

USE OF TEA FUNGUS ISOLATE AS STARTER CULTURE FOR OBTAINING OF KOMBUCHA

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ABSTRACT

Kombucha is a beverage with special therapeutic properties produced by metabolic activity of yeasts (*Saccharomyces sp.*, *Zygosaccharomyces sp.*, *Schizosaccharomyces sp.*, *Torulopsis sp.*, *Pichia sp.*) and acetic acid bacteria (*Acetobacter xylinum*, *Acetobacter aceti*, *Gluconobacter oxydans*). The fermentation is traditionally carried out by inoculating a previously grown culture (as cellulosic pellicle or fermentation broth) into a freshly prepared tea decoction and incubated statically under aerobic conditions for 7-10 days.

The aim of this paper was the use of yeasts and acetic acid bacteria culture isolates from local tea fungus as starter culture. During process of fermentation in liquid broth was determined: pH value, total organic acid, number of yeasts and acetic acid bacteria.

Also, carbohydrates in fermentation broth was tested by TLC. The investigation results confirmed that isolated strains of yeasts and acetic acid bacteria from tea fungus may be used as starter cultures for the obtaining of kombucha. The fermentation was faster in medium inoculated with fermentation broth compared to the fermentation with starter cultures. The fermentation time is dependent on initial count of yeast cells.

KEYWORDS:

kombucha, tea fungus, yeast, acetic acid bacteria, starter culture

1. INTRODUCTION

Kombucha is a sour, slightly sparkling beverage prepared by fermentation of sweetened black tea with tea fungus. The beverage has been claimed to be a prophylactic agent and to be beneficial to human health – as a diuretic in edemas, in arterosclerosis, in case of gout, sluggish bowels, for stones, etc. (1, 2, 3).

Experience has also shown the kombucha beverage to regulate the intestinal flora, strengthen the cells, harmonize the metabolism, act as a natural antibiotic and help maintain the pH e.g. the acid-alkaline balance of the body (3). However, many of this remains to be proved.

The consumption of kombucha was first practiced in 220 B.C. in Manchuria. It, then, spread to Russia where kombucha is called teakwas. This beverage was introduced into Germany during W. W. II, in the 50's arrived into France and France-dominated North Africa (2). Presently, kombucha is popular in the United States, due to its refreshing power and curative effects.

The tea fungus is a symbiosis of acetic acid bacteria (*Acetobacter xylinum*, *Acetobacter aceti*, *Acetobacter pasteurianus*, *Gluconobacter oxydans*) (Greenwalt, 2000) and yeast (*Saccharomyces sp.*, *Zygosaccharomyces sp.*, *Torulopsis sp.*, *Pichia sp.*, *Brettanomyces sp.* (5, 6, 7). The yeasts ferment the sugar in the cultivation medium to ethanol, which is further oxidised by the acetic acid bacteria to produce acetic acid. The result is reduced pH of medium. Besides acetic acid, the fermented liquid contains gluconic, glucuronic and lactic acid. Glucuronic acid is the main therapeutic agent in Kombucha, as a detoxification agent (Frank, 1995, Loncar 2000). Many flavour compounds, including alcohols, aldehydes, ketones, esters and amino acids have been identified.

Kombucha is traditionally prepared by fermentation of sweetened (sucrose) black tea. This medium (freshly prepared medium) is usually inoculated with cellulose pellicle formed during the previous cultivation and incubated statically under aerobic conditions for 7-10 days.

The media for tea fungus cultivation may be also successfully inoculated with fermented liquid from previous fermentation, where the concentration of cells is generally higher than those in cellulosic pellicles (6).

The aim of this work was to investigate the possible use of isolated strains of bacteria and yeasts from the local tea fungus as the starter culture for the obtaining of kombucha beverage. The justification of use of pure cultures for the obtaining of kombucha beverage is based on a number of positive experience with starter cultures, which were used for the obtaining of different food products of advanced quality.

Generally, the starter cultures are preparations containing a great number of physiologically active microorganisms, which are added to substrates to achieve desired changes. The starters, adapted to the medium, should increase the fermentation rate. Their use enables the strict control of fermentation process, hereby the predicting of product quality.

2. MATERIALS AND METHODS

2.1. Cultural conditions of the tea fungus

Tap water containing 70 g/l sucrose was boiled and 0,5 g/l echinacea was added. After 15 minutes the medium was filtered. 3 l of sweetened echinacea tea was poured into 5 l glass vessels. The inoculum was added to the cooled medium at about 30°C. The medium were incubated statically, under aerobic conditions, at 28°C.

2.2. Preparation of starter cultures for inoculation

Three yeast strains, isolated from the local tea fungus, were used for the inoculation of sweetened echinacea herba tea, and marked as 2/1, 5/3 and 7/2. The strains are kept in the collection of cultures of Department of microbiology, in the refrigerator on Novi sladni agar (10). The activation of yeast isolates of tea fungus was performed by double passage, the first on slant Sabouraud saccharose agar, and the second on the same medium in Cole's bottles. After the second passage of yeast isolates of tea fungus, the cells were suspended in sterile physiological solution.

The acetic acid bacteria isolates of tea fungus, marked as BSV 5 and BSV 9 are kept at refrigerator temperature, on a slant YMP. The mentioned strains were double passaged for physiological activation, on DeCarr medium in 300 ml Erlenmayer flasks.

2.3. Microbiological analyses

The total count of yeast cells was determined by CFU method on Novi sladni agar nutritive medium with the addition of chloramphenicol. The plates were incubated at 28°C, for 72 hours.

The determination of total count of acetic acid bacteria by CFU method was performed on YPM medium (11), where actidion (cykloheximid), as antimycotic, was added just before the pouring. The medium was incubated at 28°C, for 120 hours at least.

2.4. Chemical analyses

The pH value of fermented liquid samples was determined by electronic pH-meter (HI 9321).

The total acidity of fermented beverage samples was determined by potentiometric titration with NaOH, $c = 0,1 \text{ mol/l}$, after the removal of CO_2 (12).

The qualitative analysis of carbohydrates in the fermented medium was performed by TLC method on silica gel. Solvent mixture chloroform-acetic acid – water (3 : 6 : 1) was used for double developing of chromatograms.

3. RESULTS AND DISCUSSION

The previous investigations of the same authors showed that tea fungus can be cultivated on sweetened echinacea tea (*E. purpurea*). However, using this medium for the same cultivation time, significantly sourer kombucha is obtained compared with the beverage obtained by traditional fermentation of sweetened black tea. In addition, the antioxidative activity of kombucha prepared from echinacea tea was higher (13) and this finding confirmed the assumption that the obtained beverage is of increased pharmacologic properties. For this reason, the kombucha was cultivated on medium with echinacea.

Different volumes of yeast starter culture suspension were used for the inoculation of the medium, so the cultivation mediums contained different number of yeast cells. On the other hand, same volumes of suspension of acetic acid bacteria were added to each of four cultivation vessels (A, B, C, D). The number of yeast cells and acetic acid bacteria of tea fungus isolate in the suspension prepared for inoculation is presented in Table 1.

Table 1. Scheme of the trial

sample	medium volume (l)	inoculum
A	2	α
B	3	α
C	2	β
D	3	β
control	3	fermentation broth

The inoculation of sweetened echinacea tea was performed following the scheme presented in Table 1. The volume ratio of yeast and acetic acid bacteria for inoculation was $\alpha = 1/10 \text{ (ml/ml)}$, e.g. $\beta = 0,1/10 \text{ (ml/ml)}$, calculated on 1000 ml of medium. Medium inoculated traditionally with fermentation broth, obtained by previous fermentation of the same medium, was the control sample. The quantity of added fermentation broth was 10% (v/v), while the number of cells of yeast and acetic acid bacteria was: yeasts = $5 \times 10^6 \text{ cfu/ml}$; acetic acid bacteria = $7 \times 10^6 \text{ cfu/ml}$.

The count of yeasts and acetic acid bacteria, determined subsequently in suspension for the inoculation of sweetened *Echinacea purpurea* tea, is presented in Table 2.

Tabela 2. Count of yeast and acetic acid bacteria cells in suspensions for inoculation

yeasts		acetic acid bacteria	
isolate	cfu/ml	isolate	cfu/ml
2/1	5.75×10^5	5	2.35×10^7
5/3	3.3×10^8	9	4.5×10^6
7/2	5.72×10^7	-	-

The fermentation of mediums inoculated with starter cultures and fermentation broth from the previous cultivation of tea fungus was followed determining the total acidity and pH value (Tables 3 and 4).

Tabela 3. Change in titratable acidity (g/l) during fermentation

fermentation time (days)	sample				
	A	B	C	D	control
2	0.41	0.38	0.19	0.14	0.36
4	0.25	0.22	0.22	0.16	1.40
6	0.60	0.47	0.39	0.25	3.18
8	2.52	1.87	0.85	0.38	4.99
10	3.75	2.79	1.24	0.74	-
12	-	-	3.04	3.56	-

The pH value of sweetened noninoculated echinacea tea is 7.75. The significant decrease of pH value after the inoculation was expected, and the determined pH values for mediums inoculated with fermentation broth and suspensions of starter cultures was 5.03 e.g. 5.18, respectively. At the same time, the total acidity of mediums after the inoculation was 0,19 g/l (starter cultures) e.g. 0.33 g/l (fermentation broth). The higher acidity of fermentation broth (calculated on total acidity 3.65 g/l) resulted in increased acidity of the medium inoculated with it. This increased acidity is suitable for the yeasts and acetic acid bacteria cells and this could stimulate the fermentation proces of the medium.

Tabela 4. Change in pH value during fermentation

fermentation time (days)	sample				
	A	B	C	D	control
2	5.16	5.20	5.23	5.31	4.78
4	5.05	5.11	5.14	5.27	4.11
6	4.52	4.61	4.89	5.02	3.76
8	3.81	4.00	4.35	4.79	3.57
10	3.74	3.89	4.21	4.55	-
12	-	-	3.92	3.99	-

The biotransformation process was interrupted when the desired level of total acidity was achieved. On the basis of previous investigations and long-standing consumption experience of the beverage this value is between 3 and 4 g/l (15).

The pH value of the medium decreases during the cultivation of tea fungus, due to the formation of organic acids (acetic in the first place), as the result of physiological activity of yeasts and acetic acid bacteria. The change of total acid content is not following linearly the change of pH. After the sudden decrease of the initial pH value, the change of total acidity is minimal, even though the synthesis of organic acids continued (14, 15).

The fermentation rate in the control bottle is the biggest, and the critical values of kombucha acidity are reached after 6 – 8 days of cultivation of tea fungus. The biotransformation of mediums inoculated with starter cultures of tea fungus is generally slower, and the beverage is obtained after 10 – 12 days. The acidity of the medium inoculated with higher number of yeast cells (vessels A and B) is higher compared to vessels C and D, where the number (count) of yeast cells was ten times smaller. So, the assumption that the count of yeast cells is of vital significance for the fermentation of sweetened tea. Their number is limiting the process is justified, having in mind the role of yeast cells in the symbiotic union with acetic acid bacteria and the fact that the first reaction in the main course of the biotransformation depends on the yeasts.

The fermentation was somewhat slower in the vessels A and B though the total count of isolate of tea fungus cells was of the same order as in the control vessel. The explanation may be the physiological state of starter culture cells which were not in the exponential or stationary phase in the moment of inoculation.

The ratio of the cultivation vessel volume and amount of medium used is also affecting the fermentation process. Namely, the fermentation with tea fungus is faster if the amount of the medium in the vessel of given volume is smaller at unchangeable process parameters. This phenomenon is most probably connected with the more efficient distribution of air oxygen in lower volume mediums.

The presence of carbo-hydrates (glucose, fructose, saccharose) in fermentation liquid during tea fungus cultivation, determined by chromatographic analysis, confirms the faster transformation of medium inoculated with the fermentation broth. After 6 days of fermentation saccharose was not found in medium samples inoculated with fermentation broth. Saccharose was hydrolized under the influence of enzymes, forming glucose and fructose, as the result of metabolic activity of tea fungus yeasts. The presence of saccharose was registered in samples of fermentation liquid inoculated with starter cultures (samples A, B, C, D, figure 1 – left).

The presence of monosaccharides was not detected after 8 days of the process in samples of fermented medium inoculated with greater count of yeasts cells (samples A and B). This finding is in accordance with the acidity of this medium. The fermentation in vessels C and D is the slowest, and monosaccharides were found even after eight days of cultivation.

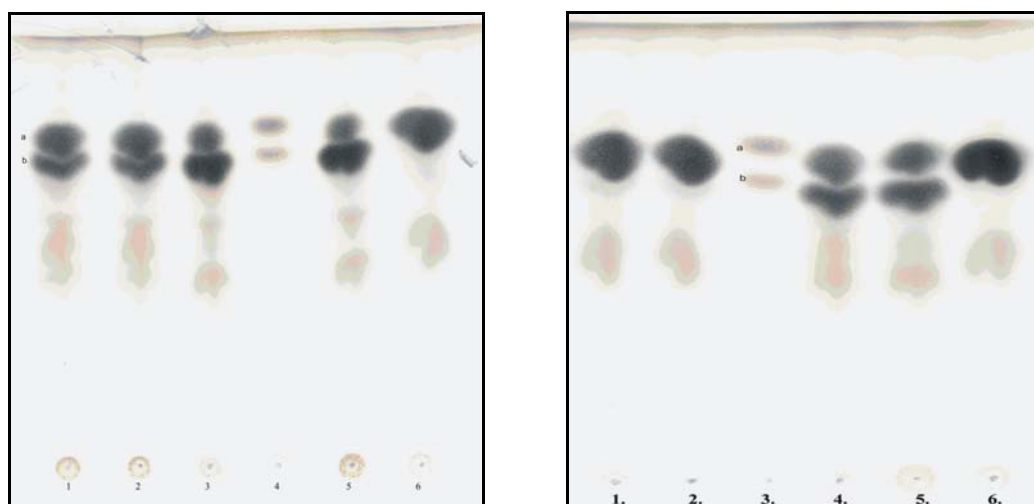


Figure 1. Chromatogram of carbohydrates in the fermented medium after 6 days (on the left) and 8 days of cultivation (on the right); 1 – sample A; 2 – B; 3 – C; 4 – standard (a-glc. and fru.; b-sac.); 5 – D; 6 – control sample.

4. CONCLUSION

The investigation results confirmed that isolated strains of yeasts and acetic acid bacteria from tea fungus may be used as starter cultures for the obtaining of kombucha. At the investigated conditions the fermentation was faster in medium inoculated with fermentation broth (liquid obtained during the previous production of kombucha beverage), compared to the fermentation with starter cultures. It is possible to influence the physiological state of inoculum with starter cultures and improve the process rate or compensate with the amount of inoculum.

The volume of the medium affects the biotransformation rate of sweetened tea. The smaller the medium volume, the faster the biotransformation process.

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