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EXPERIMENTAL ARTICLES

Fractions of Barley Spent Grain as Media for Growth of Probiotic Bacteria

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Abstract—The application of the protein and polysaccharide fractions of barley spent grain as a basis of growth media for probiotic bacteria was studied. High values of biomass yield, cell viability, and organic acid production were observed in the variants of media containing the barley spent grain supplemented with lactose, ascorbic acid, yeast extract, and mineral salts. Cells of lactic acid bacteria and bifidobacteria had the typical rod-shaped morphology.

Key words: bifidobacteria, lactic acid bacteria, fractions of barley spent grain, growth, acid formation, probiotics.

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At present, probiotics and prebiotics are the subjects of increasing interest to researchers. Lactic acid bacteria and bifidobacteria are known as probiotic microorganisms characterized by valuable functional and technological properties [1–4]. Technologies for probiotic production require not only active strains, but also low-cost media for their cultivation. Components of growth media act as sources of carbon, nitrogen, and phosphorus and can also have beneficial effects on human health. The products of grain processing are known to be efficient substrates for cultivation of lactic acid bacteria and bifidobacteria; they provide for high growth potential, metabolite production, and cell viability during a long-term storage [5–8]. These products can act as prebiotics which selectively stimulate the growth of lactic acid bacteria and bifidobacteria in the intestines. Cereal grains contain water-soluble carbohydrate polymers (β-glucan and arabinoxylan), oligosaccharides (galacto- and fructooligosaccharides), and water-insoluble polysaccharides (xylan, cellulose, and starch), which presumably act as prebiotics. Moreover, these dietary fibers can be included in the diet as sources of carbohydrates with multiple beneficial physiological effects [5]. Recently, food additives containing the species of *Lactobacillus* and *Bifidobacterium* and the products of cereal grain processing, which serve as prebiotic components, have appeared in the market. Germinated barley products and their polysaccharide fraction are known to prevent diarrhea and enteritis and can be used for prophylaxis of colitis [6].

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The barley spent grain containing the protein and polysaccharide fractions is a waste material obtained after removal of wort after brewing beer [9, 10]. Its protein and polysaccharide fractions are of growing interest as dietary additives for the treatment of dyspepsia and as alternative sources of protein and carbohydrates for animals [11, 12]. Earlier, we recommended the use of the protein fraction from the barley spent grain as the main component of the media for the isolation and cultivation of actinobacteria, production of biologically active substances, and intense sporulation [13].

The aim of this work was to study the physiological, biochemical, and morphological characteristics of lactic acid bacteria and bifidobacteria grown in media containing the protein and polysaccharide fractions of barley spent grain.

MATERIALS AND METHODS

Bacterial strains. The strain *Bifidobacterium adolescentis* 94 BIM was obtained from the Belarus Collection of Nonpathogenic Microorganisms (Institute of Microbiology, National Academy of Sciences of Belarus). The culture of lactic acid bacteria *Lactobacillus* sp. was isolated from commercial preparation of *Lactobacterinum siccum* (Immunopreparat, Russia).

Composition of media. The barley spent grain was separated into the coarse polysaccharide fraction (8-mesh size) and the fine protein fraction (50-mesh size) by sifting through a BoMill AB sieve (Sweden) [10, 13]. To prepare the medium, 2 g of an appropriate fraction was added per 100 ml of distilled water, mixed, and

Table 1. Composition of media

Medium designation	The main component of the medium	Additives, %
BHB	Brain heart broth	Lactose, 0.8; ascorbic acid, 0.05; ammonium acteate, 0.3; sodium acetate, 0.2; trisoduim citrate, 0.3; $MgSO_4 \times 7H_2O$, 0.012; $MnSO_4$, 0.005
PF-1	Protein fraction of the barley spent grain	No additives
PF-2	The same	Lactose, 1.0
PF-3	The same	Lactose, 1.0; ascorbic acid, 0.05
PF-4	The same	Lactose, 1.0; ascorbic acid, 0.05; yeast extract, 0.5
PF-5	The same	Lactose, 1.0; ascorbic acid, 0.05; yeast extract, 0.5; ammoium acetate, 0.3; sodium acetate, 0.2; trisodium citrate, 0.3; $MgSO_4 \times 7H_2O$, 0.012; $MnSO_4$, 0.005
FF-1	Polysaccharide fraction of the barley spent grain	No additives
FF-2	The same	Lactose, 1.0
FF-3	The same	Lactose, 1.0; ascorbic acid, 0.05
FF-4	The same	Lactose, 1.0; ascorbic acid, 0.05; yeast extract, 0.5
FF-5	The same	Lactose, 1.0; ascorbic acid, 0.05; yeast extract, 0.5; ammoium acetate, 0.3; sodium acetate, 0.2; trisodium citrate, 0.3; $MgSO_4 \times 7H_2O$, 0.012; $MnSO_4$, 0.005

Note: pH of the media was adjusted to 7.2; then the media were autoclaved at 121°C for 15 min; BHB, brain heart broth (Merck, Germany); fractions of the barley spent grain, PF-1and FF-1, were obtained as described in [13]; semisolid media BHB, PF-5, and FF-5 contained 0.2% agar.

autoclaved at 121°C for 15 min. The suspension was then cooled and filtered through a paper filter; pH was adjusted to 7.2 with 0.5 M NaOH. The composition of the media applied for investigation of growth, acetic acid production, and morphology of bifidobacteria and lactic acid bacteria is presented in Table 1.

Cultivation of bacteria. A cell suspension (4.5 ml) containing 10^9cells/ml was inoculated into 100-ml flasks with 90 ml of the medium. Bacteria were cultivated at 37°C for 24–48 h under static conditions. The number of viable cells was determined by plating serial dilutions onto a selective semisolid thioglycollate medium with 0.2% agar [14]. The results were expressed in colony-forming units (CFU) per 1 ml of cell suspension. To characterize the colonial growth of lactic acid bacteria and bifidobacteria, tenfold dilutions of culture suspensions (10^{-6}–10^{-8}) were plated onto semisolid media containing protein or polysaccharide fractions of the barley spent grain.

The biomass was determined by measuring the optical density of bacterial suspensions at 590 nm; pH was analyzed as described earlier [14].

Fatty acid formation. Acetate was analyzed by gas chromatography on an HP-FFAP column at 80–140°C; the injector temperature was 180°C; the temperature of the flame ionization detector was 260°C. Nitrogen carrier gas flow was 67 ml/min. After filtration of the sample, an aliquot (0.9 ml) was mixed with 0.1 ml of 10% phosphoric acid; 1 μl of this mixture was injected into the column in a pulse mode. The concentration of lactic acid was determined by the Lauer method [15].

Cell morphology. Cell morphology of probiotic bacteria was studied as described earlier [3]. Specimens were examined under a LABORHOT-2 microscope (Nicon, Japan) at a magnification of 1000×.

RESULTS

The media used in this study contained either the brain heart broth medium (BHB, Merck, Germany) [16] or protein and polysaccharide fractions of the barley spent grain (Table 1). Characteristics of the BHB medium are comparable with those of the media based on non-fat milk that are usually recommended for the cultivation of lactic acid bacteria and bifidobacteria. Except for the PF1 and FF1 media, all the other media were supplemented with various additives. The criteria used to assess the culture growth (cell viability, biomass yield, production of organic acids, and pH changes in the course of cell cultivation) are given in Tables 2 and 3. The media containing the polysaccharide and protein fractions of the barley spent grain without any additives were suitable for the cultivation of bifidobacteria and lactic acid bacteria. The addition of yeast extract to PF4 and FF4 media resulted in better growth of bifidobacteria, biomass yield, and acetate formation as compared with those parameters in the case of PF3 and FF3 media. Supplementation of the media containing protein or polysaccharide fractions of the barley spent grain with lactose, ascorbic acid, and mineral salts was favorable for the growth of bacteria. The media BHB, PF5, and FF5 supported the highest cell growth and the maximum biomass yield. An increase in

Table 2. Growth of bifidobacteria and acid formation in the media containing the fractions of barley spent grain

	Cell viability, CFU/ml		*OD,* 590 nm		Biomass, mg/ml		pH		Acetic acid, mg/l	Lactic acid, mg/l
Medium	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	24 h
BHB	5×10^9	5×10^9	0.79	0.79	1.20	1.20	4.55	4.55	11580	7720
PF-1	2×10^8	4×10^8	0.24	0.28	0.37	0.42	4.75	4.71	820	547
PF-2	1×10^9	2×10^9	0.38	0.40	0.57	0.6	4.02	4.03	1360	907
PF-3	1×10^9	3×10^9	0.37	0.41	0.56	0.62	4.05	4.01	2470	1647
PF-4	3×10^9	4×10^9	0.53	0.56	0.81	0.85	4.01	4.00	3215	2143
PF-5	6×10^9	5×10^9	0.82	0.85	1.24	1.29	4.55	4.56	5180	3453
FF-1	8×10^7	9×10^7	0.12	0.13	0.18	0.20	4.52	4.43	1415	943
FF-2	5×10^8	8×10^8	0.25	0.26	0.37	0.39	4.01	4.01	2890	1927
FF-3	5×10^8	9×10^8	0.25	0.30	0.37	0.46	4.02	4.01	3090	2060
FF-4	1×10^9	2×10^9	0.44	0.49	0.66	0.74	4.01	4.01	3690	2460
FF-5	2×10^9	3×10^9	0.73	0.73	1.10	1.10	4.02	4.01	5080	3387

Note: Designations of the abbreviations as in Table 1; the formation of acetate in acetate-containing media (Table 1) was calculated by subtracting its initial concentration; the production of acetic and lactic acids was determined after 24 h of cell cultivation.

Table 3. Growth of lactic acid bacteria and acid formation in the media containing the fractions of barley spent grain

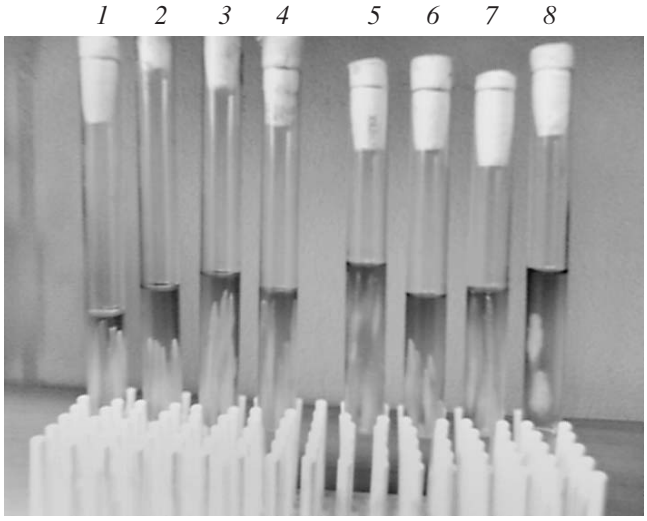
	Cell viability, CFU/ml		*OD*, 590 nm		Biomass, mg/ml		pH		Acetic acid, mg/l
Medium	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h
BHB	5×10^9	6×10^9	0.76	0.82	1.20	1.30	4.64	4.61	7940
PF-1	2×10^8	2×10^8	0.27	0.27	0.42	0.42	4.87	4.84	890
PF-2	1×10^9	3×10^9	0.34	0.38	0.53	0.60	4.21	4.03	950
PF-3	1×10^9	3×10^9	0.35	0.38	0.56	0.60	4.25	4.01	1065
PF-4	3×10^9	4×10^9	0.56	0.58	0.88	0.92	4.01	4.00	1125
PF-5	4×10^9	5×10^9	0.76	0.77	1.20	1.21	4.55	4.53	6260
FF-1	2×10^7	3×10^7	0.08	0.09	0.13	0.14	4.65	4.62	890
FF-2	4×10^8	5×10^8	0.14	0.20	0.23	0.31	4.01	4.01	890
FF-3	5×10^8	6×10^8	0.16	0.21	0.25	0.34	4.02	4.01	1085
FF-4	1×10^9	2×10^9	0.43	0.43	0.68	0.68	4.01	4.01	1285
FF-5	3×10^9	3×10^9	0.55	0.57	0.87	0.88	4.02	4.01	6160

Note: Designations of the abbreviations as in Table 1; the formation of acetate in acetate-containing media (Table 1) was calculated by subtracting its initial concentration; the production of acetic acid was determined after 24 h of cell cultivation.

the cell growth was accompanied by enhanced acid formation. In the case of bifidobacteria, the concentration of acetic acid was the highest (11580 mg/l) in BHB medium and amounted to 5080 and 5180 mg/l in FF5 and PF5 media, respectively. Acid production remained unaltered after cell cultivation for 24 or 48 h. To study cell and colony morphology, bifidobacteria and lactic acid bacteria were grown on semisolid PF5 and FF5 media. As seen from the Figure, bacteria formed large cream-white colonies of different morphology. Bifidobacteria were represented by rods branched at the ends; after cell division, typical polymorphic daughter cells detached. Lactobacilli under study consisted of typical rod-shaped cells. The experimental data revealed good growth of bacteria on the media tested and, consequently, their good adaptation to new media.

DISCUSSION

Formulation of media with protein and carbohydrate components easily utilized by bifidobacteria and lactic acid bacteria, which will allow for the recycling of a waste product, is of great importance for the development of probiotic production. A waste product of brew-



Growth of bifidobacteria (tubes *1–4*) and lactic acid bacteria (tubes *5–8*) on semisolid medium PF-5.

ing, barley spent grain, is known to contain proteins, lipids, and poorly soluble polysaccharides with the properties of fiber. These components of barley spent grain have traditionally been used as additives to ruminant fodder and components of bakery products. Protein content of the barley spent grain varies from 8 to 55% depending on the initial protein level in the barley and subsequent processes of malting and wort preparation [9, 10, 13–25]. The protein fraction is mainly composed of hydrophobic peptides and proteins, which are poorly soluble in water. It is known that some species of *Bifidobacterium* and *Lactobacillus* are able to produce extracellular proteinases, which allow the cells to utilize casein, albumin, and some immunoglobulins [14]. Probiotic bacteria were shown to decompose difficultle hydrolysable protein compounds [25]. These bacteria were characterized by the synthesis of proteolytic enzymes, which break peptide bonds between an amino acid and proline or glutamic acid in the P1 position. Our results demonstrated the production of similar enzymes by the lactic acid bacteria and bifidobacteria under study [17]. These results agree well with the data on the use of media containing the protein fraction of the barley spent grain for the isolation of *Xanthomonas* sp., which produced proline-specific endopeptidase [17].

The results obtained in this study indicate that the protein and polysaccharide fractions of barley spent grain can be used as components of media for cultivation of probiotic bacteria. Since both fractions are suitable for bacterial growth, ungraded barley spent grain can also be applied for probiotic production. The fraction containing poorly soluble polysaccharides (food fibers) supplemented with the protein from yeast extract can be used as a food additive for prophylaxis of diarrhea and colitis [6]. The media tested supported active bacterial growth, high biomass accumulation, and formation of organic acids that offer possibilities to use barley spent grain to refine the technology for low-cost probiotic production. Thus the biomass of probiotic bacteria can be a promising source of immunostimulant components, such as polysaccharides and glycolipids for vaccine production. A method for the isolation of immunostimulant substances with the use of liquefied carbon dioxide has recently been devised [18]. The results obtained can be used for the development of low-cost production of a whole complex of preparations, including probiotics, prebiotics, and vaccines. Moreover, the use of brewing wastes may be an integral part of a closed cycle of utilizing industrial bioresources [19–24].

To conclude, the results of this work can be used for further studies of the mechanisms of the prebiotic and probiotic activity of components of cereals, lactic acid bacteria, and bifidobacteria, which serve as promising biotherapeutic agents.

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