Foods and Raw Materials. 2023;11(1)

ISSN 2308-4057 (Print)
ISSN 2310-9599 (Online)

Review Article
Open Access

Available online at http://jfrm.ru/en
https://doi.org/10.21603/2308-4057-2023-1-563
https://elibrary.ru/CVKROO

Medusomyces gisevii: cultivation, composition, and application

Elena A. Flyurik*, Olga S. Ermakova

Belarusian State Technological University, Minsk, Republic of Belarus

* e-mail: flurikE@mail.ru

Received 11.04.2022; Revised 07.09.2022; Accepted 04.10.2022; Published online 30.01.2023

Abstract:
Tea fungus (*Medusomyces gisevii*) is a natural symbiotic consortium of yeast-like fungi and bacteria. Scientific literature provides a lot of information about the consortium, but it is largely fragmentary. We aimed to review and systematize the information on the research topic.

We studied scientific publications, conference proceedings, intellectual property, regulatory documents, and Internet resources on the *M. gisevii* consortium using Scopus, Web of Science, e.LIBRARY.RU, and Google Academy. The methods applied included registration, grouping, classification, comparative analysis, and generalization.

We described the origin and composition of tea fungus, specifying the microorganisms that make up its symbiotic community depending on the place of origin. Then, we reviewed the stages of fermentation and cultivation conditions in various nutrient media and presented the composition of the culture liquid. Finally, we analyzed the antimicrobial effect of *M. gisevii* on a number of microorganisms and delineated some practical uses of the fungus.

The data presented in this article can be used to analyze or develop new methods for the cultivation and application of *M. gisevii*. We specified some possibilities for using not only the culture liquid but also the fruit body of the fungus in various industries.

Keywords: Kombucha, *Medusomyces gisevii*, composition, cultivation, application

Please cite this article in press as: Flyurik EA, Ermakova OS. *Medusomyces gisevii*: cultivation, composition, and application. Foods and Raw Materials. 2023;11(1):152–161. https://doi.org/10.21603/2308-4057-2023-1-563

INTRODUCTION

Kombucha, a fermented tea drink, is reported to have been first mentioned as early as 220 BC [1]. Originating in Manchuria (China), it was brought to Japan in 414 AD, and then to Eastern Europe and America. Kombucha appeared in Russia and Ukraine during the Russo-Japanese War of 1904–1905 [2]. During the Second World War, the drink was brought to Germany, and in the 1950s, to France, as well as North Africa, where it became quite popular. In the post-war years, its popularity reached its peak in Italy and Switzerland, where the drink was found as beneficial as yogurt [3].

Literature offers a variety of names for the kombucha drink based on *Medusomyces gisevii*, including "Manchurian mushroom", "Japanese mushroom", "Japanese sponge", "sea mushroom" or simply "mushroom", "kvass" or "tea kvass", "fango", "kombuha", "Indian tea mushroom", "miraculous mushroom", "tea fungus", "kam-boo-ha", "Scoby", "Hongo", and many others [1, 4, 5]. The book *Kombucha* by Gunter W. Frank boasts as many as 86 synonyms for the drink's name.

Historically, the kombucha culture liquid has long been used in traditional medicine not only as a refreshing drink, but also to heal various diseases.

Today, kombucha is sold in retail stores, and the *M. gisevii* culture can be purchased online [1, 6].

According to State Standard STB 1818-2007 of the Republic of Belarus, "Functional Food Products. Terms and Definitions", a functional food product is a product that is intended for systematic use as part of a diet in all age groups of a healthy population to reduce the risk of developing diseases associated with nutrition, as well as to maintain and improve health due to the presence of physiologically functional ingredients.

Functional food products can be divided into several groups (Fig. 1).

According to Fig. 1, a drink based on the *M. gisevii* microbiological community can be classified as a functional product.

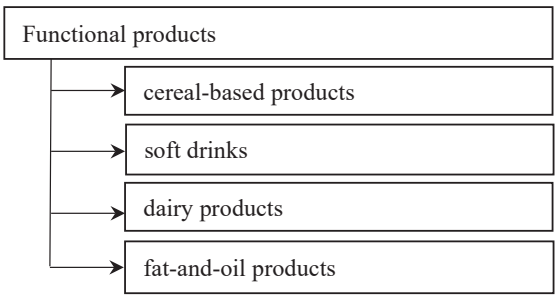

Figure 1 Classification of functional products

M. gisevii (medusomycete) is a symbiotic community of yeast-like fungi and bacteria that forms a thick, leathery, amorphous layered film of tea fungus on the surface of fermentable nutrient solutions (tea extract, juice, etc.) [7].

It is quite common to cultivate tea fugus at home and use its culture liquid, or fermentation product, as a drink (kombucha). This drink is widely known as a valuable preventative and medicinal remedy for various diseases. Its popularity is fueled by recommendations given in popular science articles and publications on traditional medicine. However, scientific literature also reports its negative impact on the body [1, 8]. Therefore, we found it important to study the chemical composition of the *M. gisevii* fermented culture liquid and to systematize the materials available on it.

STUDY OBJECTS AND METHODS

Our study was carried out at the Department of Biotechnology, the Faculty of Technology of Organic Substances, Belarusian State Technological University. We studied scientific articles, conference proceedings, intellectual property, regulatory documents, and Internet resources over a period from 1989 to 2022. They were selected from the bibliographic databases of Scopus, Web of Science, eLIBRARY.RU, and Google Academy by using *Medusomyces gisevii* and kombucha as keywords. We analyzed the data by employing such methods as registration, grouping, classification, as well as comparative analysis and generalization.

RESULTS AND DISCUSSION

Composition. The first scientific information about the *Medusomyces gisevii* microflora appeared in the articles of Gustav Lindau (1913). Its species composition is diverse and dependent on the conditions, place, and time of cultivation. The *M. gisevii* symbiont is composed of culture liquid, zooglea, mesoglea, and sediment [7, 9, 10].

The zooglea of *M. gisevii* is a complex structural formation of bacterial cellulose in which various microorganisms are immobilized. It is based on the colonies of acetic acid bacteria *Gluconacetobacterim*, *Acetobacter*, *Lactococcus*, *Lactobacillus*, and *Clostridium*, as well as yeasts *Saccharomyces*, *Bretanomyces*, *Torulopsis*, *Zygosaccharomyces*, *Schizosaccharomyces*, *Candida*, and others [4, 7, 9–11].

Amarasinghe *et al.* identified the following components in the zooglea: *Acetobacter xylinum*, *Acanthodica xylinoides*, *Acetobacter aceti*, *Acetobacter pausterianus*, *Bacterium gluconicum*, *Kloeckera* spp., *Schizosaccharomyces pombe*, *Saccharomyces ludwigii*, *Saccharomyces cerevisiae*, *Torulaspora* spp., *Zygosaccharomyces bailii*, and *Pichia* spp.

In the study by Savary *et al*., the zooglea was composed of *Dekkera bruxellensis*, *Hanseniaspora uvarum*, *Acetobacter okinawensis*, and *Liquorilactobacillus nagelii* [13]. The patent by Chekasina *et al.* described the following consortium: *Saccharomyces mandshuricus*, *Hausemaspora* sp., *Torulopsis globosa*, *Torulopsis* sp., *S. ludwigii*, *Saccharomyces lactis*, *A. xylinum*, *A. aceti*, *Gluconobacter subaxydans*, and other microorganisms [14].

Bayramaliyeva *et al.* isolated thirteen strains of yeast from the *M. gisevii* fruit body and culture liquid, as well as characterized their morphological and cytological features [15].

Thus, the *M. gisevii* symbiosis can have a very diverse composition.

Quorum sensing is a mechanism that establishes a balance between the microorganisms of a symbiont [9]. It enables a symbiotic community to form, depending on the combination of certain factors. In addition, the symbiotic community contains about 1–10% of so-called "persister" microorganisms. These are cells which are at rest and represent a protective and adaptive life form.

The color of the zooglea is determined by the tea extract or other coloring components of the nutrient medium. The zooglea is kept on the surface of the culture liquid due to carbon dioxide and partly due to the edge adhesion to the vessel, with a film growing at rest without layers. When the film gets damaged, it forms a new layer on the surface of the old one, resulting in layers. The synthesis of bacterial cellulose begins after the symbiont is placed in the nutrient medium and proceeds through several stages: 1) consolidation of bacteria with associates forming on the surface of the culture liquid; 2) synthesis of cellulose microfibrils and the formation of multilayer structures of bacterial cellulose on the surface of the medium; 3) their colonization by microorganisms; and 4) the activity of symbiont microorganisms. Bacterial cellulose forms throughout the entire cultivation period, although it depends on many factors (substrate, extract, inoculum, temperature, pH, etc.).

Therefore, the metabolic activity of *M. gisevii*'s enzymatic systems depends on the activity of microorganisms in the culture liquid that are immobilized in bacterial cellulose. Bacterial cellulose is synthesized by the acetic acid bacteria *Gluconacetobacter xylinus* with the help of monosaccharides (glucose or its phosphorylated forms) [9, 16]. It is

formed by Gram-negative bacteria of the genera *Komagataeibacter* (*Gluconacetobacter*), *Agrobacterium*, *Achromobacter*, *Enterobacter*, *Rhizobium*, *Pseudomonas*, *Salmonella*, *Azotobacter*, and *Alcaligenes*, as well as by Gram-positive bacteria *Sarcina ventriculi* and *Rhodococcus*. The best known producer of bacterial cellulose is the acetic bacterium *Komagataeibacter xylinus* (*G. xylinus*, *A. xylinum*, *Acelobacter aceti* ssp. *xylinum*, *Acetobacter xylinus*).

The inner part of bacterial cellulose is a multilayer structure of microfibrils, in which nutrient substrates, enzymes, microorganisms and their metabolic products move due to diffusion.

Mesoglea is made of filamentous formations of bacterial cellulose containing symbiont microorganisms. It is most actively formed in extracts of black and green teas at low positive temperatures (< 10°C) [9].

The sediment of the culture medium is usually localized at the bottom of the vessel as a dense brown mass [9, 10]. It consists of resins, tannins, tea dust, yeasts, and bacteria that stop growing in the presence of 4–5% of sugars. This is due to the accumulation of microbial products (e.g., organic acids) in the culture liquid.

The fungal biomass contains crude protein (45–53%), carbohydrates (37–43%), including polysaccharides (23–26%), lipids (8–11%), nucleic acids (7–9%), minerals (8–10%), vitamins (C, B_1, PP – 0.11–0.54%), amino acids (28–33%), and microelements (2.1–3.7%). The microelements (mg/kg) include potassium (14025), sodium (5790), magnesium (1379), iron (1017), copper (31.7), zinc (70.5), manganese (342), and chromium (10.5) [17].

The amino acids in the biomass protein (%) include lysine (2.01), histidine (1.75), arginine (2.05), aspartic acid (3.54), trionine (1.41), serine (1.39), glutamic acid (4.07), proline (1.97), alanine (1.97), cystine (0.21), valine (1.73), methionine (0.45), isoleucine (1.57), leucine (2.23), tyrosine (1.23), and phenylalanine (1.93) [17].

Fermentation process. Kombucha is produced by three types of fermentation: lactic acid, alcoholic, and acetic [4, 7]. During lactic acid fermentation, glucose is decomposed to lactic acid under the action of lactic acid bacteria. Alcoholic fermentation leads to the decomposition of glucose to ethyl alcohol by yeast, with carbon dioxide emitting during the process. Acetic fermentation converts ethyl alcohol into acetic acid and water by oxygen and acetic bacteria [18].

Fermentation also produces several intermediate substances, including phosphoric acid. It plays an important role in the formation of phosphoric acid esters, which are converted into free pyruvic acid.

In addition, fermentation leads to an increase in polyphenols and flavonoids in the culture liquid. Thearubigin turns into theaflavin and the culture liquid changes its color from dark to light during the cultivation of the fungus [19, 20].

Catechins present in tea can be converted by symbiont microorganisms to simpler components, which increases the antioxidant power of the culture liquid [19–21]

Cultivation conditions. Since the *M. gisevii* microorganisms can be found on the surface of plants, they can be grown on artificial nutrient media, using extracts of various types of tea and other plants [9]. Their normal growth requires a nutrient medium that satisfies the needs of the symbiotic community. Such a medium may include leaves of black and green tea, rooibos (*Aspalathus linearis*), fireweed (*Epilobium*), lemon balm (*Melissa officinalis*), common oak (*Quercus robur*), common blueberry (*Vaccinium myrtillus*), fragrant callisia (*Callisia fragrans*), herniaria (*Herniaria*), acacia (*Acacia*), gray myrobalan (*Phyllanthus emblica*), Bengal quince (*Aegle marmelos*), woolly erva (*Aerva lanata*), cassia (*Cassia auriculata*), common barley (*Hordeum vulgare*), mint (*Mentha*), common thyme (*Thymus vulgaris*), nettle (*Urtica*), savory (*Satureja*), turmeric (*Curcuma xanthorrhiza*), and other plants [1, 19, 22–26].

The main substrates of *M. gisevii* are carbohydrates represented mainly by mono- and oligosaccharides (glucose, fructose, galactose, mannitol, xylose, sucrose, maltose, etc.), alcohols, organic acids, and other substances [27]. Galactose is less preferable as a nutrient substrate, while sucrose and lactose are better assimilated. The use of lactose results in minimal formation of bacterial cellulose. Glucose promotes the synthesis of bacterial cellulose and gluconic acid, especially at a concentration of 10–20 g/L. Replacing glucose with maltose leads to a 10-fold decrease in bacterial cellulose [9].

Voon *et al.* analyzed tea fungus cultivation with white refined sugar, coconut sugar, and sugar molasses [28]. They reported a significantly larger fungal biomass when using refined sugar. The culture liquid based on sugar molasses contained the largest amount of organic acids, while the culture liquid based on coconut sugar showed better antioxidant activity and a higher phenol content.

During cultivation, yeast oxidizes carbohydrates to ethyl alcohol and carbon dioxide, and bacteria complete the oxidation of ethanol to acetic acid. The acid accumulates during bacterial cultivation and affects the pH value. During symbiont cultivation, the nutrient medium is saturated with ethanol and acetic acid, thus protecting the symbiotic community from contamination by foreign microflora [9].

When microorganisms actively consume the nutrient substrate, the culture medium becomes acidified. This process depends on the temperature and the presence of various additives in the medium, such as alcohols (ethanol and glycerol). Glycerol in low concentrations serves as a source of carbon for the synthesis of bacterial cellulose, but in high concentrations (> 20%) it inhibits this process [9]. Ethanol (1.0–1.5%) can accelerate

the synthesis of bacterial cellulose and be used as an alternative carbon source [29].

In addition to the above alcohols, ethylene glycol, propanol, butanol, methanol, and mannitol are used to stimulate the biosynthesis of bacterial cellulose. These additives can increase the yield of bacterial cellulose by 13.4–56.0%. However, carbohydrates at concentrations of 5–10% are preferable for production purposes. Also, food industry wastes such as molasses and distillery waste are recommended as cheap substrates [9]. Organic acids (lactic, pyruvic, malic, acetic, succinic, and citric) added to the main substrate increase the yield of bacterial cellulose by 1.4–1.9 times [30]. Specialized media have proven effective for tea fungus cultivation, which, in addition to carbohydrates, contain salts ($(NH_4)_2SO_4$, KH_2PO_4, $MgSO_4$, $FeSO_4$, $CaCl_2$, $NaMoO_4$, $ZnSO_4$, $MnSO_4$, $CuSO_4$, etc.), vitamins (PP, B_1, B_2, B_3, B_6, H), and aminobenzoic acid [9].

Gladysheva *et al.* produced bacterial cellulose by bioconversion of *M. gisevii* on a synthetic nutrient medium containing sucrose, black tea extract, starch hydrolysate, and enzymatic miscanthus hydrolysate [31, 32]. Although this medium is not optimal for the biosynthesis of bacterial cellulose, IR spectroscopy established that the bacterial cellulose obtained was a chemically pure compound containing only cellulose. This indicated a high adaptive potential of the symbiotic culture of *M. gisevii*.

According to Skiba *et al.*, the highest yield of bacterial cellulose (7.5–8.0%) was provided at an inoculum amount of 10–20% vol., although all the amounts under study produced bacterial cellulose samples with the same three-dimensional microfibrillar structure [33]. The authors found that the inoculum amounts and the duration of biosynthesis affected the degree of polymerization. Thus, the process of biosynthesis can be controlled to synthesize bacterial cellulose with a given degree of polymerization.

Pribilsky *et al.* pointed to the importance of water pretreatment for fungus cultivation. In their study, filtered water was disinfected and treated with natural minerals (flint, carnelian, opal chalcedony, quartz) [34]. The minerals were placed in water for the entire fermentation process to promote fungal growth and inhibit foreign microorganisms.

The patent by Skripitsyna described a method for obtaining drinks from fermented vegetable extracts with spices and salt [6]. For this, vegetable juice was fermented with the culture liquid of *M. gisevii*.

Another method of cultivating fungus to obtain a soft drink was presented by Ogarkov *et al.* [35]. The authors mixed sugar, tea fungus concentrate, water-soluble melanin, and a water-alcohol solution of lemon balm with water in the presence of carbon dioxide and kept the mixture at 7–10°C.

Fungus zooglea can also be cultivated anaerobically, resulting in a drink with maximum biological activity [36, 37]. However, this method has two disadvantages, namely a decrease in dry matter and an increase in the cultivation time up to 150 days [36].

In addition, Skripitsyna and Zajtsev described methods for preparing beverages by successively fermenting sugar-containing products (water with sugar, jam, or honey) with yeast cultures (baking, wine, etc.) and cultivating tea fungus [6, 38].

Rogozhin *et al.* studied the effect of low temperatures on the cultivation of *M. gisevii* [10]. It is a fact that low temperatures inhibit the body's metabolism. A short-term effect of low temperatures usually leads to a higher metabolic and functional activity than under normal conditions. However, their prolonged action, especially with temperatures below 4°C, can cause death in some living organisms. In another study, Rogozhin *et al.* cultivated *M. gisevii* in a black tea extract [39]. Low temperatures completely suppressed the activity of symbiont microorganisms, which manifested in stable pH values and electrical conductivity for 30–240 days. However, raising the temperatures increased the symbiont's productivity, as shown by changes in the above indicators.

In the same study [39], *M. gisevii* was cultivated in coffee extracts at 8°C. During the first 30 days, symbiont microorganisms were at rest and then, during the following 60–240 days, their metabolic activity was increased by lower pH values and higher electrical conductivity of the culture liquid. This indicated that the coffee extract contained components that could activate microorganisms even at low temperatures. However, prolonged exposure to –20°C had a negative effect on the viability of *M. gisevii*, showing individual cryoprotective properties of black tea and coffee extracts. Furthermore, negative temperatures had different effects on the ability of *M. gisevii* to synthesize bacterial cellulose in black tea and coffee extracts. In the black tea extract, the symbiont's enzymatic systems were less active, while in the coffee extract, they exhibited high activity, manifesting in a 1.84–3.92-fold increase in the zooglea mass [39].

Tea fungus should not be stored at low temperatures since it can lead to a so-called "malolactic transformation". This means that malic acid, which is beneficial for the body, is converted into lactic acid, whose excess can cause muscle pain and fatigue. However, the study by Jayabalan *et al.* showed that heat treatment was not appropriate either for preserving tea fungus [40].

Marchenko and Sotnikov calculated the productivity of the fungus film during its cultivation [41]. The acid formation rate of the culture liquid was 0.03–0.08 ΔK/h, where K was the acidity of the fermented drink taken as the volume (cm^3) of 0.1 mol/dm^3 of sodium hydroxide solution used to neutralize 100 cm^3 of a culture liquid sample. Therefore, various methods are proposed to stimulate fungal growth, e.g., 8–10 g of dead bees (a source of chitin) per 1 liter of nutrient medium [15, 42].

This additive accelerates the production of bacterial cellulose, increases fungal biomass, and improves the taste of the resulting product.

A culture with hydrophilic properties immobilized on a carrier with a rough surface (chopped twigs, wood chips) can be used to reduce the cultivation time, increase productivity, and improve the quality of kombucha [41]. There is also a method for preparing a non-alcoholic beverage with a pear flavor based on tea fungus and fruit waste used as a substrate [43].

Composition of the *Medusomyces gisevii* culture liquid. During the symbiont cultivation, the culture liquid accumulates a large number of various components, including nutrient substrate residues and products of microbial activity moving due to diffusion. The culture liquid contains organic (acetic, gluconic, citric, oxalic, lactic, kojic, tartaric, pyruvic, L-lactic, D-sugar, usnic, malonic, malic, and succinic) acids, inorganic (phosphoric) acids, proteins, lipids (sterols, phosphatides, fatty acids), carbohydrates, vitamins (C, B, PP), pigments (chlorophyll, xanthophyll), enzymes (catalase, lipase, protease and carboxylase, amylase, tryptic enzymes), nucleic acids, nitrogenous bases, chitin, caffeine, amino acids, purine bases, polyphenols, ethanol, as well as various elements (zinc, copper, iron, manganese, nickel, cobalt) and even a natural antibiotic, jellyfish [4, 7, 17, 21, 23, 25, 26, 42, 44, 45].

During the cultivation of *M. gisevii*, the nutrient medium is saturated with ethanol and acetic acid, creating favorable conditions for natural protection of the symbiotic community from contamination by foreign microflora. However, at the initial stage of cultivation, the emerging bacterial cellulose can be damaged by various types of mold due to a high content of carbohydrates and tea extracts in the medium [9]. Therefore, it is recommended to add a small amount of the fermented culture liquid during the initial period of symbiont growth.

Ivanov *et al.* found glucuronic acid (0.037–1.390%) in the first 5 days of fungal growth, which was not detected later [17]. During 20 days of growth, the culture liquid (100 cm^3) contained citric and malic acids (4 mg) and volatile acids (12 mg). The content of ethyl alcohol ranged from 0.15 to 0.7%. However, alcohol was not detected on the 30th day of growth. In addition, the culture liquid contained vitamins C and B, tannins (0.08%) and purine bases, a large number of resinous and fat-like substances insoluble in water and alcohol, proteins and nucleoproteins (5.24%), and a number of enzymes (amylase and catalase).

Antimicrobial properties of the culture liquid. *M. gisevii* has antimicrobial activity due to the presence of antibacterial substances in its culture liquid, which have both bacteriostatic and bactericidal properties. The culture liquid increases the size and volume of bacterial cells, which leads to changes in their shape, vacuolization, and the appearance of granular inclusions. Further, it decreases the intensity of redox processes in microbial cells, reduces their virulence, and increases immunogenicity [46, 47]. In the study [48] associated the antimicrobial activity of *M. gisevii* with the action of acetic acid (the main product of fermentation) on microorganisms. Many researchers [5, 25, 45, 46, 48–53] established the antibacterial efficacy of the *M. gisevii* culture liquid against various microorganisms (Table 1).

Noteworthily, not only acetic acid and large proteins, but also other molecular structures can be active components of the *M. gisevii* culture liquid [45].

Thus, although numerous studies have shown the antimicrobial effect of the *M. gisevii* culture liquid against a number of pathogenic microorganisms, they have not fully explained the exact mechanism of this effect.

Practical use. Depending on the nutrient substrate, the *M. gisevii* culture liquid can be used as a soft drink to prevent a wide range of diseases, such as hypertension, atherosclerosis, sleep disorders, liver problems, gastrointestinal disorders, and others [1, 2, 4, 5, 9, 12, 55–60]. Kombucha stimulates the endocrine and immunocompetent systems, limits atherosclerotic plaques, corrects body weight, increases the body's resistance to carcinogenic factors, has a sedative effect, prevents and alleviates headaches, reduces alcohol dependence, as well as has antitumorous and probiotic effects in combination with ginger [1, 61, 62]. There is a formulation of a fermented beverage based not only on tea fungus but also on birch chaga fungus [37].

Bondareva *et al.* developed a biologically active substance with a prebiotic effect based on *M. gysevii* [57, 63]. This culture liquid is also used as a plant base for medicines with a pronounced healing and anti-inflammatory effect [2, 64, 65]. The systematic intake of the fungal cultural liquid can improve the well-being of elderly people with severe symptoms of atherosclerosis and have a beneficial effect on intestinal atony and gastrointestinal diseases. The culture liquid can also reduce blood cholesterol levels due to a high content of gluconic acid. However, quite a few publications [8, 26] point to its negative effects on human health.

M. gisevii can be used as a biosorbent to remove heavy metals through the ion exchange mechanism [66, 67]. The symbiont is used to produce acetic acid on an industrial scale for the food industry, as well as to produce starter cultures for fermented milk products [10, 68]. Kombucha enriched with sea grapes (*Caulerpa racemosa*) has been proposed as a functional drink to combat obesity [69].

Bacterial cellulose is widely used in the production of pulp, paper, and paints, as well as in the fine chemical industry and electronics [10, 11, 32, 70]. Fine powders obtained from bacterial cellulose are used in the food industry as thickeners and gelling agents [9, 10]. Unlike plant cellulose, bacterial cellulose is pure and free of lignin, hemicellulose, and other impurities [53]. Therefore, a biofilm can serve as a matrix for immobilizing various inorganic compounds (ions of silver, selenium, magnesium, cobalt, manganese, etc.)

Table 1 Antibacterial efficacy of *Medusomyces gisevii* culture liquid

Microorganism	Source of information	Microorganism	Source of information
Aspergilus niger	[46, 50]	*Helicobacter pylori*	[45]
Bacillus anthracis	[52]	*Pasteurella multocida*	[52]
Bacillus aureus	[46]	*Pasteurella avium*	[52]
Bacillus cereus	[45, 46, 52]	*Penicillium aurantiogriseum*	[50]
Bacillus mycoides	[52]	*Proteus vulgaris*	[52]
Bacillus sp.	[50]	*Pseudomonas aeruginosa*	[45, 46, 50, 52]
Bacillus pumilus	[52]	*Pseudomonas fluorescens*	[52]
Bacillus subtilis	[25, 52]	*Salmonella abortusequi*	[52]
Brucella abortus	[52]	*Salmonella sp.*	[5, 54]
Brucella melitensis	[52]	*Salmonella dublin*	[52]
Brucella suis	[52]	*Salmonella enteritidis*	[50]
Campylobacter jejuni	[45]	*Salmonella gallinaran*	[52]
Candida albicans	[45, 46]	*Salmonella paratyphi*	[52]
Candida tropicalis	[46]	*Salmonella pullorus*	[52]
Candida parapsilosis	[46]	*Salmonella typhimurium*	[25, 45–50]
Candida glabrata	[46]	*Sarcina lutea*	[50]
Candida dubliniensis	[46]	*Sarcina maxima*	[52]
Candida sake	[46]	*Serratia marcescens*	[52]
Clostridium botulinum	[52]	*Staphylococcus aureus*	[5, 45, 46, 48–50, 53, 54]
Clostridium novyi	[52]	*Staphylococcus epidermidis*	[46, 52]
Clostridium histolyticum	[52]	*Staphylococcus saprophyticus*	[52]
Clostridium tetani	[52]	*Streptococcus caseolyticus*	[52]
Clostridium septicum	[52]	*Streptococcus cremoris*	[52]
Escherichia coli	[5, 25, 45, 46, 49, 50, 52–54]	*Streptococcus pyogenes*	[52]
Leoconostoc monocytogenes	[55]	*Shigella dysenteriae*	[25, 46]
Listeria monocytogenes	[46, 48]	*Shigella flexneri*	[52]
Malassezia sp.	[46]	*Shigella sonnei*	[45]
Micrococcus sp.	[52]	*Vibrio cholerae*	[25]
Micrococcus luteus	[46]	*Yersinia enterocolitica*	[45]
Microsporum gypseum	[46]		

and biogenic molecules (peptides, amino acids, proteins, enzymes, vitamins, hormones, antibiotics, etc.). Depending on the immobilized components, the film can then be used in medicine and/or pharmaceutics, e.g., to treat burns and ulcers, as well as postoperative, purulent, and traumatic wounds [16, 56, 59, 71]. Biofilms with immobilized bioactive substances can also be applied in cosmetology to restore skin elasticity. The Siberian Federal University in cooperation with the Institute of Biophysics (Siberian Branch of the Russian Academy of Sciences) use bacterial cellulose as a substrate for growing various tissue engineering structures. Since bacterial cellulose is non-toxic and non-allergic, as well as has a high absorption capacity, it can be used as an adsorbent to stimulate digestion.

In bakery, the *M. gisevii* culture liquid is added to the dough to activate its maturation and increase the calorie content of the finished product [10, 72, 73].

Tea fungus can be used as a feed additive for animals. For example, in Tatarstan, a preparation containing *M. gisevii* biomass and/or culture liquid is used for feeding birds [17]. Dried biomass can also be applied for the same purpose [13].

The fruit body of tea fungus is used to create eco-friendly clothing and to produce valuable bacterial cellulose by recovering hydrocarbon waste [74].

The Department of Biotechnology at the Belarusian State Technological University (Minsk, Republic of Belarus) has been developing a preparation based on the fruit body of tea fungus to stimulate plant growth.

CONCLUSION

Based on the above, we can conclude that the *Medusomyces gisevii* culture liquid is an excellent prophylactic against cardiovascular and gastrointestinal diseases. It can help treat atherosclerosis, acute tonsillitis, arterial hypertension, and other diseases. However, there is still a lack of research into the composition and properties of the culture liquid, as well as methods for its cultivation. Therefore, new works are published each year that open up new possibilities for the use of *M. gisevii*. We analyzed the prospects for using not only the culture liquid but also the fruit body of *M. gisevii* in the food, pharmaceutical, and other industries. In addition, the materials collected from numerous studies can be used to create an optimal technology for its cultivation.

CONTRIBUTION

O.S. Ermakova collected information and E.A. Flyurik processed it.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

1. Dutta H, Paul SK. *Kombucha* drink: Production, quality, and safety aspects. In: Grumezescu AM, Holban AM, editors. Production and management of beverages. Volume 1: The science of beverages. 2019. pp. 259–288. https://doi.org/10.1016/B978-0-12-815260-7.00008-0
2. Karomatov ID, Kahhorova SIK. Medical properties kombucha (literature review). Biology and Iintegrative Medicine. 2018;18(1):381–394 (In Russ.).
3. de Miranda JF, Ruiz LF, Silva CB, Uekane TM, Silva KA, Gonzalez AGM, *et al.* Kombucha: A review of substrates, regulations,composition, and biological properties. Journal of Food Science. 2022;87(2):503–527. https://doi.org/10.1111/1750-3841.16029
4. Jakubczyk KJ, Piotrowska G, Janda K. Characteristics and biochemical composition of kombucha – fermented tea. Medycyna Ogólna i Nauki o Zdrowiu. 2020;26(2):94–96. https://doi.org/10.26444/monz/118887
5. Ali SA, Shivanna GB. Physico-chemical characteristics of russian tea fungus: *Kombucha*. International Journal of Pharmacy and Pharmaceutical Sciences. 2017;9(4):161–165. https://doi.org/10.22159/ijpps.2017v9i4.16753
6. Skripitsyna MA. Microorganism culture, method for obtainment of fermented base for kvasses production, tea fungus culture liquid obtainment method, tea fungus culture liquid, beverages obtainment method. Russia patent RU 2552485C2. 2015.
7. Villarreal-Soto SA, Beaufort S, Bouajila J, Souchard J-P, Taillandier P. Understanding kombucha tea fermentation: A review. Journal of Food Science. 2018;83(3):580–588. https://doi.org/10.1111/1750-3841.14068
8. Soysal AU, Akman Z, Koroğlu AE, Yalman H, Koca D. An unexpected cause of cardiotoxicity: Kombucha tea. The Anatolian Journal of Cardiology. 2022;26(6):492–494.
9. Rogozhin VV, Rogozhin YuV. *Medusomyces gisevii*: structure, function, and use. Proceedings of Universities. Applied Chemistry and Biotechnology. 2017;7(4):24–35. (In Russ.). https://doi.org/10.21285/2227-2925-2017-7-4-24-35.
10. Rogozhin VV, Rogozhin YuV. Influence of low positive temperatures on the viability and productivity of *Medusomyces gisevii*. Bulletin of Altai State Agricultural University. 2018;160(2):59–65. (In Russ.).
11. Skiba EA, Gladysheva EK, Budaeva VV, Aleshina LA, Sakovich GV. Yield and quality of bacterial cellulose from agricultural waste. Cellulose. 2022;29(3):1543–1555. https://doi.org/10.1007/s10570-021-04372-x
12. Amarasinghe H, Weerakkody NS, Waisundara VY. Evaluation of physicochemical properties and antioxidant activities of kombucha "Tea Fungus" during extended periods of fermentation. Food Science and Nutrition. 2018;6(3):659–665. https://doi.org/10.1002/fsn3.605
13. Savary O, Mounier J, Thierry A, Poirier E, Jourdren J, Maillard M-B, *et al.* Tailor-made microbial consortium for Kombucha fermentation: Microbiota-induced biochemical changes and biofilm formation. Food Research International. 2021;147. https://doi.org/10.1016/j.foodres.2021.110549
14. Chekasina EV, Nikitina MB, Aften'ev PV. K-17 consortium of yeasts and bacteria – a producer of vitamin complex, organic acids, enzymes and cellulose and a method of preparing weakalcoholic drink using thereof. Russia Patent RU 2081911C1. 1997.
15. Bayramaliyeva EO, Sidyakin AI, Reshetnik GV. Study of morphological-cultural and cytological features of the strains of yeast of kombucha (Medusomyces gisevii Lindau). Scientific Notes of V.I. Vernadsky Crimean Federal University. Biology. Chemistry. 2019;5(71):11–22. (In Russ.).
16. Revin VV, Liyas'kina EV, Sapunova NB, Bogatyreva AO. Isolation and characterization of the strains producing bacterial cellulose. Microbiology. 2020;89(1):88–98. (In Russ.). https://doi.org/10.31857/S0026365620010139
17. Ivanov AV, Nizamov RN, Konjukhov GV, Ivanov AA, Sharifullina DT, Tukhfatullov MZ, *et al.* Feed additive for poultry husbandry and method of poultry breeding. Russia Patent RU 2574691C1. 2016.
18. Rimareva LV, Serba EM, Overchenko MB, Shelekhova NV, Ignatova NI, Pavlova AA. Enzyme complexes for activating yeast generation and ethanol fermentation. Foods and Raw Materials. 2022;10(1):127–136. https://doi.org/10.21603/2308-4057-2022-1-127-136
19. Gaggia F, Baffoni L, Galiano M, Sandris Nielsen D, Riemer Jakobsen R, Castro-Mejia JL, *et al.* Kombucha beverage from green, black and rooibos teas: A comparative study looking at microbiology, chemistry and antioxidant activity. Nutrients. 2019;11(1). https://doi.org/10.3390/nu11010001

20. Bortolomedi BM, Paglarini CS, Brod FCA. Bioactive compounds in kombucha: A review of substrate effect and fermentation conditions. Food Chemistry. 2022;385. https://doi.org/10.1016/j.foodchem.2022.132719
21. Jayabalan R, Malbasa RV, Sathishkumar M. Kombucha tea: Metabolites. In: Mérillon J-M, Ramawat KG, editors. Fungal Metabolites. Cham: Springer; 2017. pp. 965–978. https://doi.org/10.1007/978-3-319-25001-4_12
22. Leonarski E, Guimarães AC, Cesca K, Poletto P. Production process and characteristics of kombucha fermented from alternative raw materials. Food Bioscience. 2022;49. https://doi.org/10.1016/j.fbio.2022.101841
23. Aleshina LA, Gladysheva EK, Budaeva VV, Skiba EA, Arkharova NA, Sakovich GV. X-ray diffraction study of bacterial nanocellulose produced by the *Medusomyces gisevii* Sa-12 culture in enzymatic hydrolysates of oat hulls. Crystallography Reports. 2018;63(6):955–960. https://doi.org/10.1134/S1063774518050024
24. Zubaidah E, Dea EC, Sujuti H. Physicochemical and microbiological characteristics of kombucha based on various concentration of Javanese turmeric (*Curcuma xanthorrhiza*). Biocatalysis and Agricultural Biotechnology. 2022;44. https://doi.org/10.1016/j.bcab.2022.102467
25. Herwin F, Siska N. Production of kombucha from *Muntingia calabura* L. leaves and evaluation of its antibacterial activity and total flavonoid content. Journal of Applied Pharmaceutical Science. 2022;12(8):187–192. https://doi.org/10.7324/JAPS.2022.120819
26. Coelho RMD, de Almeida AL, do Amaral RQG, da Mota RN, de Sousa PHM. Kombucha: Review. International Journal of Gastronomy and Food Science. 2020;22. https://doi.org/10.1016/j.ijgfs.2020.100272
27. Tran T, Grandvalet C, Verdier F, Martin A, Alexandre H, Tourdot-Marechal R. Microbiological and technological parameters impacting the chemical composition and sensory quality of kombucha. Comprehensive Reviews in Food Science and Food Safety. 2020;19(4):2050–2070. https://doi.org/10.1111/1541-4337.12574
28. Muhialdin BJ, Osman FA, Muhamad R, Che Wan Sapawi CWNS, Anzian A, Voon WWY, *et al.* Effects of sugar sources and fermentation time on the properties of tea fungus (kombucha) beverage. International Food Research Journal. 2019;26(2):481–487.
29. Dobrynya YuM, Bondareva NI, Avanesyan SS, Timchenko LD, Simecheova EI, Rzhepakovskiy IV. The influence of ozonization on ethyl alcohol content in the culture broth *Medusomyces gisevii* (tea fungus) at different temperature conditions of cultivation. Fundamental Research. 2015;(7–3):454–457. (In Russ.).
30. Lu H, Jia Q, Chen L, Zhang L. Effect of organic acids on bacterial cellulose produced by *Acetobacter xylinum*. Research and Reviews: Journal of Microbiology and Biotechnology. 2016;5(2).
31. Skiba EA, Gladysheva EK, Golubev DS, Budaeva VV, Aleshina LA, Sakovich GV. Self-standardization of quality of bacterial cellulose produced by *Medusomyces gisevii* in nutrient media derived from *Miscanthus* biomass. Carbohydrate Polymers. 2021;252. https://doi.org/10.1016/j.carbpol.2020.117178
32. Gladysheva EK, Golubev DS, Skiba EA. Investigation of bacterial nanocellulose biosynthesis by *Medusomyces gisevii Sa-12* from enzymatic hydrolyzate obtained by alkaline delignification of miscanthus. Proceedings of Universities. Applied Chemistry and Biotechnology. 2019;9(2):260–269. (In Russ.). https://doi.org/10.21285/2227-2925-2019-9-2-260-269
33. Skiba EA, Baibakova OV, Gladysheva EK, Budaeva VV. Study of the influence of *Medusomyces gisevii* Sa-12 inoculum dosage on bacterial cellulose yield and degree of polymerization. Proceedings of Universities. Applied Chemistry and Biotechnology. 2019;9(3):420–429. https://doi.org/10.21285/2227-2925-2019-9-3-420-429
34. Pribilsky VL, Ustimenko PI. A method of producing a soft drink fermentation. Patent UA 22805. 2002.
35. Ogarkov BN, Ogarkova GR, Samusenok LN, Alekseeva TN, Oreshchenko AV. Non-alcoholic bevarage “Melissoviy”. Russia Patent RU 2210952C2. 2003.
36. Khachatrjan VKh, Isaeva VS, Ivanova TV. Drink, method of producing culture liquid of tea fungus and method of drink production. Russia Patent RU 2153816C1. 2000.
37. Zajtsev DA. Bioactivive composition (variants), method for production thereof and method for production of tea fungus zooglea. Russia Patent RU 2280394C1. 2006.
38. Zajtsev DA. Method of manufacturing biologically acive beverage and beverage manufactured by method. Russia Patent RU 2337592C2. 2008.
39. Rogozhin VV, Rogozhin YuV. The influence of subfreezing temperature on viability and productivity of *Medusomyces gisevii*. Bulletin of Altai State Agricultural University. 2018;159(1):97–104. (In Russ.).
40. Jayabalan R, Marimuthu S, Thangaraj P, Sathishkumar M, Binupriya AR, Swaminathan K, *et al.* Preservation of kombucha tea – effect of temperature on tea components and free radical scavenging properties. Journal of Agricultural and Food Chemistry. 2008;56(19):9064–9071. https://doi.org/10.1021/jf8020893
41. Marchenko VV, Sotnikov VA. “Tea fungus” culture production method and fermentation beverage production method using “Tea fungus” culture. Russia Patent RU 2480519C2. 2013.

42. Ivanov AV, Nizamov RN, Konjukhov GV, Ivanov AA, Tukhfatullov MZ, Sychev KV, *et al.* Tea fungus infusion production method. Russia Patent RU 2556121C1. 2015.

43. Gasilo YuA, Strezhekurov EE, Kotousova AM, Kotousov NG. Vitaminized non-alcoholic beverage "Peknavit-1". Patent SU 1477364A1. 1989.

44. Bunyatyan ND, Samylina IA, Luneva NM, Glazova NV, Zainkova NV, Serkova AN, *et al.* Acid proteases released from the native solution *Medusomyces gisevii Lindau*. Russian Journal of Biopharmaceuticals. 2018;10(2):28–32. (In Russ.).

45. Sreeramulu G, Zhu Y, Knol W. Kombucha fermentation and its antimicrobial activity. Journal of Agricultural and Food Chemistry. 2000;48(6):2589–2594. https://doi.org/10.1021/jf991333m

46. Vukmanović S, Vitas J, Ranitović A, Cvetković D, Tomić A, Malbaša R. Certain production variables and antimicrobial activity of novel winery effluent based kombucha. LWT. 2022;154. https://doi.org/10.1016/j.lwt.2021.112726

47. Cvetkovic DD, Markov SL, Velicanski A. Antimicrobial activity of kombucha made from Rtanj tea. Hemijska Industrija. 2005;59(9–10):248–253. https://doi.org/10.2298/HEMIND0510248C

48. Abaci N, Senol Deniz FS, Orhan IE. Kombucha – An ancient fermented beverage with desired bioactivities: A narrowed review. Food Chemistry: X. 2022;14. https://doi.org/10.1016/j.fochx.2022.100302

49. Talawat S, Ahantharik P, Laohawiwattanakul S, Premsuk A, Ratanapo S. Considerations on the in-vitro inhibitor effect of kombucha on pseudomonas aeruginosa isolates from female urethral and periurethral area. Journal of Natural Sciences. 2006;40(4):925–933.

50. Nyiew K-Y, Kwong PJ, Yow Y-Y. An overview of antimicrobial properties of kombucha. Comprehensive Reviews in Food Science and Food Safety. 2022;21(2):1024–1053. https://doi.org/10.1111/1541-4337.12892

51. Danielyan LT. Kombucha and its biological features. Moscow: Meditsina; 2005. 174 p. (In Russ.).

52. Mohsin AZ, Mat Nor NA, Muhialdin BJ, Mohd Roby BH, Abadl MM, Marzlan AA, *et al.* The effects of encapsulation process involving arabic gum on the metabolites, antioxidant and antibacterial activity of kombucha (fermented sugared tea). Food Hydrocolloids for Health. 2022;2. https://doi.org/10.1016/j.fhfh.2022.100072

53. Cardoso RR, Neto RO, dos Santos D'Almeida CT, do Nascimento TP, Pressete CG, Azevedo L, *et al.* Kombuchas from green and black teas have different phenolic profile, which impacts their antioxidant capacities, antibacterial and antiproliferative activities. Food Research International. 2020;128. https://doi.org/10.1016/j.foodres.2019.108782

54. Dobrynya YuM. Morphological characteristic of the liver under the influence of the substance from *Medusomyces gisevii* (tea mushroom) in the conditions of the intestine disbacteriosis. Veterinary Pathology. 2018;65(3):34–39. (In Russ.). https://doi.org/10.25690/VETPAT.2018.65.20142

55. Leal JM, Suarez LV, Jayabalan R, Oros JH, Escalante-Aburto A. A review on health benefits of kombucha nutritional compounds and metabolites. CYTA – Journal of Food. 2018;16(1):390–399. https://doi.org/10.1080/19476337.2017.1410499

56. Bondareva NI, Timchenko LD, Alieva EV, Dobrynja YuM, Gandraburova NI, Piskov SI, *et al.* Microbiocenosis of the large intestine of rats after oral application of zoogloeas *Medusomyces gysevii* (Kombucha). Medical News of North Caucasus. 2017;12(1):87–90. https://doi.org/10.14300/mnnc.2017.12024 (In Russ.).

57. Chakravorty S, Bhattacharya S, Bhattacharya D, Sarkar S, Gachhui R. Kombucha: A promising functional beverage prepared from tea. In: Grumezescu AM, Holban AM, editors. Non-alcoholic beverages. Volume 6: The science of beverages. Woodhead Publishing; 2019. pp. 285–327. https://doi.org/10.1016/B978-0-12-815270-6.00010-4

58. Alves GD, Beverari SF, Florentino LC, Guerrero AS, Silva MAD. Biosynthesis and characterization of bacterial cellulose from the kombucha tea. Periodico Tche Quimica. 2019;16(32):395–405. (In Portug.).

59. Dobrynya YuM, Timchenko LD, Bondareva NI, Piskov SI. Influence of a biologically active substance from *Medusomyces gisevii* (tea fungus) on the phagocytic activity of blood neutrophils of white rats. Agrarian Bulletin of the Urals. 2018;168(1):8–11. (In Russ.).

60. Salafzoon S, Mahmoodzadeh Hosseini H, Halabian R Evaluation of the antioxidant impact of ginger-based kombucha on the murine breast cancer model. Journal of Complementary and Integrative Medicine. 2017;15(1). https://doi.org/10.1515/jcim-2017-0071

61. Matei B, Salzat J, Diguță CF, Cornea CP, Luță G, Utoiu ER, *et al.* Lactic acid bacteria strains isolated from Kombucha with potential probiotic effect. Romanian Biotechnological Letters. 2018;23(3):13592–13598.

62. Timchenko LD, Rzhepakovskij IV, Bondareva NI, Avanesyan SS, Dobrynya YuM, Sizonenko MN, *et al.* Method of obtaining biologically active substance with prebiotic effect based on Medusomyces gysevii. Russia Patent RU 2630457C1. 2017.

63. Areshidze DA, Kozlova MA, Semin IA, Kuznetsova YaA. Wound-healing, anti-inflammatory ointment on the basis of tea fungus (Medusomyces gisevii Lindau). Russia Patent RU 2647458C1. 2018.

64. Diez-Ozaeta I, Astiazaranc OJ. Recent advances in Kombucha tea: Microbial consortium, chemical parameters, health implications and biocellulose production. International Journal of Food Microbiology. 2022;377. https://doi.org/10.1016/j.ijfoodmicro.2022.109783

65. Najafpour A, Khorrami AR, Azara PA, Tehrani MS. Study of heavy metals biosorption by tea fungus in Kombucha drink using Central Composite Design. Journal of Food Composition and Analysis. 2020;86. https://doi.org/10.1016/j.jfca.2019.103359

66. Rana A, Sindhu M, Kumar A, Dhaka RK, Chahar M, Singh S, *et al.* Restoration of heavy metal-contaminated soil and water through biosorbents: A review of current understanding and future challenges. Physiologia Plantarum. 2021;173(1):394–417. https://doi.org/10.1111/ppl.13397

67. Gubanova EhB, Kudryavtseva TA, Arsenyeva TP, Zabodalova LA, Leonova EV. Method of preparing sour for cultured milk products with curative properties. Russia Patent RU 2165711C2. 2001.

68. Baslak C, Demirel S, Kocyigit A, Alatli H, Yildirim M. Supercapacitor behaviors of carbon quantum dots by green synthesis method from tea fermented with kombucha. Materials Science in Semiconductor Processing. 2022;147. https://doi.org/10.1016/j.mssp.2022.106738

69. Volova TG, Prudnikova SV, Kiselev EG, Nemtsev IV, Vasiliev AD, Kuzmin AP, *et al.* Bacterial cellulose (BC) and BC composites: Production and properties. Nanomaterials. 2022;12(2). https://doi.org/10.3390/nano12020192

70. Skiba EA, Budaeva VV, Ovchinnikova EV, Gladysheva EK, Kashcheyeva EI, Pavlova IN, *et al.* A technology for pilot production of bacterial cellulose from oat hulls. Chemical Engineering Journal. 2020;383. https://doi.org/10.1016/j.cej.2019.123128

71. Permatasari HK, Firani NK, Prijadi B, Irnandi DF, Riawan W, Yusuf M, *et al.* Kombucha drink enriched with sea grapes (*Caulerpa racemosa*) as potential functional beverage to contrast obesity: An *in vivo* and *in vitro* approach. Clinical Nutrition ESPEN. 2022;49:232–240. https://doi.org/10.1016/j.clnesp.2022.04.015

72. Roby BHM, Muhialdin BJ, Abadl MMT, Nor NAM, Marzlan AA, Lim SAH, *et al.* Physical properties, storage stability, and consumer acceptability for sourdough bread produced using encapsulated kombucha sourdough starter culture. Journal of Food Science. 2020;85(8):2286–2295. https://doi.org/10.1111/1750-3841.15302

73. Kaya Z, Asir Y. Assessment of instrumental and sensory quality characteristics of the bread products enriched with Kombucha tea. International Journal of Gastronomy and Food Science. 2022;29. https://doi.org/10.1016/j.ijgfs.2022.100562

74. Subbiahdoss G, Osmen S, Reimhult E. Cellulosic biofilm formation of *Komagataeibacter* in kombucha at oil-water interfaces. Biofilm. 2022;4. https://doi.org/10.1016/j.bioflm.2022.100071

ORCID IDs
Elena A. Flyurik https://orcid.org/0000-0001-9598-7693
Olga S. Ermakova https://orcid.org/0000-0001-9725-8371