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Horse Bean Extract for the Supplementation of Lactic Acid Bacteria Culture Media

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Abstract: Seven strains of lactic acid bacteria isolated from different biotopes were cultivated on media containing horse bean extract. Growth was compared to that recorded on the widely used MRS and M17 media. The feasibility of the use of a vegetal substrate (horse bean extract), namely vegetal protein, in place of peptones and meat extract for the nitrogen supplementation of culture media for LAB growth was demonstrated. Horse bean extract appears especially efficient for growth of LAB species isolated from plants, illustrating the relationship between the supplementation used and the natural biotope of the LAB strain.

Key words: Lactic acid bacteria, growth, nitrogen supplementation, horse bean extract

INTRODUCTION

Lactic acid bacteria have numerous industrial applications, especially in food industries. They play an important role in numerous natural fermentations and hence are involved in the transformation of many food products. They can naturally be present in the endogenous flora of the raw material or added as starter culture. Growth of lactic acid bacteria remain a difficult task which require complex culture media, from both a qualitative and a quantitative point of view, owing to their fastidious nutritional requirements and the variability from a strain to another[1]. Therefore, only one medium may not be convenient for a high number of LAB strains.

The media used to study the physiological and biochemical properties of LAB needed, not only to be rich and complex media, but must also allow to highlight the specific properties of the studied LAB strains. Owing to the fastidious growth factor requirements of LAB and since they are very sensitive to inhibitors, selective culture media are very difficult to formulate.

Since the fifties, the determination and evaluation of selective media for LAB remain a matter of topical interest[2,3]. Among the available specific media, MRS and M17 remain especially convenient for numerous LAB strains[4]. Often, recent media corresponded only to modified old ones. For example, for a better selectivity of the MRS medium for a given strain, some authors recommend an acidification or the addition of inhibitors or to replace glucose by another sugar[5-7]. Other authors propose the use of synthetic media, viz. chemically defined media, to examine the nutritional requirements or the metabolic pathways of LAB[8-10].

The specific media used for LAB growth usually contained peptones, yeast or meat extract, milk, casein or whey protein hydrolysates, yeast or bacteria autolysates (for a recent review see 4). Contrarily, vegetal substrates for LAB growth have not attracted similar interest and only few papers are available[11-15]. The aim of this study is therefore the evaluation of the potential of three culture media based on a vegetal Algerian horse bean broth for the growth of seven strains of lactic acid bacteria isolated from different biotopes.

MATERIALS AND METHODS

Microorganisms: Six local lactic acid bacteria strains as well as one commercial strain, *Lactobacillus rhamnosus* LBC80 10D (Danisco, Dangé Saint Romain, France) were used.

The local strains were isolated from plants, strains of *Lactobacillus plantarum* and *Leuconostoc mesenteroïdes*, from a traditional fermented milk, L'ben, strains of *Lactococcus lactis* ssp. *diacetylactis* and

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Lactobacillus acidophilus and from infant faeces and potentially probiotics, strains of *Lactobacillus casei* ssp. *rhamnosus* and *Enterococcus faecium*.

The following tests were carried out for identification of the different strains:

- Morphological characteristics and cellular arrangement.
- Gram, catalase, citratase and oxidase tests.
- Growth at various temperatures, in presence of a high NaCl concentration (6.5%) or in presence of bile (40% w/v).
- Thermoresistance test.
- Starch and esculin hydrolysis.
- Exopolysaccharide and acetoine production.

The homofermentative test was conducted in liquid modified MRS medium supplied with a Durham bell jar and covered with a semi-solid layer of white agar gel. The strain fermentation profile was determined using the API 50 CH medium (BioMérieux, Marcy l'Etoile, France). The acidification capacity was determined after growth on 10% reconstituted milk (low temperature powder).

Culture media

Plant juice preparation: Pulse flour (horse bean) (Big Cultures Institute, Guelma, Algeria) was used for culture medium preparation. This product is characterised by a high protein content (30% on the dry matter) and also contained sugars (verbascose 3%, stachyose 1% and raffinose 0.2%) and trace element (4%).

Seeds were manually husked and then powdered. 60 g of the powder were then dissolved in one litre of distilled water at pH 9, magnetically stirred during 30 min, before centrifuged at 3000 rev min^{-1} for 20 min. The supernatant was filtered and then used as a basis for the preparation of the three following media:

- **Medium A:** it contained (g L^{-1}): glucose, 15; yeast extract, 5; sodium acetate, 5; di-ammonium citrate, 2; Mg SO_4, $7H_2O$, 0.2 g; Mn SO_4, $4H_2O$, 0.05; Tween 80, 1 mL; Agar, 13 g. The horse bean supernatant was used for medium reconstitution.
- **Medium B:** similar amounts of the same components as medium A were dissolved in 900 mL of horse bean supernatant. After sterilisation, 100 mL of 10% reconstituted milk (w/v) was added to the former solution.
- **Medium C:** similar amounts of the same components as medium A were dissolved in 750 mL of horse bean supernatant. The solution was completed to 1 l with fresh carrot juice, namely the supernatant of centrifuged pulp of pounded carrot.

Before sterilisation (121°C for 20 min), the pH was adjusted to 6.4.

Experiments were also carried out on MRS and M17 media (Difco, BD, Franklin Lakes, NJ, USA) in view of comparison.

Culture: Stock cultures were maintained on sloping agar medium. They were reactivated by culture on sterile reconstituted milk (10%) at 30°C until medium coagulation. The inoculation level was 1%.

Cultures were carried out at 37 °C for 48 h, except the *L. lactis* ssp. *diacétylactis*, which was cultivated at 30°C. All the strains were cultivated on media A, B and C; the *Lactobacillus* strains and the *Leuc. mesenteroïdes* strain were also cultivated on the MRS medium, while the *L. lactis* ssp. *diacetylactis* and the *E. faecium* strains were cultivated on the M17 medium.

Cell numeration: They were carried out after duplicate dilutions (10^{-2} to 10^{-8}) in sterile peptone solution (15 g L^{-1} peptone and 5 g L^{-1} NaCl). Petri dishes were inoculated into the mass. After its solidification, the medium was covered by a layer of white gelified cysteine medium (0.25 g $^{-1}$ of cystéine chlorhydrate)[16], to reduce aerobiosis. Incubation conditions were similar to that of culture, namely 37°C for 48 h, except the *L. lactis* ssp. *diacétylactis* which was cultivated at 30°C. Only the Petri dishes containing between 25 and 250 Units Forming Colony were taken into account.

RESULTS

Cell numeration of seed cultures, just before inoculation, was approximately 10^6 UFC for all strains, leading to initial number of cells during culture of approximately 10^4. The comparison with the final cell numeration (Table 1) clearly showed that all the media tested favoured growth of the chosen strains. The duplicate cell numeration values are given in Table 1.

Potential of medium A: This medium resulted in a significant growth for all the strains tested, since the number of UFC remained always between $3.0\ 10^6$ and $3.5\ 10^8$. The comparison with the growth recorded on the reference media, showed a better growth on the medium A for the strains isolated from plants, *Lb. plantarum* et *Leuc. mesenteroides*, if compared to growth on the MRS medium Table 1. Contrarily, for the strains of *Lb. casei rhamnosus*, *L. rhamnosus LBC8010D*, *Lb. acidophilus* and *L. lactis* ssp. *diacetylactis*, higher final cell numeration were recorded on MRS and M17 medium for the *Lactococcus* strain, if compared to the medium A, in the range $2.0\ 10^9$ to $6.5\ 10^9$ and $1.4\ 10^7$ to $3.6\ 10^7$ UFC mL^{-1},

Table 1: Cell numeration after culture on the various media tested

Medium	*Leuconostoc mesenteroïdes* UFC mL^{-1}	*Lactobacillus plantarum* UFC mL^{-1}	*Lactobacillus casei rhamnosus* UFC mL^{-1}	*Lactobacillus rhamnosus LBC8010D* UFC mL^{-1}	*Lactobacillus acidophilus* UFC mL^{-1}	*Lactococcus lactis* ssp. *diacetylactis* UFC mL^{-1}	*Enterococcus faecium* UFC mL^{-1}
A	2.46-2.72 10^8	3.4-3.7 10^8	1.36-1.55 10^7	1.44-1.56 10^7	3.5-3.7 10^7	2.94-3.09 10^6	2.02-2.04 10^8
B	2.58-2.62 10^7	3.25-3.38 10^8	2.65-2.75 10^7	2.59-2.81 10^7	4.60-4.77 10^8	2.76-2.83 10^7	1.1-1.03 10^9
C	9.43-9.37 10^8	4.64-4.59 10^8	1.18-1.21 10^7	1.29-1.32 10^7	3.52-3.6 10^7	1.46-1.57 10^5	1.77-1.84 10^8
MRS	2.02-2.08 10^7	2.76-2.81 10^8	4.30-4.32 10^9	2.81-2.83 10^9	2.05-2.11 10^9	/	4.51-4.52 10^8
M17						6.50-6.57 10^9	9.15-9.20 10^9

respectively (Table 1). Similar final cell numeration were recorded for *E. faecium* growing on media A and MRS, 2-4.5 10^8 UFC mL^{-1}, while this number increased to 9.0 10^9 UFC mL^{-1} during growth on M17 medium (Table 1).

Potential of medium B: Milk addition to the horse bean solution, medium A, improved growth of the strains isolated from milk and human faeces. The milk stimulatory effect was especially significant for the strains *L. lactis* ssp. *diacetylactis*, *Lb. acidophilus* and *E. faecium*, which gained about 1 log after milk addition to the medium A. However, cell numerations remained lower than those recorded on reference media, MRS and M17 Table 1.

Potential of the medium C: Fresh carrot juice addition appears to have a positive effect only on the strains isolated from plants, *Leuc. mesenteroïdes* and *Lb. plantarum*, while it had no effect on the growth of *Lb. casei* ssp. *rhamnosus*, *Lb. rhamnosus*, *Lb. acidophilus* and *E. faecium* and a negative effect on *L. lactis* ssp. *diacetylactis* (Table 1).

DISCUSSION

The three media tested appears to be convenient for growth of all the tested strains. However, they were especially efficient for growth of the strains isolated from plants. This positive effect has to be related to the plant origin of the nitrogen supplementation of culture media, horse bean extract, leading to a better growth than that recorded on the reference medium, MRS.

To improve growth of the LAB strains isolated from milk or human faeces, animal protein (milk) had been added to the medium; growth remained however lower than that recorded on the reference media, MRS and M17.

Medium A contained 60 g L^{-1} of pulse flour from horse bean (87% dry weight), containing 30% proteins, namely 15.7 g L^{-1} of protein supplied by the horse bean extract; namely 2.46 g L^{-1} of protein nitrogen (a ratio of protein on nitrogen of 6.38 was considered). By also taking into account 0.5 g L^{-1} of protein nitrogen from yeast extract (5 g L^{-1} containing 10% of total nitrogen), medium A and C contained 2.96 g L^{-1} of protein nitrogen (the amount of proteins contained in the carrot juice has been neglected) and medium B contained 3.43 g L^{-1} of protein nitrogen (3 g L^{-1} proteins supplied by the added milk). These values should be related to the protein nitrogen content of MRS and M17 media, which contained 5 and 2.5 g^{-1} of yeast extract respectively (10% total nitrogen), 10 and 5 g L^{-1} of meat extract (12.5% total nitrogen), respectively and 10 g L^{-1} of various peptones (between 10 and 12.5% total nitrogen) for both media. The protein nitrogen contents of MRS and M17 media were therefore 3.0 and 2.0 g L^{-1}, respectively.

This study demonstrates the feasibility of the use of a vegetal substrate (horse bean extract), namely vegetal protein, in place of meat extract and peptones for the nitrogen supplementation of culture media for LAB growth. Horse bean extract appears especially efficient for growth of LAB species isolated from plants, illustrating the variability between LAB strains[1]. The major point highlighted in this study was therefore the relationship between the complex supplement added in the culture medium and the origin of the LAB strains cultivated. Indeed, horse bean extract appeared in this study more efficient than meat extract and peptones.

Many supplementations have been tested for LAB growth, such as corn steep liquor, malt sprout extract, casein hydrolysates[17], supplying LAB not only in usable nitrogen[18], but also in growth factors[19]. This preliminary work have to be followed therefore by an optimisation of culture media composition and especially the supplementation of culture medium with only horse bean extract without any additional supplementation, to examine the efficiency of horse bean extract to supply for growth factors needed for LAB growth. A screening on an important number of LAB strains may be also useful.

REFERENCES

1. Chamba, J.F., C. Duong, A. Fazel and F. et Prost, 1994. Sélection des Souches de Bactéries Lactiques in Bactéries lactiques Aspects Fondamentau. De Roisart H. et Luquet F.M Ed. Lorica
2. Rogosa, M., J.A. Mitchell and R.F. Wiseman, 1951. A selective medium for the isolation and enumeration of oral and faecal *lactobacilli*, J. Bacteriol., 62: 132-133.

3. Terzaghi, B.E. and W.E. Sandine, 1975. Improved medium for lactic streptococci and their bacteriophage, Applied Microbiol., 29: 807-813.
4. Carr, J.C., D. Chill and N. Maida, 2002. The lactic acid bacteria: A literature survey, Crit. Rev. Microbiol., 28: 281-370.
5. Dave, R.I. and N.P. Shah, 1996. Evaluation of media for selective enumeration of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* ssp. *bulgaricus*, *Lactobacillus acidophilus* and *Bifidobacteria*. J. Dairy Sci., 79: 1529-1536 .
6. Talwalkar, A. and K. Kailasapathy, 2004. Comparison of selective and differential media for the accurate enumeration of strains of *Lactobacillus acidophilus*, *Bifidobacterium* sp. and *Lactobacillus casei* complex from commercial yoghurts. Intl. Dairy J., 14: 143-149.
7. Roy, D., 2001. Media for the isolation and enumeration of bifidobacteria in dairy products. Intl. J. Food Microbiol., 69: 167-182.
8. Vinderola, C.G. and J.A. Reinheimer, 1999. Culture media for the enumeration of *Bifidobacterium bifidum* and *Lactobacillus acidophilus* in the presence of yoghurt bacteria. Intl. Dairy J., 9: 497-505.
9. Jensen, P.R. and K. Hammer, 1993. Minimal requirements for exponential growth of *Lactococcus lactis*. Applied Environ. Microbiol., 59: 4363-4366.
10. Juillard, V., D. Le Bars, E.R.S. Kunji, W.N. Konings, J.C. Gripon and J. Richard, 1995. Oligopeptides are the Main Source of nitrogen for *Lactococcus lactis* during Growth in Milk. Applied Environ. Microbiol., 61: 3024-3030.
11. Gardner, N., J. Gardner, T. Savard, P. Abernier, G. Calwell and C.P. Champagne, Selection and characterization of mixed starter cultures for lactic acid fermentation of carrot, cabbage, beet and onion vegetable mixtures. Intl. J. Food Microbiol., 64: 261-275.
12. Chalampopoulos, D., S.S. Pandiella and C. Webb, 2002. Growth studies of potentially probiotic lactic acid bacteria in cereal-based substrates. J. Applied Microbiol., 92: 851-859.
13. Chalampopoulos, D., S.S. Pandiella and C. Webb, 2003. Evaluation off the effect of malt,wheat and barley extracts on the viability of potentially probiotic lactic acid bacteria under acidic conditions. Intl. J. Food Microbiol., 82: 133-141.
14. Kontula, P., A. Von Wright and T. Sandholm, 1998. Oat branch β gluco and xylo-oligosacharides as fermentation substrates for lactic acid bacteria. Intl. J. Food Microbiol., 45: 163-169.
15. Nancib, N., A. Nancib, A. Boudjelal, C. Benslimane, F. Blanchard and J. Boudrant, 2001. The effect of supplementation by different nitrogen sources on the production of lactic acid from date juice by *Lactobacillus casei* sub sp. *rhamnosus*. Bioresour. Technol., 78: 149-153.
16. Marchal, N., J.L. Bourdon and C.I. Richard, 1982. Milieux de Cultures Pour l'isolement et l'identification Biochimiques des Bactéries. Doin (Ed.), Paris.
17. Hujanen, M. and Y.Y. Linko, 1996. Effect of temperature and various nitrogen sources on L(+)-lactic acid production by *Lactobacillus casei*. Applied Microbiol. Biotechnol., 45: 307-313.
18. Leh, M.B. and M. Charles, 1989. Lactic acid production by batch fermentation of whey permeate: A mathematical model. J. Ind. Microbiol., 4: 65-70.
19. Aeschlimann, A. and U. Von Stockar, 1990. The effect of yeast extract supplementation on the production of lactic acid from whey permeate by *Lactobacillus helveticus*. Applied Microbiol. Biotechnol., 32: 398-402.