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Development of New Culture Medium for Various Microorganisms

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Abstract: Hydrolyzed milk medium for growth of some lactic acid bacteria (*Streptococcus salivarius* subsp. *thermophilus* and *Lactococcus lactis*), *Bacillus subtilis* and yeast *Saccharomyces cerevisiae* was developed. In this study we have attempted to improve an alternative growth medium for these bacteria and yeast instead of their conventional media. The growth rates of these microorganisms in hydrolyzed milk medium had small amount lower than their conventional media in general. Therefore the investigation suggests that hydrolyzed milk medium can be used for microbiological and genetic studies.

Key Words: Hydrolyzed milk medium, bacteria, yeast, growth

Çeşitli Mikroorganizmalar İçin Yeni Kültür Ortamının Geliştirilmesi

Öz: Bazı laktik asit bakterilerinin (*Streptococcus salivarius* subsp. *thermophilus* ve *Lactococcus lactis*), *Bacillus subtilis* ve maya *Saccharomyces cerevisiae*'nin üretimi için hidrolize süt besi yeri geliştirilmiştir. Bu çalışmada, bu bakteri ve mayalar için geleneksel olarak kullanılan besi yerleri yerine alternatif bir üreme ortamı geliştirmeyi amaçladık. Genel olarak, geleneksel besi yerleri ile kıyaslandığında, bu mikroorganizmaların hidrolize süt besi yerinde üreme oranları az miktarda daha düşüktür. Bu nedenle bulunan sonuçlar hidrolize süt besi yerinin mikrobiyolojik ve genetik çalışmalarda kullanılabileceğini göstermiştir.

Anahtar Kelimeler: Hidrolize süt besi yeri, bakteri, maya, gelişme

Introduction

Culture media have been the mainstay of microbiology. The development of a new media, however, has been done in an empirical rather than scientific manner, and modern microbiologists are faced with the introduction of novel rapid techniques.

Milk contains 87.3% water (range of 85.5% - 88.7%), 3.9 % milkfat (range of 2.4% - 5.5%), 8.8% solids-not-fat (range of 7.9 - 10.0%; protein 3.25% (3/4 casein), lactose 4.6%, minerals 0.65% - Ca, P, citrate, Mg, K, Na, Zn, Cl, Fe, Cu, sulfate, bicarbonate, many others, acids 0.18% - citrate, formate, acetate, lactate, oxalate, enzymes - peroxidase, catalase, phosphatase, lipase, gases - oxygen, nitrogen, vitamins - A, C, D, thiamine, riboflavin, others) (Anonymous 2006).

Milk is an excellent medium for growing a variety of microorganisms. Natural milk, which has long been used as a medium for bacteriological growth, presents many points which detract greatly from its value in exact determinations. For several years there has been used in this laboratory artificial milk as a substitute for natural milk (Whittaker, 1910). Many microorganisms grow better in milk than nutrient broth.

The aim of the present study was to develop a new alternative culture media for some Lactic Acid Bacteria (LAB) (*Streptococcus salivarius* subsp. *thermophilus* and *Lactococcus lactis*), *Bacillus subtilis* and yeast *Saccharomyces cerevisiae*.

Material and Methods

Microorganism Strains and Conventional Growth Media: *S. salivarius* subsp*. thermophilus* FI8976 and *L. lactis* IL1403 strains were obtained from M. Sait EKİNCİ (University of Kahramanmaraş Sütcü Imam, Kahramanmaraş-Turkey). *B. subtilis* RSKK389 strain were obtained from BGSC (*Bacillus* Genetic Stock Centre, USA). Yeast *S. cerevisiae* was kindly provided by Jan Walker (Molecular and Cell Biology,

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Institute of Medical Sciences, University of Aberdeen, United Kingdom). Recombinant *S. salivarius* subsp. *thermophilus, L. lactis* IL1403 (Aşan 2002) and *S. cerevisiae* (not published) strains were created in previous study in Biotechnology and Genetic Laboratories, Department of Animal Science, Faculty of Agriculture, University of Çukurova.

S. salivarius subsp. *thermophilus* and *L. lactis* were cultured in M17+0.5% w/v glucose (pH 7.2) at 42ºC and 37ºC, respectively. *B. subtilis* strain was cultured in LB (Luria Bertani, pH 7.5) at 37ºC. *S. cerevisiae* cells were grown in YPD (1% yeast extract, 2% bacto peptone, 2% glucose) at 30ºC.

Preparation of Hydrolyzed Milk Medium: To prepare milk medium (MM), fat free milk powder was dissolved in distilled water (7% w/v) (Milk can be used instead of MM). To prepare hydrolyzed milk medium (HMM), commercial protease enzyme (1% w/v) was added into MM and stirring at room temperature for 30 minutes. The suspension was then centrifugated to remove precipitate from HMM.

HMM was adjusted to pH 7.2 and 0.5% w/v glucose was then added for Lactic Acid Bacteria (original and recombinant *S. salivarius* subsp. *thermophilus* and *L. lactis*). For *B. subtilis* strain HMM was only adjusted to pH 7.5. For original and recombinant *S. cerevisiae*, 2% w/v glucose and 1% w/v yeast extract were added into HMM and pH was not adjusted. All HMM were then sterilized.

Measurement of Growth Rate: The growth rate of bacteria and yeast strains was measured at OD_{600nm} by spectrophotometer at various hours (0, 12, 24, 36 and 48).

Results and Discussion

Results

In the present study, the growth of some LAB and yeast (*S. salivarius* subsp. *thermophilus* and *L. lactis*, *B. subtilis* and *S. cerevisiae*) in hydrolyzed milk medium (HMM) was measured and compared with their conventional laboratory media.

Figure 1 and 2 show the growth rate of original and recombinant *S. salivarius* subsp. *thermophilus* and *L. lactis* strains in the conventional medium (M17) and test medium (HMM). Our data indicated that the growth rate of the LAB in HMM was less than that in conventional medium.

Figure 3 shows the growth rate of *B. subtilis* in the conventional medium (LB) and HMM. The growth rate in HMM was lower than in conventional medium until 24 hour. However, the growth decreased in LB while it was stable in HMM with increasing culture time.

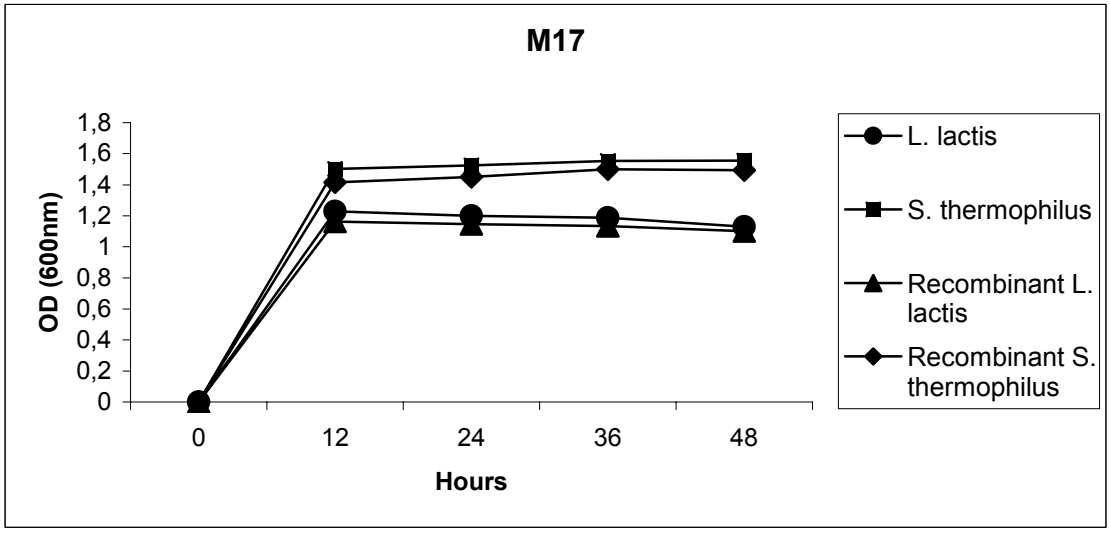

Figure1. The growth rate of some Lactic Acid Bacteria (original and recombinant *S. salivarius* subsp. *thermophilus* and *L. lactis*) in the conventional medium (M17)

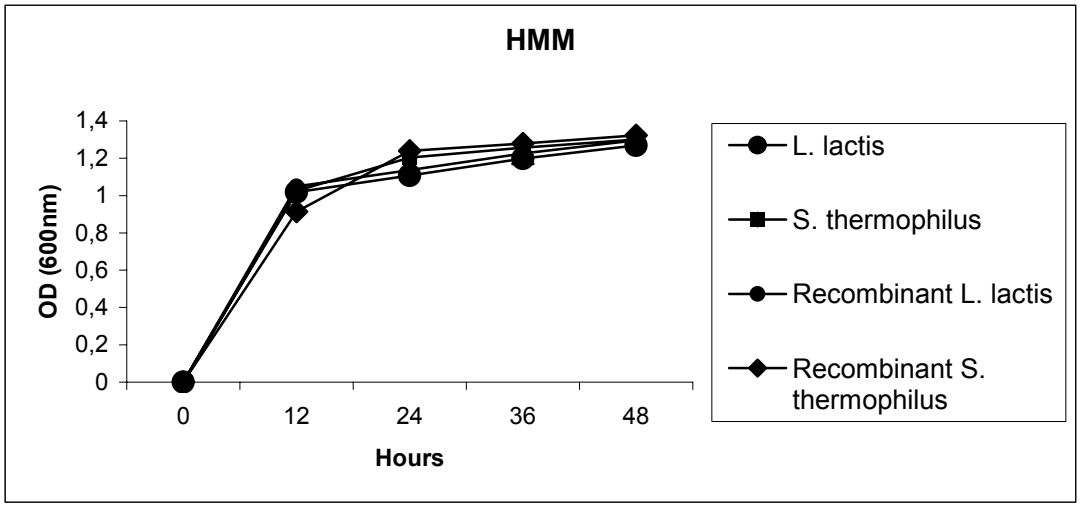

Figure2. The growth rate of some Lactic Acid Bacteria (original and recombinant *S. salivarius* subsp. *Thermophilus* and *L. lactis*) in the HMM medium

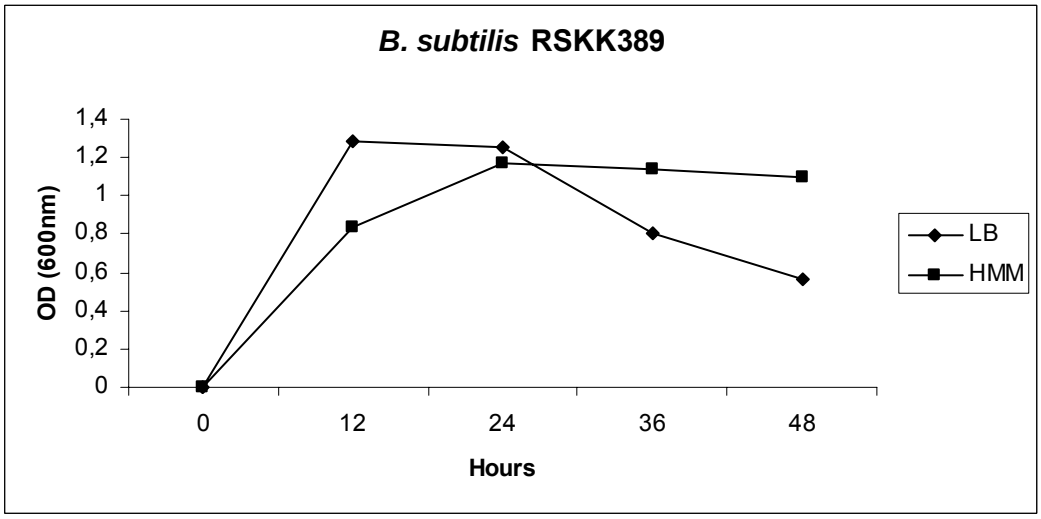

Figure3. The growth rate of *B. subtilis* in the conventional medium (LB) and HMM

Figure 4 and 5 show the growth rate of original and recombinant *S. cerevisiae* in the conventional medium (YPD) and test medium (HMM+2% glucose+1% yeast extract). Original and recombinant *S. cerevisiae* had lower growth rate in test medium with compared to conventional medium during culture time but at the end of the 48. hour, the difference in growth rate between test and conventional medium was decreased especially for recombinant *S. cerevisiae*.

Discussion

The objective of this research was to develop an alternative and practical medium for the growth of some LAB bacteria (*S. salivarius* subsp. *thermophilus* and *L. lactis*), *B. subtilis* and the yeast *S. cerevisiae*. These microorganisms are commonly used in microbiology and genetic laboratories for many researches.

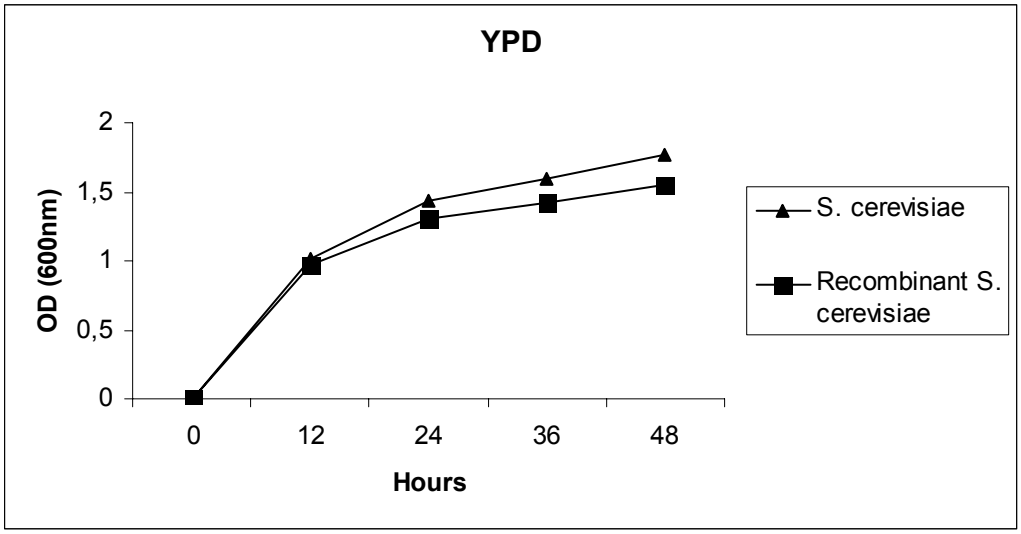

Figure4. The growth rate of original and recombinant *S. cerevisiae* in the conventional medium (YPD)

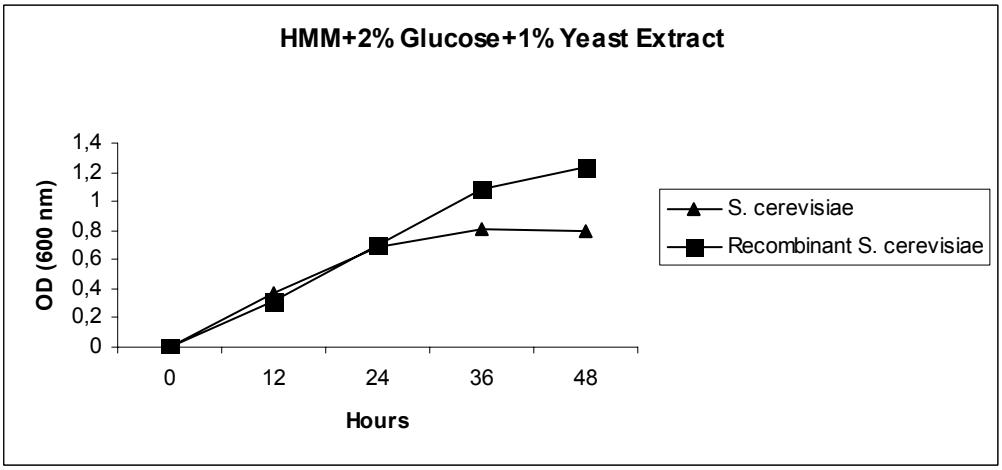

Figure5. The growth rate of original and recombinant *S. cerevisiae* in the HMM

Lactobacillus johnsonii and *L. gasseri* were grown in milk medium only when yeast extract was added (Avonts et al., 2004). The researchers indicated that the only strain of dairy origin among the seven *Lactobacillus* strains displayed the best growth in the milk medium. In other study, a new medium consisting of UHT milk, tween80 and agar was described for the development of both germ tube and chlamydoconidia by *Candida albicans* (Jitsurong et al., 1993). In comparison with the conventional medium, corn meal tween 80 agar, this medium gave a significantly higher percentage and chlamydoconidia production.

In the study, the growth rate of the bacteria and yeast in the HMM had lower than that in their conventional media but differences were small amount in general. Also, because of growing of recombinant bacteria and yeast in the HMM medium, the growth medium (HMM) may be used for developing recombinant microorganisms in biotechnological and genetic studies. On the other hand, HMM is easy to prepare and can be kept constantly on hand instead of their commercial growth media.

The results presented in this paper clearly indicated that hydrolyzed milk medium (HMM) can be used for growth of *S. salivarius* subsp. *thermophilus* and *L. lactis*, *B. subtilis* and *S. cerevisiae* instead of their conventional media.

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