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INFLUENCE OF POWDERED *CHLORELLA VULGARIS* ON ACID PRODUCTION, GROWTH, AND VIABILITY OF *ENTEROCOCCUS FAECIUM* IN MILK

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INTRODUCTION

Microalgae are photosynthetic microorganisms that can be used to produce high value compounds (Kreitlow et al., 1999). Spray-dried microalgal biomasses typically contain 3% to 7% moisture, 46% to 63% protein, 8% to 17% carbohydrates, 4% to 22% lipids, 2% to 4% nucleic acid, 7% to 10% ash, and a wide range of vitamins and other biologically active substances. Certain microalgae have been commercially produced for almost half a century now with the main species grown being *Chlorella* and *Spirulina* for health food (Borowitzka, 1999). *Chlorella vulgaris* is a green algal species that produces astaxanthin, canthaxanthin and, in minor amounts, β-carotene and luthein (Mendes et al., 2003). Particular microorganisms such as *Enterococcus faecium* have been increasingly used as probiotics in animal nutrition for nearly 20 years, and have been strictly regulated since 1993 (Vescovo et al., 1993; Becquet, 2003). *Enterococcus faecium* is significant in dairy manufacturing by having both beneficial and detrimental effects in products. Beneficial effects include desirable flavor enhancement, bacteriocin production, and probiotic impact, whereas detrimental effects include product spoilage (Flint, 2003). The main objective of this research was to study the effect of a dried *C. vulgaris* biomass, used at the rate of 3 g/L, on acid production and growth of a probiotic strain of *E. faecium* in milks with total solids contents ranging between 12% and 30%. Subsequently, it was also tested whether addition of the green algae biomass affected the survival rates of enterococci in a milk-based fermented feed stored at 4°C for 5 weeks.

MATERIALS AND METHODS

Both in the fermentation and production/storage trials, reconstituted skim milks were used as raw material, which were heated to 90°C and held for 10 min before being cooled to inoculation temperature. The freeze-dried starter culture of *E. faecium* was kindly supplied by the Department of Animal Nutrition, University of West Hungary (Mosonmagyaróvár, Hungary). Before the start of the trials, the strain was subcultured twice at 37°C for 24 h in Casein-peptone Soymeal-peptone (CASO) broth and Citrate Azide Tween® Carbonate (CATC) agar (Merck; Darmstadt, Germany). The *C. vulgaris* biomass was obtained from the Institute of Cereal Processing (Bergholz-Rehbrücke, Germany). Previous work (Springer et al., 1998) indicated that 3 g/L of microalgal biomass was optimal in regards to sensory properties and cost. The heat-treated and cooled *Chlorella*-supplemented and control milks were inoculated with *E. faecium* at the rate of 1%, corresponding to approximately 6.5×10^6 cfu/mL of milk, and were then incubated at 37°C.

During the fermentation trials, the pH value of three replicate samples from each treatment at each sampling time was measured with an HI 8521 pH-meter and combined glass electrode (Hanna Instruments, Karlsruhe, Germany). Viable cell counts of *E. faecium* were enumerated both in the fermentation and production/storage experiments with the standard pour-plate technique using CATC agar. The inoculated plates were incubated at 37°C for 24 h. The entire experimental program was repeated twice.

The influence of the *Chlorella* biomass on acid production, growth, and viability of *E. faecium* during the fermentation process and subsequent refrigerated storage was analyzed with the Student's *t*-test, by means of the STATISTICA data analysis software system, version 9.1 (StatSoft, Tulsa, OK, USA). Significance of difference was set at $P < 0.05$ in all cases.

RESULTS

The results of fermentation trials showed that acid development by and growth rate of *E. faecium* were stimulated significantly ($P < 0.05$) by the dried biomass of *C. vulgaris* in all culture media formulations tested (**TABLE 1 and 2**). Similarly, after 35 days of refrigerated storage, both the surviving numbers and viability percentages of *E. faecium* cells were found to be significantly higher ($P < 0.05$) in the *Chlorella*-enriched samples than in controls (**TABLE 3**).

TABLE 1: Effect of 3 g/L *Chlorella vulgaris* biomass on acid production* of *Enterococcus faecium* in milks with total solids contents ranging between 12% and 30%

Time (h)	Milk with							
	12% total solids		18% total solids		24% total solids		30% total solids	
	Control	*Chlorella*	Control	*Chlorella*	Control	*Chlorella*	Control	*Chlorella*
0	6.31 ± 0.07^a	6.31 ± 0.08^a	6.33 ± 0.06^a	6.33 ± 0.05^a	6.34 ± 0.07^a	6.34 ± 0.09^a	6.31 ± 0.08^a	6.31 ± 0.06^a
10	6.13 ± 0.08^a	5.64 ± 0.12^b	6.06 ± 0.09^a	5.20 ± 0.07^b	5.95 ± 0.06^a	5.15 ± 0.12^b	5.84 ± 0.06^a	5.23 ± 0.08^b
12	5.76 ± 0.07^a	5.37 ± 0.10^b	5.80 ± 0.08^a	4.92 ± 0.09^b	5.83 ± 0.09^a	4.91 ± 0.11^b	5.64 ± 0.07^a	4.89 ± 0.06^b
14	5.40 ± 0.09^a	5.04 ± 0.08^b	5.42 ± 0.08^a	4.55 ± 0.11^b	5.70 ± 0.08^a	4.50 ± 0.08^b	5.43 ± 0.09^a	4.55 ± 0.06^b
17	5.32 ± 0.06^a	4.44 ± 0.08^b	5.36 ± 0.06^a	4.48 ± 0.05^b	5.41 ± 0.07^a	4.19 ± 0.07^b	5.39 ± 0.09^a	4.52 ± 0.06^b
20	5.13 ± 0.07^a	4.10 ± 0.07^b	5.14 ± 0.08^a	4.16 ± 0.07^b	5.14 ± 0.06^a	4.07 ± 0.09^b	5.21 ± 0.10^a	4.51 ± 0.09^b
22	5.07 ± 0.05^a	4.04 ± 0.06^b	5.08 ± 0.05^a	4.06 ± 0.10^b	5.06 ± 0.08^a	3.92 ± 0.06^b	5.15 ± 0.06^a	4.49 ± 0.10^b

* Values are pH means ± SD based on 6 observations (3 samples, 2 replicates).
[a,b] Means bearing different superscript letters within a row in the same total solids subcolumns differ significantly ($P < 0.05$).

TABLE 2: Effect of 3 g/L *Chlorella vulgaris* biomass on growth* of *Enterococcus faecium* in milk with 12% total solids content

Time (h)	Control	*Chlorella*-enriched
	milk inoculated with *Enterococcus faecium*	
0	6.83 ± 0.10^a	6.93 ± 0.09^a
8	8.26 ± 0.09^b	8.66 ± 0.08^a
12	8.41 ± 0.11^b	8.96 ± 0.06^a
22	8.72 ± 0.10^b	9.08 ± 0.07^a

* Values are $\log_{10}$ CFU/mL means ± SD, based on 6 observations (3 samples, 2 replicates).
[a,b] Means bearing different superscript letters within a row differ significantly ($P < 0.05$).

TABLE 3: Viability of *Enterococcus faecalis* in *Chlorella vulgaris*-enriched fermented milk during refrigerated storage at 4°C

Storage time (d)	Control		*Chlorella*-enriched	
	$\log_{10}$ CFU/ml*	%	$\log_{10}$ CFU/ml*	%
0	9.18 ± 0.05^b	100.0	9.41 ± 0.11^a	100.0
7	9.28 ± 0.02^a	126.7	8.92 ± 0.03^b	32.3
14	8.51 ± 0.10^a	22.0	8.54 ± 0.08^a	13.5
21	9.29 ± 0.11^a	133.3	9.23 ± 0.04^a	65.4
28	$9.06 \pm 0.19^{a,b}$	80.0	9.10 ± 0.17^a	50.0
35	8.50 ± 0.13^b	21.3	9.13 ± 0.02^a	53.8

* Values are means ± SD, based on 6 observations (3 samples, 2 replicates).
[a,b] Means bearing different superscript letters within a row differ significantly ($P < 0.05$).

CONCLUSIONS

The beneficial effects of the *C. vulgaris* biomass on acid production, growth, and survival of *E. faecium* are of practical importance because, thus, shorter time is needed for the manufacture of the same amount of fermented feed and, consequently, productivity will increase in addition to an improvement in health-promoting capacity of the product. It is also worth mentioning that a rapid rate of acid production prevents the growth of undesirable microorganisms. All things considered, the powdered biomass of *C. vulgaris*, which is rich in biologically active substances, may be suitable for use in cost-effective production of dairy-based probiotic feeds fermented by *E. faecium*.

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