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Original scientific paper

BETA-GLUCANS FROM BIOMASS OF PLANT AND MICROBIAL ORIGIN

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ABSTRACT:
The aim of the present study is to explore the transformation of (1→3)(1→4)-β-D-glucans of rye biomass by *Aspergills niger* and accumulation of (1→3)(1→6)-β-D-glucans in the microbial cell wall.
Biomass from rye grain was obtained as a result of enzymatic hydrolysis of grain grinding of Omsk region of non-standard quality with grain impurity content of 45 ± 2 % by preparations (1→4)-β-glucanolytic, (1→3)-β-glucanolytic, (1→4)-xylanolytic and (1→4)-amylolytic action. Fermentation of hydrolysates, sucrose-mineral and molasses medium by *A. niger* was carried out by a batch process under aerobic conditions. Determined the content of β-glucans, amino-nitrogen, glucose, disaccharides in grinding grain rye, rye biomass, the biomass of *A. niger*, the supernatants by colorimetric methods. Determination of chitin in biomass and qualitative determination of chitosan in supernatants of hydrolysates was carried out using chitosan sulfate sample and subsequent microscopy.
The results of the research showed that (1→3)(1→4)-β-D-glucans in grain grinding are 10.2 ± 0.2 % in terms of dry matter, which exceeds the content of polysaccharide in the grain of standard quality by 1.5 – 3 times. In rye biomass revealed their smaller amount, 6.4 ± 0.5 %, apparently, due to the action of (1→4)- and (1→3)-β-glucanase, (1→4)-xylanase and (1→4)-amylase. In microbial mass *A. niger* content of (1→3)(1→6)-β-D-glucans were at the level of 21.7 ± 0.7 %.
On the basis of the obtained results, it was concluded that it is possible to use rye grain of non-standard quality, with a high content of grain impurities and a low proportion of starch polysaccharides, as a source of β-glucan-containing substrate for biosynthesis (1→3)(1→6)-β-D-glucans by *A. niger* having advantages over (1→3)(1→4)-β-D-glucans of plant origin. They are functionally more active and have a wide range of applications, namely as food additives in the manufacture of a wide range of products: for the enrichment of fibers, increasing the shelf life of products due to its water-binding properties, as thickeners, emulsifying and fat-reducing micro-ingredients, stabilizers of creamy emulsions, textureformers, flavor enhancers.

1. Introduction

Traditional raw materials for food micro-ingredient technologies are plant polymers and agricultural wastes containing them. The peculiarity of agricultural raw materials in the Russian Federation is strictly seasonal nature of production and often – not standard quality. There are difficulties with his delivery in regards to the remote location of the manufacturer. A cost-effective alternative is raw materials of microbiological origin, namely the biomass of industrial producers.

Biomass as a waste of the technological cycle can be used as a raw material for the production of a number of food micro-ingredients in one technological process, which increases the profitability of production as a whole [1,2,3]. Microbial mass is a source of important food micro-ingredients, the production of which is absent or developing in the Russian Federation. Among them are the technologies of dietary fibers, the raw material base for which in the Russian Federation is represented mainly by beet pectin and pectin-cellulose derivatives, dietary fiber from wheat and other natural raw materials [4,5,6,7].

The peculiarity of polymer fibers from the cell wall of the microbial mass is their chemical structure, represented mainly by the active form of glucans, namely (1→3)(1→6)-β-D-glucans. In other natural sources, such as cereals (oats, barley, rye) or, for example, chitin-glucan complex (HGC) in the crab shell, higher fungi, there are 1→4-forms that are functionally less active [8,9,10].

The aim of the present study is – based on the principles of biocatalysis of plant and microbial biomass to study the transformation of β-glucans of rye by *A. niger* to predict the composition of microbial glucan-containing polymers – potential food micro-ingredient.

2. Materials and methods

The object of the study was:

• crushed (grinding, particle size 750 ± 50 microns) grain rye winter Omsk region of non-standard quality, namely, does not meet the requirements for "mass fraction of moisture" – 17 ± 2 % (more than 14 %), "weed admixture" – 8 ± 2 % (more than 5 %), "grain admixture" – mostly sprouted grains and grains of other crops 45 ± 2 % (more than 15 %), with a starch content below 50 %;

• biomass from rye grain obtained as a result of enzymatic hydrolysis of grinding with preparations (1→4)-β-glucanolytic, (1→3)-β-glucanolytic, (1→4)-xylanolytic and (1→4)-amylolytic action;

• biomass *A. niger* L-4 obtained in laboratory conditions during fermentation of rye grain biomass hydrolysate [11].

For Biocatalysis used:

• Cellovyridin G3X ("SIBBIOFARM", Russia), standardized for cellulose activity 50 units CA/g and containing enzymes synthesized by the culture *Trichoderma viride*, cellulase (1,4-β-D-glucan-glucanohydrolase, KE 3.2.1.4), exo-glucanase and endo-glucanase (1,4-β-D-glucan-glucanohydrolase, KE 3.2.1.74) and cellobiase (exo-1,4-β-D-glucocerebrosidase, KE 3.2.1.91), xylanase (endo-1,4-xylanase (3.2.1.8 KE)), for the hydrolysis of colloidal compounds of pentosans and hexosans, pH = 3.9 ± 0.1; t = 37 ± 2 °C, τ = 60 min, a dosage of 2 units CA/g of dry matter (d. m.) [11];

• Amilosubtilin G3X ("SIBBIOFARM", Russia), standardized for amylase activity 1500 units AU/g and containing enzymes synthesized by the culture *Bacillus subtilis*, namely α-amylase (diastase, α-1.4-D-glucanohydrolase, KE 3.2.1.1), xylanase and cellulase catalyses the hydrolysis of difficultly digestible polysaccharides of the microorganisms at pH = 6.5 ± 0.1, t = 92 ± 2 °C, τ = 60 min, the dosage of 1 units AA/g [11];

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• preparation of β-glucanase (endo-(1→3)(1→4)-β-glucanase) *Trichoderma longibrachiatum* (Sigma-Aldrich, USA; 3100 units β-GlA/g), which has the specificity of action on β-glucans of the cell wall of mycelial fungi, pH = 4.7 ± 0.1; t = 48 ± 2 °C, τ = 60 min, dosage 50 units β-GlA/g.

Fermentation of carbohydrate-containing substrates by *A. niger* was carried out under the conditions of shaker - incubator Multitron (INFORS, Switzerland): capacity of rocking flasks 750 ml, periodic method, aerobic conditions with stirring 160 – 220 rpm, t = 32 ± 1 °C, τ = 120 h [11 – 14].

The composition of the medium, g/L: carbohydrate substrate – 130 (in terms of sugar); ammonium nitrate – from 1.0 to 2.5 (depending on the carbon source); magnesium sulfate – 0.25; potassium phosphate monosubstituted – 0.16 [11,12,13,14].

Determined the content of β-glucans, amino-nitrogen, glucose, disaccharides in grinding grain rye, rye biomass, the biomass of *A. niger*, the supernatants by colorimetric methods [15]. Determination of chitin in biomass and qualitative determination of chitosan in supernatants of hydrolysates was carried out using chitosan sulfate sample and subsequent microscopy [15]. The results were processed using the program Origin 61 ($p \leqslant 0.05$).

3. Results

The results of studies have shown that the content of (1→3)(1→4)-β-D-glucans in grain grinding and biomass from rye grain exceeds the content of polysaccharide in grinding of standard quality grain and its biomass by 1.5 – 3 times (Table 1,2).

Table 1.

β-Glucan content in plant and microbial sources

Name source's β-glucan	Name of enzyme preparation	Dosage of enzyme preparation, u/g	Mass fraction β-glucans, % d.m.
Standard quality rye grain grinding	–	0	3.7 ± 0.5
Rye grain grinding not of standard quality	–	0	10.2 ± 0.2
Biomass from grain ryes standard quality	Celloviridin G3X	2	6.4 ± 0.5
	β-glucanase Amylosubtilin G3X	0	
		1	
	Celloviridin G3X	2	5.4 ± 0.2
	β-glucanase Amylosubtilin G3X	50	
		1	
Biomass from grain ryes not standard quality	Celloviridin G3X	2	7.2 ± 0.2
	β-glucanase Amylosubtilin G3X	0	
		1	
	Celloviridin G3X	2	6.5 ± 0.2
	β-glucanase Amylosubtilin G3X	50	
		1	
Biomass of *A. niger* L-4: – standard raw material qualities	–	0	20.4 ± 0.3
– raw is not a standard qualities	–	0	21.7 ± 0.7

The grain revealed a smaller number of insoluble forms of glucans, apparently, due to the action of (1→4)- and (1→3)-β-glucanase, (1→4)-xylanase and (1→4)-amylase during enzymatic hydrolysis of rye grain grinding. In the liquid fraction of the hydrolyzed mass, the proportion of soluble forms of glucans was 2.8 – 4.6 %.

In the microbial mass *A. niger* L-4 during fermentation of biomass from grain of both standard and non-standard quality, the content of β-glucans was at the same level and was 3 – 3.6 times higher compared to the substrate, that is, grain biomass.

The results of enzymatic hydrolysis of biomass *A. niger* and HGC by β-glucanase *T. longibrachiatum* showed that the amount of β-glucan in biomass hydrolysate was 5.1 and 1.6 times higher compared, respectively, with the biomass hydrolysates obtained during fermentation of sucrose-mineral and molasses media (Table 2).

Table 2.

Results of enzymatic hydrolysis of biomass A. niger and HGC by β-glucanase *T. longibrachiatum*

Source name β-glucan, the enzymatic environment of the carb	Carbohydrate content, %			Chitin content, %
	glucose	disaccharides	β-glucans	
Biomass hydrolysate – starch-containing medium	not founded	31 ± 1	36 ± 2	8 ± 1
– sucrose-mineral medium		45 ± 3	7 ± 1	20 ± 1
– molasses medium		34 ± 3	20 ± 1	32 ± 1
Hydrolysate HGC – starch-containing medium	15 ± 1	22 ± 2	50 ± 1	17 ± 1
– sucrose-mineral medium	15 ± 1	42 ± 1	41 ± 2	68 ± 1
– molasses medium	14 ± 1	51 ± 1	34 ± 1	72 ± 1

The level of the indicator for the hydrolysate HCG also decreased in a number of carbon sources: starch → crystalline sugar → beet molasses.

The content of chitin in the residual, non-hydrolyzed mass was significantly lower and, apparently, due to the greater susceptibility of chitin chains of the acidic medium (pH = 4.7 ± 0.1) during enzymatic hydrolysis to transformation into a soluble form – chitosan.

The mass fraction of amine nitrogen in the supernatants of hydrolysates was within 2 – 3 %. Glucose in the hydrolysates of microbial biomass is not detected. In HGC, compared with biomass *A. niger*, the amount of glucose increased due to hydrolysis of the more accessible glucan chain.

Determination of chitin in biomass and qualitative determination of chitosan in supernatants of hydrolysates was carried out using chitosan sulfate sample and subsequent microscopy. It was founded that crystals forms are different and depend of carbon source kind using on fermentation stage (Figure. 1, 2).

4. Discussion

It is known from the literature that the final formation of the polymer framework of the cell wall falls on the final stage of physiological development of the microorganism, often associated with the stationary phase of the biotechnological process [15,16,17,18]. The above experimental data were obtained for biomass after 120 hours of cultivation of *A. niger*

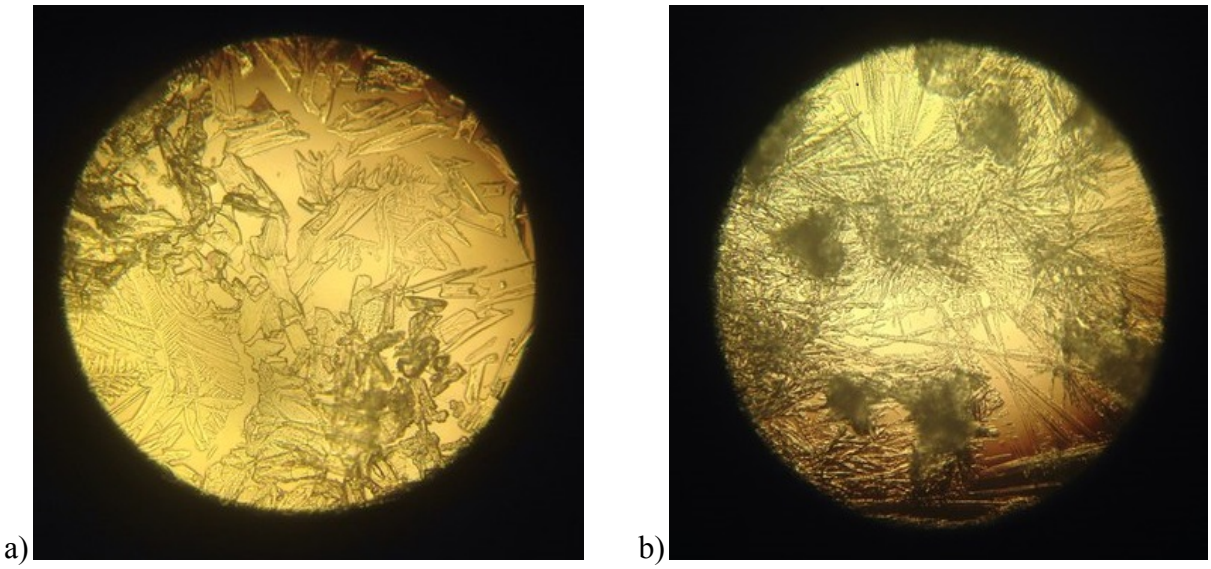

Figure 1. HGC after the action of β-glucanase *T. longibrachiatum* (10x10 magnification)
(a) HGC from biomass during fermentation molasses medium;
(b) – HGC from biomass during fermentation sucrose-mineral medium

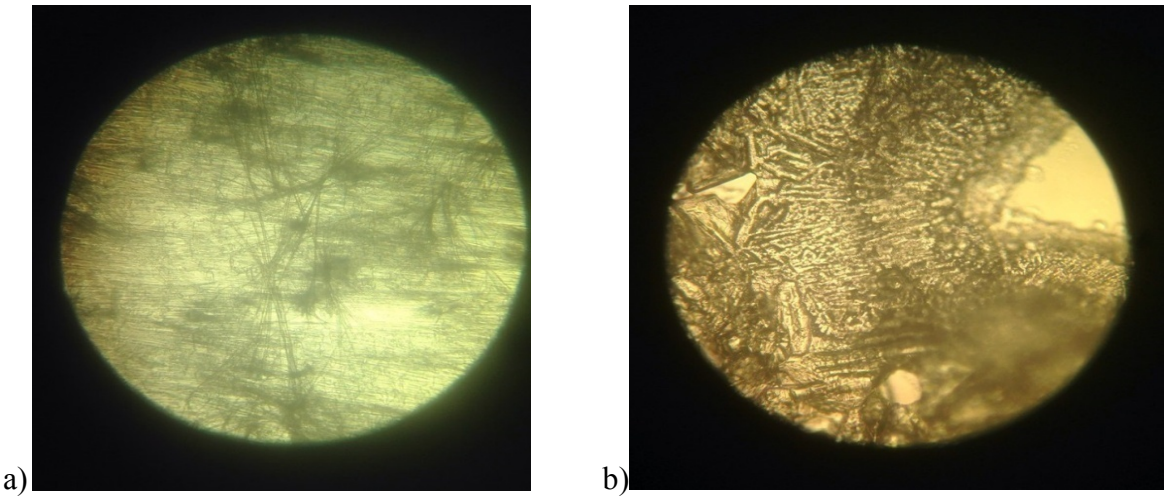

Figure 2. HGC after the action of β-glucanase T. longibrachiatum (10x10 magnification)
(a) – HGC from biomass during fermentation of starch hydrolysate;
(b) – HGC from biomass during fermentation of biomass from rye grain

L-4. It is possible that at an earlier stage of the process the content of β-glucans is different and, accordingly, the ratio of glucan and chitin components of HGC, occupying 40 – 50 % of the dry matter of the cell wall, also changes. Earlier studies have shown that successive alkaline and acidic effects on *Aspergillus* biomass lead to deproteinization, deacetylation and ensure the availability of chitin and glucan copolymers of HGC for enzymes with different specificity of action [19]. Comparatively, exposure to endo-(1→3)(1→4)-β-glucanase *T. Longibrachiatum*, which has the specificity of action to non-starch polysaccharides (hemicelluloses and cellulose cleavage products), is more pronounced in HGC from biomass obtained during fermentation of starch-containing medium.

The presence of amino groups in the supernatants of hydrolysates confirms the assumption about the transition of chitin aminopolysaccharide to chitosan containing glucosamine. The absence of glucose in microbial biomass hydrolysates seems to be associated with simultaneous hydrolysis and transglycosylation reactions with β-glucanase.

5. Conclusion

Based on the experimental data obtained, a conclusion was made about the influence of the nature and structure of the carbon source on the formation of glucan copolymers of HGC in the cell wall. β-D-glucans of rye biomass containing (1→3)(1→4)-the bonds are exposed to (1→4)- and (1→3)-β-glucanase, (1→4)-xylanase and (1→4)-amylase of microbial origin. Accumulation of β-D-glucans containing (1→3)(1→6)-in the microbial cell wall of the *A. niger* in the stationary phase of growth and development varies quantitatively depending on the raw material (biomass from rye grains of standard and non-standard quality, starch, crystalline sugar, beet molasses).

Features of the chemical structure of polymer fibers from the cell wall of the microbial mass, namely the presence of β-1,3/1,6-bonds, indicates the active form of glucans of biomass *A. niger*. This property distinguishes microbial glucans from glucans from other natural sources, such as cereals, in particular, rye, or HGC in the shell of crab, higher fungi. They contain 1,4-forms, which are functionally inactive. Most methods for producing various forms of β-glucans are multistage, using organic solvents, concentrated acids and alkalis and the yield is often low (15 – 30 %).

Therefore, it is necessary to develop simpler and more effective isolation schemes, in particular on the basis of biocatalysts techniques.

Due to the multiple forms of β-glucans, the choice of identification methods is important.

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