J. Agric. Sci. Mansoura Univ., 28 (9): 6767 - 6773, 2003

EFFECT OF SUPPLEMENTAION OF WHEY AGAR (AS BASAL MEDIUM) WITH SOME NUTRITIONAL INGREDIENTS ON THE GROWTH OF PURE CULTURES OF LACTIC ACID BACTERIA.

Nassib,T. A. and H. El-Ganainy
Dairy Department, Fac. of Agric., Mansoura Univ., Egypt.

ABSTRACT

This experiment was carried out as an attempt to improve the nutritional value of whey agar medium as a cheap and suitable medium for growing some strain of lactic acid bacteria with the addition of peptone, yeast, tomato, or carrot extract either separately or in combination. Applying whey agar (WA) medium gave no growth with any of the examined pure cultures of lactic acid bacteria. The addition of peptone and carrot extract (WPC) to the whey agar resulted in higher numbers of all cultures, compared with whey peptone (WP) agar. The addition of peptone and tomato extract (WPT) further increased the numbers of lactic acid strains. The highest numbers were gained with *Str. lactis*, whereas the lowest were detected with *L. casei. L. bulgaricus* behaved similarly on this medium with *Str. lactis.* Rather closely numbers were detected with *Str. cremoris* and *Str. thermophilus.* Further increase of growth was observed by enriching the (WA) with peptone and yeast extract (WPY). The incorporation of peptone and both yeast extract and carrot extract (WPYC) or peptone and both of yeast and tomato extract (WPYT) resulted in more intensive growth of all examined cultures. Finally, supplementing the (WA) medium with peptone together with both yeast and tomato and carrot extracts yielded almost the highest stimulatory effect on all of the examined cultures of lactic acid bacteria.

INTRODUCTION

The complexity of the growth factor requirements reflects a certain deficiency in the enzymatic mechanism of lactic acid bacteria as compared with many other organisms, which are capable of synthesizing most of these growth factors themselves. Consequently, several of the metabolic pathways, *i.e.* the sequences of biosynthetic reactions of vitamins, amino acids and other growth factors which are present in other organisms are lacking or imperfect in these bacteria (Nurmikko, 1964; Stanier *et al*.,1964 and Mitsouka, 1969).

The stimulatory effect of whey syrup was studied by Zhang-ShaoHui; *et al*. (1997) on growth of lactic acid bacteria. Whey syrup, prepared by immobilized beta- glactosidase and whey were added separately to 10% skim milk culture media, and then incubated with 2% of a fresh culture starter of *Lactobacillus delbrueckii* subsp. *bulgaricus* and change in acidity were observed during incubation. The addition of whey

syrup and whey stimulated growth of *L. delbrueckii* subsp*. bulgaricus* in the skim milk culture media. However, addition of whey syrup was more effective than the whey. The lag phase growth of *L. delbrueckii* subsp*. bulgaricus* with addition of whey syrup was 2h shorter than that with addition of whey. When whey syrup and whey were added to skim milk culture media, the acidities were higher than the control sample during incubation, suggesting that whey syrup and whey had good stimulatory effect on the growth of lactic acid cultures. The results showed that the whey syrup was a better substrate than whey for stimulating lactic acid cultures.

Whey permeate was used by Christopherson *et al.* (1989) as a medium for cultivation of mesophilic lactic acid streptococci at their optimal growth temp. Results were reasonably promising when permeate containing 0.1% yeast extract was used.

Bury,D *et al*.(1998) found that the addition of 1 or 2% of a whey protein concentrate (WPC) to a whey- based medium used for fermentation with *Lactobacillus delbrueckii* subsp *bulgaricus* 11842 or *Str. thermophilus* ST20 has produced significantly higher bacterial counts and much faster acidity development than the control whey or whey UF permeate media.

Whey or whey permeate has the potential as a culture medium for the propagation of dairy cultures (Parente and Zottola, 1991). Whey or UF of whey permeate are cheap and readily available sources for fermentation media, but require supplementation with a complex additive such as yeast extract (Gupta and Gandhi, 1995 and Parente and Zottola, 1991) or corn steep liquor (Cox and MacBean, 1977). With various potential supplements, it was observed that the addition of a whey protein concentrate significantly increased both the cell numbers and lactic acid production of *Lactobacillus delbrueckii* subsp *bulgaricus* 11842 or *Str. thermophilus* ST20. Whey broth is better growth medium than whey UF permeate broth for growth which hints that some growth factors, which might be removed by ultrafiltration. Whey protein concentrates stimulated the growth of lactic acid bacteria in whey or UF whey Permeate broths. The components responsible for the increase of growth are heat stable as the stimulatory effect is not lost after heating to 121˚C for 15 min. The heat stable components might be nucleotides, non protein nitrogen, or some specific heat stable peptides not present in Bacto-peptone.

This study was, therefore, carried out using whey as based medium which has several nutritional and economical point of view, the use of cheap whey to replace the expensive ingredients would reduce the cost, and in the same time, offer a feasible way for the utilization of whey. The high biological value of whey proteins would improve the nutritional value of the medium. Eight different media composed from cheaper and nourishing substances in addition to whey agar medium were examined to study their effect on the growth of certain pure cultures of lactic acid bacteria.

MATERIALS AND METHODS

1- Microorganisms

Five pure cultures of lactic acid bacteria were obtained from the collection of the Faculty of Agriculture, Assiut Univeristy. These cultures are:

Streptococcus lactis (11552)
Streptococcus cremoris (11332)
Lactobacillus bulgaricus (11102)
Lactobacillus casei.
Streptococcus thermophilus.

These cultures were examined for their ability to grow on the whey medium (WA) either in the absence or presence of peptone (WP); peptone and tomato extract (WPT); peptone and carrot extract (WPC); peptone and yeast extract (WPY), peptone, yeast and carrot extract (WPYC); peptone, yeast and tomato extract (WPYT); and peptone , yeast, tomato and carrot extracts (WPYTC).

2-Maintenance of lactic acid bacterial cultures:

The 5 pure cultures were maintained on slants of M.R.S. agar (De Man *et al.*, 1960) which have the following composition.

Peptone	10.0
Meat extract	10.0
Yeast extract	5.0
Glucose	20.0
Tween_80	1 ml
K_4HpO_2	2.0
Sodium acetate	5.0
Diammonium citrate	2.0
$MgSO_4 \times 7H_2O$	0.2
$MnSO_4 \times 7H_2O$	0.05
Distilled water	1000.0ml
pH 6.8	

The incubation was carried out at 30ºC for streptococci and 40ºC for lactobacilli.

3-Preparation of the extracts :

3-1 Tomato juice:

The unconcentrated liquid was extracted from mature tomatoes of red or reddish varieties, followed by draining. Such liquid is then strained free from skins, seeds and other coarse or hard substances, Homogenization of the resultant extract is carried out, followed by filtration and steaming for 30 min. (Jacobs,1951)

3-2Carrot extract:

Carrots were first thoroughly washed, scraped to remove the soil residues, squeezed and the extract was then filtered and steamed for 10 minutes.

4-Preparation of whey.

Fresh cow's milk was pasteurized at 72˚C for 10 min., followed by cooling to 32˚C. The pH was adjusted to 7.0 - 7.2, $Cacl_2$ was added (5 ml. of 40% solution /L.) and enzymatically coagulated by the addition of calf rennet. After complete coagulation, the whey was separated from the curd by cutting it. The whey was then filtered and to precipitate whey proteins, the filtrate was eventually heated at 1 atm. for 1 min., and the whey proteins were filtered off, and the resultant filtrate (whey) was sterilized at 1.2 atm. For 15 min.

The following media were prepared in order to investigate the ability of the examined cultures of lactic acid bacteria to grow on them:

1-Whey Agar medium(WA)

This medium was used as a basal (control) medium, and consisted of whey (500 ml) and agar (7.5g.)

2-Whey peptone agar (WP) medium

The same as the (WA) with the addition of peptone (5g).

3-Whey peptone tomato agar (WPT)

Of the same constituents of the WA with the addition of peptone (5g.) and tomato juice (100 ml).

4-Whey peptone carrot agar (WPC).

Of the same composition of WA with the addition of peptone (5g.), carrot extract (50 ml).

5-whey peptone yeast agar (WPY).

Of the same composition of WA with the addition of peptone (5g.) and yeast extract (2.5g).

6- whey peptone yeast tomato agar (WPYT).

Of the same composition of WA with the addition of peptone (5g.), yeast extract (2.5g.) and tomato extract (100 ml.).

7- whey peptone yeast carrot agar (WPYC).

Of the same composition of WA with the addition of peptone (5g.), yeast extract (2.5g.), carrot extract (50 ml).

8- whey peptone yeast tomato carrot agar (WPYTC).

Of the same composition of WA with the addition of peptone (5g.), yeast extract (2.5g.), carrot extract (50 ml), tomato extract (100 ml).

All of above mentioned media were prepared, the final pH was adjusted at 6.8, sterilized at 1.2 atm. for 15 min. The incubation of the cultivated with lactic acid bacteria was carried out at 30˚C for mesophilic streptococci and at 40˚C for the thermophilic streptococci and lactobacilli.

RESULTS AND DISCUSSION

In the present work, efforts were directed in an attempt to improve the whey agar medium by increasing its nutritional value with the addition of peptone, yeast, tomato or carrot extracts either separately or in combination to meet the requirements of lactic acid bacteria. Five pure cultures of lactic acid bacteria, namely *Streptococcus lactis* (11552); *Streptococcus cremoris* (11332), *Lactobacillus bulgaricus* (11102), *Lactobacillus casei* and *Streptococcus thermophilus* were used. Cultivated media were incubated at 30˚C for the mesophilic streptococci and at 40˚C for the thermophilic streptococci and lactobacilli for 24 hours before the enumeration.

Results presented in table (1) indicated that no growth was observed on the whey agar (WA) medium with any of the examined pure cultures of lactic acid bacteria.

It could also be appeared that the addition of peptone to WA slightly improved the nutritional value of this medium (WP) as the bacterial numbers were in the range of $3X10^6$ cfu/ml (*L. casei*) to $9.9\ X10^8$ cfu/ml (*Str. lactis*). Variation was also observed between the examined cultures grown on the WP agar medium.

Incorporation of both peptone and carrot extract (WPC) with whey agar resulted in higher numbers of all of the examined cultures as compared with those attained on WP. The same trend was also observed as the examined cultured varied in their ability to grow on this medium, and the highest numbers were attained with *Str. lactis*, whilst the lowest with the culture of *L. casei*.

The addition of peptone and tomato extract (WPT) further increased the obtained numbers of lactic acid strains. Meanwhile, variation was noticed between the investigated cultures and, on the other hand, the highest numbers of $11.5X10^8$ cfu/ml were gained with *Str. lactis,* whereas the lowest of $5\ X10^6$ cfu/ml were detected with *L. casei. L. bulgaricus* behaved similarly on this medium with *Str. lactis* (2 and $11.5\ X10^8$ cfu/ml., resp.), whereas rather closely numbers were found with *Str. cremoris* ($27\ X10^7$ cfu/ml), compared with *Str. thermophilus* ($15.5\ X10^7$ cfu/ ml.).

Further increase of growth , however, was observed by enriching the WA with peptone and yeast extract (WPY). The most intensive growth ($12\ X10^8$ cfu /ml) was registered with *Str. lactis*, whilst the lowest of $1\ X10^6$ cfu was found with *L. casei.* Again, both of *Str. lactis* and *L. bulgaricus* were of closely similar numbers (12 and $2.03\ X10^8$ cfu/ml, resp.), and *Str. cremoris* and *Str. thermophilus,* which resulted in 30 and $16.2\ X10^7$, in the same order.

The incorporation of peptone and both of yeast and carrot extract (WPYC) from one side or peptone and both of yeast and tomato extract (WPYT) resulted in more intensive growth for all of the examined cultures, compared with that observed with the previously mentioned media. Slight variation was found between WPYC and WPYT, although the highest growth was achieved with *Str.lactis* 13 and $17.4\ X10^8$ cfu/ml, while the lowest of 2 and $3\ X10^6$ cfu/ml. was found with *L. casei*, in the same order.

Finally, supplementing the WA medium with peptone together with both yeast, tomato and carrot extracts yielded almost the highest stimulatory effect on all of the examined cultures of lactic acid bacteria. Slight variation, however, was observed between *Str. lactis* and *L. bulgaricus* (18 and 4.7 $X10^8$ cfu / ml., resp.)., and between *Str. cremoris* and *Str. thermophilus* (43.2 and 27.5 $X10^7$ cfu / ml), whilst the lowest effect of 2 $X10^6$ cfu /ml. was registered when examining the ability of *L. casei* to grow on this medium.

In conclusion, the present data came in harmony with the preceding results during the course of these investigation, El-Ganiny,H. (2002) as the supplementation of basal medium (digested milk or whey agar) with peptone, yeast extract or natural extracts such as tomato or carrot enhanced the growth of lactic acid bacteria as a results of improvement of the nutritional value of these media.

Table (1): Effect of supplemented (WA) medium with some of nutrient factors on the bacterial numbers of Five lactic acid strains.

Media	*Str. Lactis*	*Str..cremoris*	*Lactob acillus bulgaricus*	*Lactobacillus casei*	*Streptococcus.T hermophilus*
WPYTC	18 ◌ 10^8	43.2 ◌ 10^7	4.7 ◌ 10^8	2 ◌ 10^6	27.5 ◌ 10^7
WPYT	17.4 ◌ 10^8	38 ◌ 10^7	4.3 ◌ 10^8	3 ◌ 10^6	22.8 ◌ 10^7
WPYC	13 ◌ 10^8	33.8 ◌ 10^7	3.1 ◌ 10^8	2 ◌ 10^6	21.1 ◌ 10^7
WPY	12 ◌ 10^8	30 ◌ 10^7	2.03 ◌ 10^8	1 ◌ 10^6	16.2 ◌ 10^7
WPT	11.5 ◌ 10^8	27 ◌ 10^7	2 ◌ 10^8	5 ◌ 10^6	15.5 ◌ 10^7
WPC	10.4 ◌ 10^8	18 ◌ 10^7	1.99 ◌ 10^8	4.7 ◌ 10^6	7.3 ◌ 10^7
WP	9.9 ◌ 10^8	17 ◌ 10^7	1.76 ◌ 10^8	3 ◌ 10^6	5.6 ◌ 10^7
WA	N0 growth	_	_	_	_

REFERENCES

Bury, D.; P. Jelen and K. Kimura (1998). Whey protein concentrate as a Nutrient supplement for lactic acid bacteria. Int. Dairy., J. 8: 149-151.

Christopherson-AT and Zottola-EA. (1989). Whey permeate as a medium for mesophilic lactic acid streptococci. J. Dairy Sci., 72 (7): 1701-1706.

Cox, G. C. and R. D. MacBean (1977). Lactic acid production by *Lactobacillus bulgaricus* in supplemented whey ultrafiltrate. The Australian J. Dairy. Tech., 32: 19-22.

De Man, J. C.; M. Rogosa and M. E. Sharpe (1960). A medium for the cultivation of lactobacilli. J. Appl. Bact., 23:130.

El-Ganainy,H. (2002). Media used for isolation and enumeration of lactic acid bacteria. M.Sc. Thesis, Fac. of Agric., Mansoura Univ., Egypt.

Gupta, R. and D.N. Gandhi (1995). Effect of supplementation of some nutrients in whey on the production of lactic acid. Indian J. Dairy. Sci., 48: 636-641.

Jacobs, M.B. (1951). The Chemical Analysis of Foods and Food Products . Pub. By Van Nostrand Co., Inc.

Mitsouka, T. (1969). Vergleichende Untersuchungen über *Lactobazillen aus den faeces* von Menschen, Schweinen und Hühnern – Zb1. Bakt.1. Orig. ,210: 32-51

Nurmikko, V. (1964). Growth Chemistry of lactic acid bacteria. Final report on investigation of the growth- promoting factors of lactic acid bacteria, Finland

Parente, E. and E. A. Zottola (1991). Growth of Thermophilic starters in whey permeate media. J. Dairy. Sci., 74: 20 – 28.

Stanier, R.Y.; M. Doudoroff and E. A. Adelberg (1964). General Microbiology . 2d . Ed., MacMillan and Company Ltd., London WC2.

Zhang – Shaohui.; Pan-Daodang.; Ohashi-T.; Zhang-SH. and Pan-Do. (1997). Stimulatory effect of whey syrup on growth of lactic acid bacteria and lactic acid fermentation. Milk- Sci ., 46 (3): 183 – 188.

تأثيراستخدام الشرش المدعم ببعض العناصر الغذائية المتاحة ورخيصة الثمن كبيئة لنموبكتريا حامض اللاكتيك.

طه عبد الحليم نصيب و هانى موسى الجنايني

قسم الألبان – كلية الزراعة – جامعة المنصورة

تعتبر أفراد بكتريا حامض اللاكتيك من أكثر البكتريا تعقيدا في احتياجاتها الغذائية. و تعد البيئات المستخدمة سواء لعزل تلك البكتريا أو لإجراء العد عليها مرتفعة جدا في أسعارها و خاصة تلك البيئات الانتقائية (selective) والهدف الرئيسي للدراسة هو محاولة التوصل إلى مصادر متاحة ورخيصة للفيتامينات والأحماض الأمينية والأملاح المعدنية واختبارها سواء بصورة منفردة أو متحدة وذلك بغرض التوصل إلى تركيب بيئة لعزل وعد هذه البكتريا. وعلى ذلك فقد بذلت محاولة لتدعيم أجار الشرش كبيئته لبكتريا حامض اللاكتيك عن طريق زيادة قيمتها الغذائية بإضافة أي من البيتون أو أحد مستخلصات الخميرة، الطماطم، الجزر سواء بطريقة فردية أو في صورة مشتركة وذلك لتغطية احتياجات تلك البكتريا من الناحية الغذائية. وتشير النتائج إلى عدم نمو أي من تلك المزارع النقية من البكتريا على بيئة أجار الشرش (WA) في حين أن إضافة البيتون ومستخلص الجزر إلى أجار الشرش (WPC) نتج عنة أعداد اكبر لتلك المزارع البكتيرية مقارنة بالنمو على بيئة أجار الشرش المضاف إلية البيتون (WP). كما أن إضافة البيتون ومستخلص الطماطم (WPT) أدى إلى زيادة اكبر في معدل نمو تلك السلالات النقية وان أعلى الأرقام تم الحصول عليها كانت بالنسبة للنوع *Str.lactis*. في حين اقل الأعداد بالنسبة للنوع *L.casei*. كما أن مزرعة *L. bulgaricus* تماثلت على هذه البيئة مع *Str. lactis*. أمكن تقدير أعداد متقاربة بالنسبة لكل من النوعين *Str. cremoris* and *Str.thermophilus*. لوحظ كذلك زيادة أكبر في النمو بتدعيم أجار الشرش (WA) بالبيتون ومستخلص الخميرة (WPY). كما أن إضافة كل من البيتون ومستخلص الخميرة والجزر (WPYC) أدى إلى النمو بدرجة اغزر بالنسبة لجميع المزارع المختبرة. وأخيرا فان تدعيم أجار الشرش (WA) بالبيتون وكل من مستخلصات الخميرة والطماطم والجزر أدى إلى أعلى درجات التشجيع للنمو بالنسبة لجميع المزارع المختبرة لبكتريا حامض اللاكتيك.