss*) was provided by the aquaculture company Isidro de la Cal (Galicia, Spain). In all cases, the visceral mass extracted was maintained at –20ºC until used, for a maximum period of 15 days.

In previous experiments with different hydrolysis times with pepsin, or autohydrolysis with the endogenous proteases of the visceral mass, the best results with lactic bacteria corresponded to those materials homogenised with 10% (v/w) distilled water without modifying the natural pH (in all cases within pH 6.0-6.5) and stabilised by steam flow (101ºC/1 h) immediately after homogenisation. Therefore, identical initial treatment was applied in this work. Thereafter, the homogenates were fractionated in a centrifugal decanter (small volumes can be paper-filtered in a Büchner flask) to obtain the corresponding sediments (potentially useful as substrates of biological ensilages) and supernatants. These supernatants (or fish peptones), the basic composition of which are shown in Table 1, were stored at –20ºC until application to the preparation of culture media.

Microbiological methods

The microorganisms used are shown in Table 2. Stock cultures of all species were stored in commercial marine medium with 25% glycerol at –50ºC. Table 3 describes the culture media. It should be noted that each fish peptone was used at two levels: one exactly substituting the protein concentration (Lowry) present in the commercial marine medium (MM), and another containing double concentration. In both cases 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. Similarly, it should equally be noted that the MRS medium is, by far, the richest in nutrients. Besides MRS, a medium termed MRS^{-} was used, prepared by omitting the normal concentration of 5 $g.l^{-1}$ sodium acetate, a common inhibitor of bacterial growth. In all cases, initial pH was adjusted to 7.5 and solutions sterilised at 121ºC for 15 min. Inocula (1% v/v) consisted of cellular suspensions from 16 h aged cultures on marine medium, adjusted to an absorbance (700 nm) of 0.900. Cultures were grown in 300 ml Erlenmeyer flasks with 150 ml of medium, at 22ºC with 200 rpm orbital shaking. All assays were carried out in triplicate.

At pre-established times, each culture sample was divided into two aliquots. The first aliquot was centrifuged at 5,000 rpm for 15 min, and the sediment washed twice and resuspended in distilled water to the adequate dilution for measuring absorbance at 700 nm. The dry weight was estimated from a previous calibration curve. The corresponding supernatant was used for the determination of proteins. The second aliquot was used for quantify viable cells by means of a

plate count technique on Marine Agar. Serial, tenfold dilutions were prepared in peptone-buffered solutions and 0.1 ml samples were plated in quadruplicate, incubated at 22ºC overnight, and manually counted. Results were expressed in colony-forming units per ml (cfu.ml^{-1}).

Analytical methods

Proteins were determined by the method of Lowry et al. [17]. Total nitrogen followed the method of Havilah et al. [18], applied to digests obtained by the classic Kjeldahl procedure. Total sugars were measured with the phenol-sulphuric reaction [19] according to the application by Strickland and Parsons [20] with glucose as a standard.

Numerical methods

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.

RESULTS AND DISCUSION

Preliminary tests

The preliminary tests compared the variations in pH, protein consumption and biomass production, at three incubation times between cultures of the 6 species in the 11 media defined in Table 3:

MM: Marine medium, recommended for the culture of bacteria of marine origin.
MRS (also MRS^{-}, equal to MRS without acetate to avoid possible inhibition): Commonly used in the culture of lactic acid bacteria, which are especially demanding in diverse peptidic sources.
YT (f and 2f): Fish waste medium with 1 g.l^{-1} yeast extract and 2.6 (f) or 5.2 (2f) g.l^{-1} protein (Lowry) from yellowfin tuna peptone in seawater.
SQ (f and 2f): As previously, but prepared with squid peptone.
SF (f and 2f): As previously, with swordfish peptone.
TR (f and 2f): As previously, with rainbow trout peptone.

Figure 1 shows the results corresponding to *Roseobacter* (Rb1), which sufficiently represents the group tested. The first aspect worth highlight is the capacity of all fish peptones for preparing a substitutive MM, producing analogous profiles in the three variables studied in all cases. The maximum biomasses (Table 4) were generally higher (often considerably) to those obtained in MM, which was only the most productive medium for *Vibrio* V2. However, even the most moderate growth, predominantly associated with the squid peptone, resulted in acceptable biomass. In addition, the media prepared from fish peptones promoted protein consumption far higher than those detected in MM, which suggests unbalanced nutrient concentrations of this last

one. Finally, it should be noted that the production of media 2f were not always higher than those of medium f. Although a definite explanation cannot be proposed, it is reasonable to assume the presence of compounds (perhaps lipidic peroxides) with slight inhibitory effects for the corresponding microorganisms.

With the exception of *Vibrio* V3, the results of the MRS^{-} medium were generally of the order of those prepared from viscera, and substantially higher than those obtained on MRS, in which three of the six species scarcely produced appreciable values of biomass. This confirms our previous caution about the inhibitory effects of acetate. Moreover, the effectiveness of MRS^{-} questions the generality of the common postulate of a obligatory seawater base for the culture of the microbiota isolated from marine habitats.

Kinetic approach

For a more detailed study of growth, each of the microorganisms was cultivated on the four fish peptones to concentrations (f or 2f, see Table 4) leading to highest biomass in each species, as well as on MM. The variables measured were pH, protein consumption, biomass (dry weight) and colony forming units ($cfu.ml^{-1}$). The results (figure 2 shows a representative case) confirmed the order established in Table 4.

To evaluate more formally the microbial growth, the two following models were used:

1: Logistical equation:

$$Y = \frac{K}{1+e^{c-\mu_m t}} \quad ; \text{where } c = \ln\left(\frac{K}{Y_0}-1\right) \tag{1}$$

Y biomass (X: $g.l^{-1}$) or cell number (N: $cfu.ml^{-1}$)
Y_0 initial biomass (X_0: $g.l^{-1}$) or cell number (N_0: $cfu.ml^{-1}$)
t time (hours)
μ_m maximum specific growth rate (dimensions of t^{-1})
K maximum biomass, or maximum cell number at infinite time (dimensions of Y)

2: Gompertz equation, reparameterised by Zwietering et al. [21]:

$$\ln\frac{Y}{Y_0} = A\exp\left\{-\exp\left[\frac{\mu_{\max}(\lambda - t)\exp(1)}{A}+1\right]\right\} \; ; \text{ where } Y_m = Y_0 \exp(A) \tag{2}$$

Y biomass (X: $g.l^{-1}$) or cell number (N: $cfu.ml^{-1}$)
Y_0 initial biomass (X_0: $g.l^{-1}$) or initial cell number (N_0: $cfu.ml^{-1}$)
t time (hours)
μ_m maximum specific growth rate (dimensions of t^{-1})

λ lag phase (dimensions of t)
A maximum biomass, or maximum cell number at infinite time (dimensionless)
Y_m maximum biomass, or maximum cell number at infinite time (dimensions of Y)

Both models are typically used for describing microbial growth, although usually applied to quantifications based on different criteria which are often not explicitly declared. Equation (1), versatile, with clear pseudokinetic structure and significant parameters, is useful for describing biomass (dry weight) and cell numbers. Equation (2) satisfactorily fits data with clear lag phases (λ), such as observed in processes requiring adaptation of an inoculum to the medium, and is typical for describing a logarithmic increase in relative cell number. However, the structure of (2) is more complex and employs a logarithmic normalization, which essentially eliminates the significance of μ_{max} as a maximum specific growth rate.

As an example of the group of microorganisms and media tested, figure 3 shows fits to both models of the experimental results obtained with a species of *Roseobacter*, in terms of dry weight and cell numbers (significant coefficient in all cases, with p=0.05; n=3). The maximum biomasses and maximum specific growth rates corresponding to all species and media considered are shown in Tables 5 to 12, calculated with models (1) and (2) applied to data measured both in terms of dry weights and cell numbers. The attribution of a general index to each medium inherently implies a certain degree of arbitrariness since the results depend on the species considered. The sum of the maximum biomasses (in %) corresponding to all species constitutes a reasonable criterion on which to base a ranking, and is also included in Tables 5 to 12. Moreover, for each species a maximum of the maximum biomasses (or supreme value) can be defined for a particular medium, not necessarily coincident in all species. Accordingly, to facilitate comparisons the supreme value of each species is specified in Table 13 as absolute value, and the rest of the values are presented as percentage of the supreme.

From practical point of view (especially in the case of potential probiotics) the objectives of a specific culture may be diverse, such as shortening the lag phase, accelerating the growth rate or increasing the final biomass as measured by one of the above criteria (not necessarily coincident: dry weight depends on the cell number and of its individual weights). The indices applied to the maximum biomasses can themselves be applied to the other parameters in equations (1) and (2), as show in Tables 5-12. Table 7 excludes the parameter λ from model (2), which in some cases produced artefactual negative values for the lag phase. In fact, the preparation of the inocula described in Methods eliminated the lag phase in all cultures due to the essential equivalence of all media employed.

The parametric estimates deduced from the calculations summarised in Tables 5-12 are notably similar. Nevertheless, for interpretation of the few discrepancies detected, the dry weight should take priority over those based on cell numbers. This is a consequence of the greater experimental error that (despite quadruplicate analyses) affects the plate counting methods.

Anyway, the group of comparisons demonstrates that the conventional marine medium is (i) clearly inferior to that prepared with peptones from tuna viscera, (ii) approximately equivalent to those prepared with trout and sword fish, and (iii) slightly superior to that from squid. This

confirms the possibility of using marine media derived from residual raw materials (which often provoke environmental pollution problems) for obtaining high microbial yields by simple, rapid and economical procedures. However, these results do not allow to suppose the presence in fish hydrolysates of very specific growth factors, but rather that peptidic sources from aquatic animals (especially fishes) are more appropriate than those from terrestrial animals for the culture of marine bacteria.

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TABLE 1: Main composition ($g.l^{-1}$) of peptones from fish viscera. YT: yellowfin tuna; SQ: squid; SF: sword fish; TR: rainbow trout.

	Proteins (Lowry)	Total sugars	Total nitrogen
YT	68.27	4.07	13.13
SQ	43.34	2.36	8.19
SF	54.02	2.26	11.06
TR	61.49	3.41	12.01

TABLE 2: Microorganisms employed

	Origin	Abbreviated key
Roseobacter sp.	DIFR 27-4	Rb1
Roseobacter sp.	DIFR 8-1	Rb2
Pseudomonas fluorescens	DIFR AH-2	Ps1
Vibrio splendidus	DMC 1	V1
Vibrio sp.	DIFR 10-2	V2
Vibrio anguillarum	SSF 287	V3

DIFR: Danish Institute for Fisheries Research
DMC: University of Glasgow
SSF: Stolt Sea Farm

TABLE 3: Composition of the culture media tested (g.l^{-1}, unless other units specified).

	FP[1] f	FP[1] 2f	MRS$^-$	MRS	MM
Ferric Citrate	-	-	-	-	0.10
Sodium Chloride	-	-	-	-	19.45
Magnesium Chloride	-	-	-	-	5.90
Sodium Sulfate	-	-	-	-	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}
Glucose	-	-	20.00	20.00	-
Sodium acetate	-	-	-	5.00	-
Ammonium citrate	-	-	2.00	2.00	-
Dipotassium Phosphate	-	-	2.00	2.00	-
Magnesium Sulfate	-	-	0.20	0.20	-
Manganesium Sulfate	-	-	0.05	0.05	-
Tween 80	-	-	1.00	1.00	-
Peptone	-	-	-	-	5.00
Meat extract	-	-	8.00	8.00	-
Yeast Extract	1.00	1.00	4.00	4.00	1.00
Bactopeptone	-	-	10.00	10.00	-
Protein (Lowry) from FP [1]	2.60	5.20	-	-	-
Sea water	1 liter	1 liter	-	-	-
Distilled water	-	-	1 liter	1 liter	1 liter

([1]) FP: Media prepared from fish peptones, as defined in Table 1.

TABLE 4: Maximum biomass (in descending order) obtained after preliminary tests with the different media. Media selected for the kinetic experiments are shown in bold type.

Rb1	YT 2f >	TR 2f >	SF 2f >	TR f >	MRS$^-$ >	YT f >	SF f >	MM >	MRS >	**SQ f** >	SQ 2f
Rb2	TR 2f >	SF 2f >	YT 2f >	YT f >	SF f >	TR f >	MRS$^-$ >	MM >	**SQ f** >	SQ 2f >	MRS
Ps1	TR 2f >	SF 2f >	YT 2f >	MRS$^-$ >	SQ 2f >	SF f >	YT f >	TR f >	MM >	SQ f >	MRS
V1	YT 2f >	**TR f** >	YT f >	MRS$^-$ >	MM >	TR 2f >	**SQ f** >	**SF f** >	SF 2f >	SQ 2f >	MRS
V2	MM >	MRS$^-$ >	SQ 2f >	TR 2f >	MRS >	TR f >	YT 2f >	**SF f** >	SQ f >	YT f >	SF 2f
V3	MRS >	MRS$^-$ >	YT 2f >	TR 2f >	SF 2f >	YT f >	SQ 2f >	MM >	**SF f** >	SQ f >	TR f

TABLE 5: Maximum biomasses estimated as K from model (1) applied to dry weigths. In each microorganism (rows Rb1-V3), the values of K on the different media are given as percentages of the maximum, the absolute value ($g.l^{-1}$) of which is shown bracketed.

	MM	SQ	TR	SF	YT
Rb1	41.5	31.5	51.7	32.9	100 (2.410)
Rb2	51.5	31.3	42.2	33.5	100 (1.954)
Ps1	100 (1.354)	41.6	58.1	97.8	87.7
V1	86.8	36.5	86.5	38.1	100 (0.622)
V2	100 (1.449)	57.6	38.7	67.2	28.6
V3	79.4	72.7	82.6	38.3	100 (2.619)
Sum	459.2	271.2	359.8	307.8	516.3
Ranking	2	5	3	4	1
% of the best medium	88.9	52.5	69.7	59.6	100

TABLE 6: Maximum specific growth rates estimated as μ_m from model (1) applied to dry weigths. As in Table 5, values in percentage of the maximum of each row, the absolute value ($hours^{-1}$) of which is shown bracketed.

	MM	SQ	TR	SF	YT
Rb1	100 (0.270)	55.6	89.3	75.2	85.2
Rb2	86.1	99.3	64.1	90.5	100 (0.295)
Ps1	16.8	32.7	25.7	18.4	100 (0.868)
V1	55.9	48.3	100 (0.635)	79.5	39.6
V2	41.5	97.2	59.1	100 (0.501)	20.8
V3	59.1	29.2	53.3	100 (0.672)	52.1
Sum	359.4	362.3	391.5	463.6	397.7
Ranking	5	4	3	1	2
% of the best medium	77.5	78.1	84.4	100	85.8

TABLE 7: Maximum cell numbers estimated as *K* from model (1) applied to cell numbers. As in Table 5, values in percentage of the maximum of each row, the absolute value (cfu.ml^{-1}) of which is shown bracketed.

	MM	SQ	TR	SF	YT
Rb1	13.1	6.9	10.0	1.6	100 (1.3×10^{12})
Rb2	43.1	43.1	43.1	4.6	100 (6.5×10^{12})
Ps1	11.1	6.9	8.9	14.1	100 (9.9×10^{13})
V1	22.7	7.7	11.0	3.3	100 (3.0×10^{11})
V2	87.5	87.5	8.8	100 (2.4×10^{11})	4.6
V3	100 (2.1×10^{11})	19.0	61.9	27.6	66.7
Sum	277.5	171.1	143.7	151.2	471.3
Ranking	2	3	5	4	1
% of the best medium	58.9	36.3	30.5	32.1	100

TABLE 8: Maximum specific growth rates estimated as μ_m from model (1) applied to cell numbers. As in Table 5, values in percentage of the maximum of each row, the absolute value (hours^{-1}) of which is shown bracketed.

	MM	SQ	TR	SF	YT
Rb1	74.0	72.5	77.8	100 (0.535)	79.3
Rb2	75.3	81.9	90.5	86.2	100 (0.304)
Ps1	59.8	65.5	68.4	60.0	100 (0.595)
V1	77.2	56.4	86.4	99.6	100 (0.530)
V2	100 (0.388)	97.4	68.0	93.8	30.4
V3	52.7	45.1	100 (0.636)	68.4	95.3
Sum	439.0	418.8	491.1	508.0	505.0
Ranking	4	5	3	1	2
% of the best medium	86.4	82.4	96.7	100	99.4

TABLE 9: Maximum biomasses estimated as $X_m=X_0 \exp(A)$ from model (2) applied to dry weights. As in Table 5, values in percentage of the maximum of each row, the absolute value ($g.l^{-1}$) of which is shown bracketed.

	MM	SQ	TR	SF	YT
Rb1	52.2	32.1	83.1	36.1	100 (1.914)
Rb2	50.0	30.5	42.8	32.7	100 (1.935)
Ps1	46.2	59.3	80.7	86.4	100 (1.194)
V1	95.5	38.7	100 (0.530)	42.6	87.9
V2	100 (1.377)	59.9	40.3	68.9	58.1
V3	77.7	66.8	85.2	39.4	100 (2.499)
Sum	421.6	287.3	432.1	306.1	546.0
Ranking	3	5	2	4	1
% of the best medium	77.2	52.6	79.1	56.1	100

TABLE 10: Maximum specific growth rates estimated as μ_m from model (2) applied to dry weights. As in Table 5, values in percentage of the maximum of each row, the absolute value (h^{-1}) of which is shown bracketed.

	MM	SQ	TR	SF	YT
Rb1	68.0	53.9	37.2	70.4	100 (0.594)
Rb2	94.5	92.9	55.7	87.9	100 (0.379)
Ps1	38.6	42.8	100 (1.188)	49.2	33.8
V1	50.7	41.8	73.7	49.2	100 (1.217)
V2	58.6	100 (0.635)	61.9	75.3	11.7
V3	67.8	55.5	41.2	99.5	100 (0.966)
Sum	378.2	386.9	369.7	431.5	445.5
Ranking	4	3	5	2	1
% of the best medium	84.8	86.8	82.9	96.9	100

TABLE 11: Maximum cell number estimated as $N_m=N_0 \exp(A)$ from model (2) applied to cell numbers. As in Table 5, values in percentage of the maximum of each row, the absolute value (cfu.ml^{-1}) of which is shown bracketed.

	MM	SQ	TR	SF	YT
Rb1	10.7	5.3	7.3	1.4	100 (1.5×10^{12})
Rb2	16.3	26.7	100 (8.6×10^{12})	5.8	77.9
Ps1	10.0	12.1	18.6	12.9	100 (1.4×10^{14})
V1	26.2	5.7	12.9	4.1	100 (2.1×10^{11})
V2	96.6	100 (2.9×10^{11})	8.3	82.8	72.4
V3	71.4	15.7	100 (1.4×10^{11})	38.6	92.9
Sum	231.2	165.5	247.1	145.6	543.2
Ranking	3	4	2	5	1
% of the best medium	42.6	30.5	45.5	26.8	100

TABLE 12: Maximum specific growth rates estimated as μ_m from model (2) applied to cell numbers. As in Table 5, values in percentage of the maximum of each row, the absolute value (h^{-1}) of which is shown bracketed.

	MM	SQ	TR	SF	YT
Rb1	51.7	60.2	45.6	100 (0.831)	72.9
Rb2	96.3	63.1	52.7	46.5	100 (0.493)
Ps1	53.6	72.6	64.9	71.2	100 (0.826)
V1	54.6	52.4	65.3	95.9	100 (0.691)
V2	78.8	79.2	79.2	100 (0.477)	30.8
V3	63.0	54.2	77.7	68.4	100 (0.722)
Sum	398.0	381.7	385.4	482.0	503.7
Ranking	3	5	4	2	1
% of the best medium	79.0	75.8	76.5	95.7	100

TABLE 13: Parametric values (percentages of the supreme, SUP. See text) obtained from equations [1] and [2] applied to biomasses (X) and cell numbers (N).

		MM		SQ		TR		SF		YT	
		% SUP	ranking	% SUP	ranking	% SUP	ranking	% SUP	ranking	% SUP	ranking
K	[1] X	88.9	2	52.5	5	69.7	3	59.6	4	100	1
μ_m	[1] X	77.5	5	78.1	4	84.4	3	100	1	85.8	2
K	[1] N	58.9	2	36.3	3	30.5	5	32.1	4	100	1
μ_m	[1] N	86.4	4	82.4	5	96.7	3	100	1	99.4	2
Y_m	[2] X	77.2	3	52.6	5	79.1	2	56.1	4	100	1
μ_m	[2] X	84.8	4	86.8	3	82.9	5	96.9	2	100	1
Y_m	[2] N	42.6	3	30.5	4	45.5	2	26.8	5	100	1
μ_m	[2] N	79.0	3	75.8	5	76.5	4	95.7	2	100	1

FIGURE CAPTIONS

Figure 1: Preliminary cultures of *Roseobacter sp.* (Rb1) in the eleven media described in Table 3, at four incubation times (0, 8, 23 and 47 h). ▼: pH; ●: proteins (Pr); ○: biomass (X).

Figure 2: Time-course of *Roseobacter sp.* (Rb1) cultures in MM (●), TR 2f (◇), SF 2f (▽), YT 2f (△) and SQ f (□) media. X: biomass, N: cell number.

Figure 3: Cultures of *Roseobacter sp.* (Rb1) in MM (●) and YT 2f (○) media. Top: Experimental results (points) and fits (lines) to equation (1), with measured biomass in terms of dry weight (left) and cell number (right). Bottom: As top, applying equation (2).

Figure 1

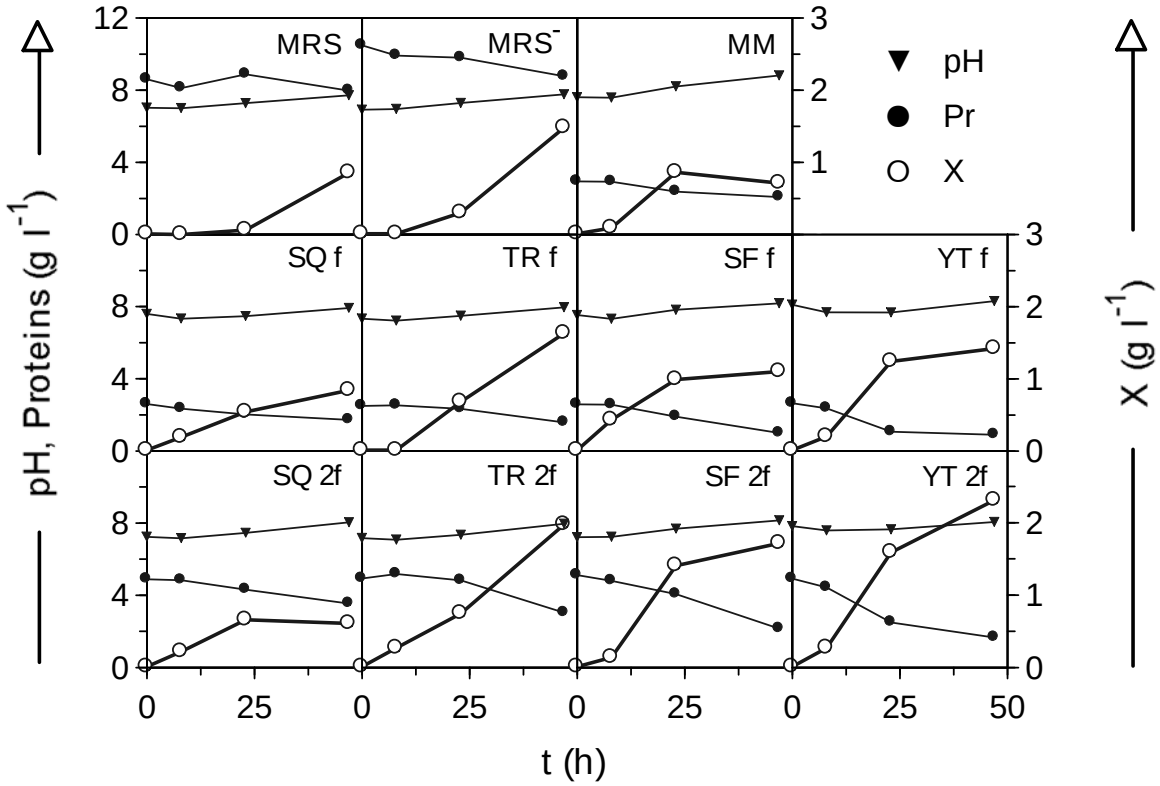

MRS
MRS-
MM
SQ f
TR f
SF f
YT f
SQ 2f
TR 2f
SF 2f
YT 2f
pH
Pr
X
pH, Proteins (g l-1)
X (g l-1)
t (h)

Figure 2

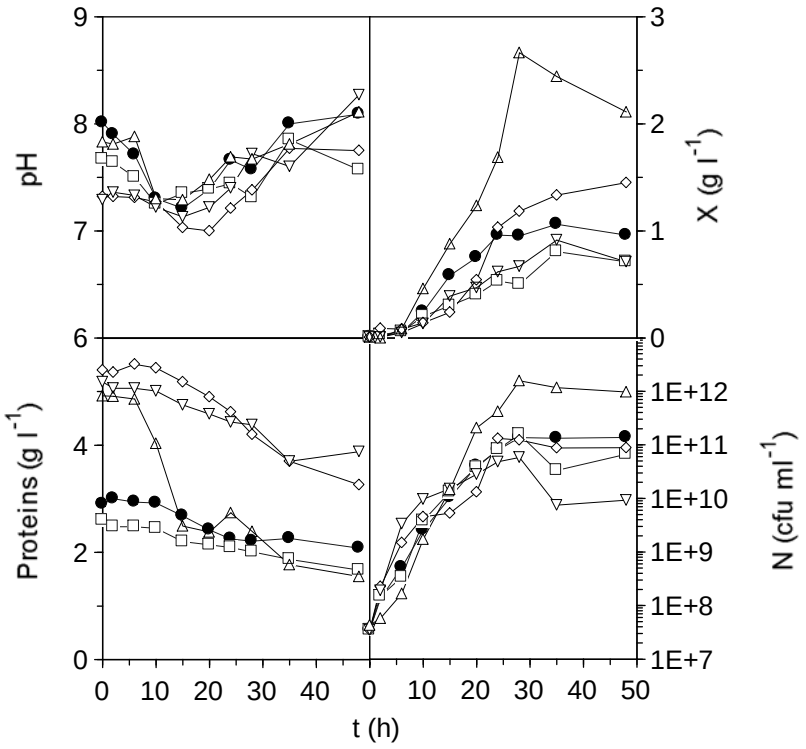
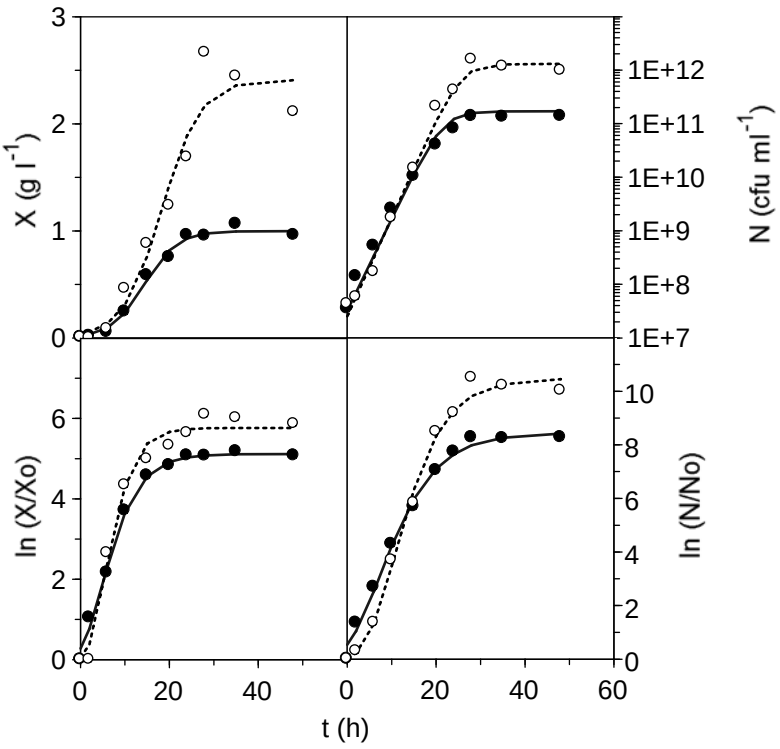

pH
X (g l-1)
Proteins (g l-1)
N (cfu ml-1)
t (h)
9
8
7
6
4
2
0
3
2
1
0
1E+12
1E+11
1E+10
1E+9
1E+8
1E+7
0
10
20
30
40
0
10
20
30
40
50

Figure 3


X (g l-1)
N (cfu ml-1)
ln (X/Xo)
ln (N/No)
t (h)
0
1
2
3
1E+7
1E+8
1E+9
1E+10
1E+11
1E+12
0
2
4
6
8
10
0
20
40
60