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Chapter 16

Saccharomyces cerevisiae var. *boulardii* – Probiotic Yeast

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1. Introduction

The discovery and study of the budding yeast *Saccharomyces cerevisiae var. boulardii* (*Sb*) is strictly related to the concept of health promoting microorganisms from food. The first most well-known and popularized throughout Europe assumption of health promoting food containing living microorganisms was yogurt. Appointed in 1888 by Louis Pasteur, Ilya Ilyich Metchnikov working in Paris developed a theory that aging is caused by toxic bacteria in the gut and that lactic acid could prolong life which resulted in popularization of yogurt consumption. Metchnikov received with Paul Ehrlich the Nobel Prize in Medicine in 1908 for his previous work on phagocytosis, which probably promoted his idea of today's so called functional food further and triggered subsequent research on this subject. Scientists started to look for traditional, regional food products considered good for health. One of them was French scientist Henri Boulard who was in IndoChina in 1920 during cholera outbreak. He observed that some people chewing the skin of lychee and mangosteen or preparing special tea did not develop the symptoms of cholera. This observation lead Henri Boulard to the isolation of a tropical strain of yeast named *Saccharomyces boulardii* (*Sb*) from lychee and mangosteen fruit, which is nowadays the only commercialized probiotic yeast.

At the beginning Metchnikov's theory that lactic acid bacteria (LAB) can prolong life was disputable and some researchers doubted it. For example, Cheplin and Rettger (1920)[1] demonstrated that Metchnikov's strain, today called *Lactobacillus delbrueckii subsp. bulgaricus*, could not live in the human intestine. A scientific discussion to be constructive needs to forge and define new argued ideas. Such a new term was probiotic (*pro* Lat. "for" and *biotic* Greek adjective from *bios* "life") used by Werner Kollath [2] in 1953 to denote, in contrast to harmful antibiotics, all good organic and inorganic complexes. It is attributed to Lilly and Stillwel [3] who in 1965 defined the probiotic as "a substance produced by one microorganism stimulating the growth of another microorganism". The significance of probiotics evolved

over time. In 1974 Parker [4] defined it as "organisms and substances which contribute to intestinal microbial balance", in 1989 Fuller [5] defined it as "a live microbial feed supplement, which beneficially affects the host animal by improving its intestinal microbial balance", in 1996 Sanders [6] wrote "Probiotics, simply defined, are microbes consumed for health effect. The term probiotic is used in food applications. The term biotherapeutic is used in clinical applications". To distinguish between the beneficial effect of living microorganism from organic compounds the term prebiotic was introduced for the latter. However, living microorganism during their growth always affect the chemical composition of the environment, thus it is very difficult to differentiate the influence of microorganisms alone from the impact of organic compounds resulting from microorganisms metabolism. Unfortunately, there is still no general agreement to clear-cut definition of the probiotic.

Irrespectively of the assumed probiotic definition, during over half of the last century the conducted research showed that *Sb* may be beneficial for human health [7]. As mentioned before, the history of probiotic strain started in 1920. Henri Boulard after his return to France patented isolated strain and in 1947 sold it to Biocodex company created for its production. *Sb* was registered as a drug for the first time in 1953 and so far it is the only registered eukaryotic probiotic microorganism.

While commercial application of *Sb* in diarrhea treatment has been steadily growing since 1953, the scientific interest measured in number of publications was in a "lag phase" during next 30-40 years. While searching year by year Scholar Google for "boulardii" it has been found out that there were no articles after 1953, with the first appearing in 1977. From 1977 to 1986 only 17 publications were found.

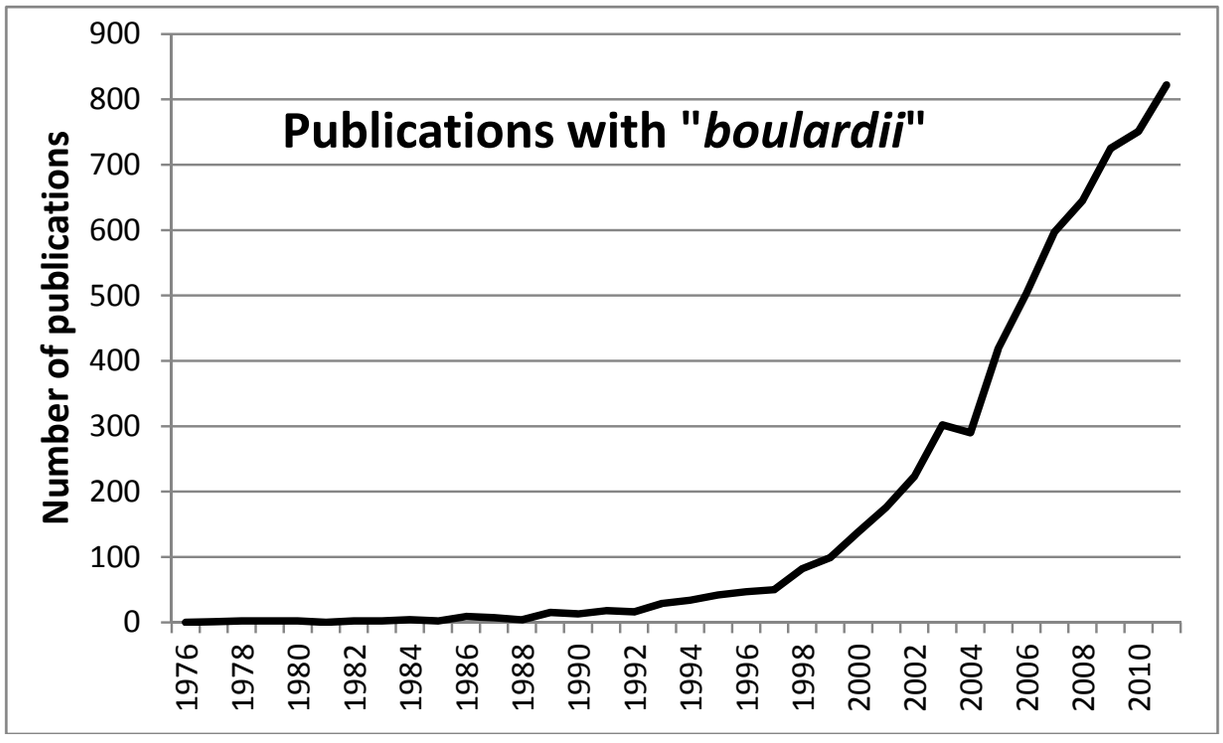

Figure 1. Number of peer-reviewed publications mentioning *Sb* from 1976 to 2010.

The publication of two successive patents in 1986 "Method for preventing or treating pseudo-membranous colitis" [8] and in 1987 "Method for the treatment of amoebiasis" [9], was probably the turning point. Thus, while in 1987 there were only 7 publication in 2011 there were already 822.

Why has *S. boulrdii* been so extensively studied in recent years? Diarrheal diseases are of various origin and continue to represent a major threat to global health. In developing countries, mortality due to acute diarrhea, especially in children, is alarmingly high. In contrast, in developed countries, mortality caused by diarrheal diseases may be considered marginal, yet these disorders are burdensome and widespread, having important economic impact on the society. While the majority of physicians regard probiotics as a very effective therapy they still criticize the lack of useful clinical guidelines [10]. Indeed, beside various origins of diarrheal diseases there are various mechanisms of action of *Sb* and the fields of its potential application are growing.

2. Systematic classification

Sb is a close relative to baker's yeast *Saccharomyces cerevisiae*, the most wildly used organism in industrial microbiology for various foodstuff products. The most obvious difference between them is unusually high optimal growth of *Sb* in the temperature (37 °C) which fits very well with the temperature of human body. Another important feature is better survival at acid pH. Yeast classification was traditionally based on their physiological and biochemical profiles. However, it fails to distinguish between several yeast species or cultivars and it resulted in a discussion whether *Sb* should remain as species or subspecies of *S. cerevisiae*. Thus, molecular methods have been developed and applied to yeast strain typing and identification.

Table 1. summarizes some results of the investigation on differences and similarities between *Sb* and *S. cerevisiae*. Although *S. boulardii* strains differ significantly from laboratory strains of *S. cerevisiae* [11], finally according to current nomenclature like International Code of Botanical Nomenclature (ICBN) *Sb* yeasts should be referred to as *S. cerevisiae var. boulardii* [16]. It should, however, be pointed out, that strongly reduces ability to mate with other strains puts *Sb* on the evolutionary way of becoming a separate species.

Taxonomy attempts to achieve two aims: first the classification that reflects the evolution and phylogenetic relationships and second the development of procedures enabling identification of individual species. Thus, independently of discussion on the systematic classification, very important issue concerns identification of species which affect human health. *S. cerevisiae* appears to be an emerging pathogen [17-19]. Thus, recent research concentrates on unravelling features determining the pathogenicity. It has been shown that yeast pathogenicity correlates with survival in oxidative stress [20] which could be triggered by transcription factor Rds2 [21] or activation of MAP kinases and variability in the polyglutamine tract of Slt2 [22]. Probiotic properties are also strain specific, which is the case for *Sb* used as probiotic [11, 12]. Thus there is a need for a valuable molecular markers able

to distinguish among strains and establish appropriate methods for the identification of probiotic strains of the *Sb. Such a method could be, for example,* microsatellite length polymorphism, having a discriminatory power of 99% [15, 23], restriction fragment length polymorphism [24], full genome hybridization [14], randomly amplified polymorphic DNA [25], GeneChip hybridization [11], artificial neural network–assisted Fourier-transform infrared spectroscopy [26] or multilocus enzyme electrophoresis [27]. These identification methods enable the discrimination between various strains but are not necessarily related to mechanisms of probiotic activity. Metabolic footprinting using mass spectrometry may be useful in this regard. Using gas chromatography–time of flight–mass spectrometry there was good correlation with genetic method of strains classification. Probiotic strains of *Sb* showed tight clustering both genetically and metabolically. The major discriminatory metabolites were: trehalose, myo-inositol, lactic acid, fumaric acid and glycerol 3-phosphate [28]. Next very important step is very to find out a functional relationship between specific DNA and probiotic action.

Sb	***S. cerevisiae***
Higher optimal growth temperature (~37 °C)	Lower optimal growth temperature (~30 °C)
Higher resistance to low pH [11]	Lower resistance to low pH [11]
The karyotypes of *Sb* are very similar to those of *S. cerevisiae*	Typing RFLPs or PCR- (ex 5.8S rDNA) failed to distinguish *Sb* from *S. cerevisiae* [12]
Do not use galactose [13]	Use galactose
Asporogenous in contrast to S. cerevisiae but may produce fertile hybrids with of *S. cerevisiae* strains [11]	Sporogenous
Lost all intact Ty1/2 elements [14].	Contains several Ty1/2 elements
Microsatellite typing shows genotypic differences [15]	
Trisomic for chromosome IX	There are stable strains with various ploidy

Table 1. Summary of some differences and similarities between *Sb* and *S. cerevisiae*.

3. Medical applications of *Sb*

Several published medical studies have shown the efficacy and safety of *Sb* for various disease indications both in adults and children. Regarding the medical use, different indications of *Sb* could be listed: prevention of antibiotic-associated diarrhea, recurrent *Clostridium difficile*-associated diarrhea and colitis, Travellers' diarrhea, acute bacterial and viral diarrhea, diarrhea in patients with total enteral feeling, anti-inflammatory bowel diseases, supplement to hydration in adults and children, against diarrhea associated with the use of antibiotics. [29-32]. There is an increasing number of publications showing the

results of double blind clinical trials, clinical guidelines including new applications of the usage of *Sb* and new potential fields. While the number of different possible application of *Sb* in prevention and treatment of health disorders is growing, it is crucial to determine mechanisms of its action. This is an extremely difficult task due to a high number of factors involved in the observed health benefits.

Use for disease	Dose (mg/d)	Duration	Adjunct to
Prevention of antibiotic associated diarrhea	500-1000	During antibiotics with additional 3 days to 2 weeks after	Nothing
Prevention of Traveller's diarrhea	250-1000	Duration of trip (3 weeks)	Nothing
Enteral nutrition-related diarrhea	2000	8-28 days	Nothing
H. pylori symptoms	1000	2 weeks	Standard triple therapy
Treatment of *Clostridium difficile* infections	1000	4 weeks	Vancomycin or metronidazole
Acute adult diarrhea	500 - 750	8-10 days	Nothing
Inflammatory bowel disease	750-1000	7 weeks to 6 months	Mesalamine
Irritable bowel syndrome	500	4 weeks	Nothing
Giardiasis	500	4 weeks	Metronidazole
HIV-related diarrhea	3000	7 days	Nothing

Table 2. Summary of recommendations for clinical use of *Sb* in adults [7]

Mechanisms of action of *Sb*

While *Sb* has been proven effective in several double-blind studies and yeast preparation is sold in several countries as both a preventive and therapeutic agent, not all mechanisms of its action have been studied [7, 33] and the new ones are still being discovered. Figure 2 summarizes most of the postulated mechanism of *Sb* activity which are :

a. antimicrobial effect,
b. nutritional effect,
c. inactivation of bacterial toxins,
d. quorum sensing,
e. trophic effects,
f. immuno-modulatory effects
g. anti-inflammatory effects,
h. cell restitution and maintenance of epithelial barrier integrity.

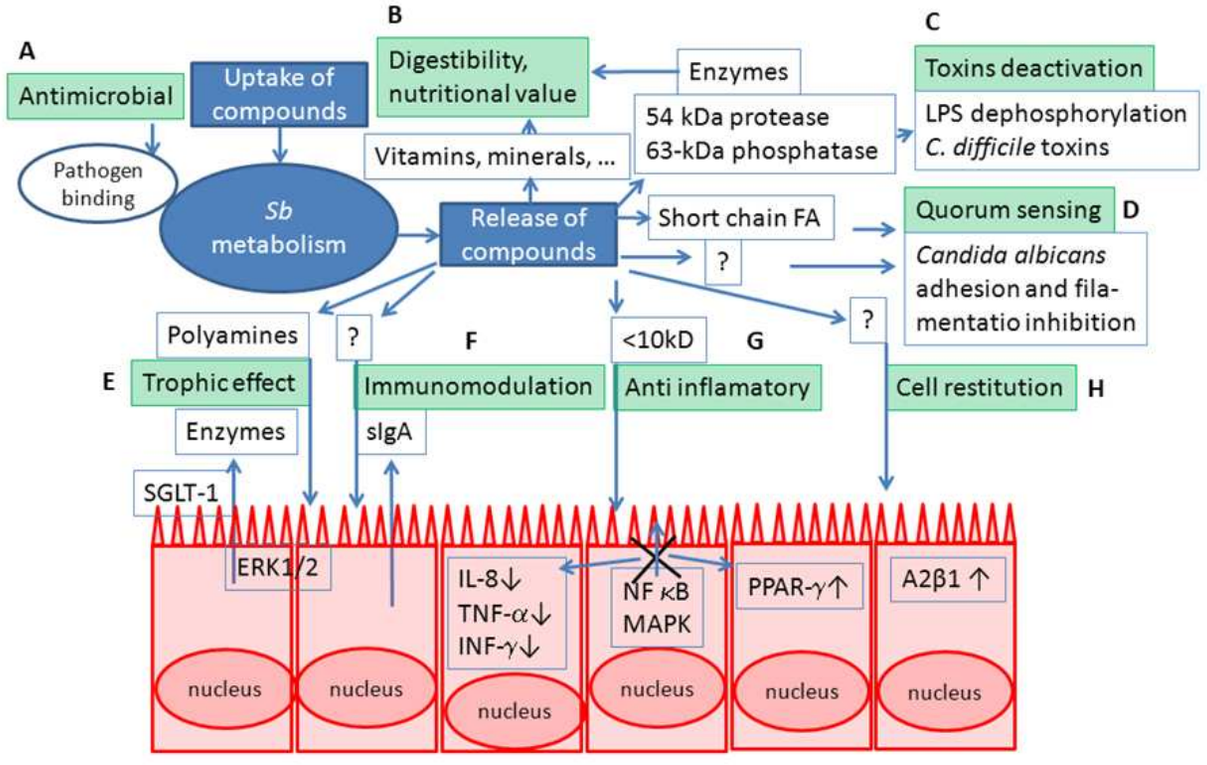

Figure 2. *Sb* possible probiotic mechanisms of action.

This enumeration is somehow artificial because one factor may play multiple roles and various processes may act synergistically.

Antimicrobial effect may be exerted through several mechanisms. One of them is irreversible binding of bacteria to the yeast surface, preventing their adhesion to the mucous membranes and subsequent elimination by the flow Fig. 2A. It has been shown that *Sb* has the ability to bind enteric pathogens to mannose as a receptor [34]. That yeast viability was not necessary for the adhesion phenomenon. Furthermore it has been shown that in the binding beside process beside mannose-containing glycoprotein other proteins are involved [35]. On the other hand, Tasteyre et al. [36] showed that the yeast could inhibit adherence of *C. difficile* to cells, thanks to its proteolytic activity and steric hindrance. This is exerted trough the modification the eukaryotic cell surface receptors involved in adhesion of *C. difficile.* Other mechanisms exerting antimicrobial effect are utilization of substrates, modification of the environment and release of various compounds.

Some of the released compounds are **quorum sensing** molecules Fig. 2D. They influence metabolism and properties of microorganisms, for example, reducing the ability to adhesion or filamentation, which are both important factors of strains pathogenicity [37, 38].

Sb may inhibit pathogens through action on microbial virulence factors. Invasive properties of *Salmonella enterica* serovar Typhimurium is closely related to the flagellum-associated motility. Study performed on human colonic cells infected by the *S. enterica* showed that in in presence of *Sb* the pathogen motility was reduced [39]. *Sb* also acts by **inactivation of**

bacterial toxins (Fig. 2C). For example, it has been shown that the 63-kDa protein phosphatase from *Sb* is able to dephosphorylate and partially inactivate the endotoxin (LPS) of *Escherichia coli*. Furthermore, *Sb* releases *in vivo* a 54-kDa serine protease that digests toxins A and B of *Clostridium difficile* and the BBM receptor of toxin A [40].

Sb also influences the growth of gut microflora and the host by its metabolism (uptake of substrates and release of products or multitude of cell components by dying cells). Yeast from *Saccharomyces* genus has been used in human and animal **nutrition** (Fig 2 B) for many centuries and new applications in agro-industries are being developed [41]. They are of high nutritional value and are used as food additive or to obtain some products such as white or "living" beer. Yeast cells are also a well-known source of proteins, B-complex vitamins, nucleic acids, vitamins and minerals, including a biologically active form of chromium known as glucose tolerance factor [42]. In some countries a mixture of a small amount of baker yeast with water and sugar was prepared as a drink for children as supplementation with B-complex vitamins. *Sb* releases during its passage through gastrointestinal track at least 1500 various compounds [43]. While vitamins are necessary exogenous organic compound which must be ingested, enzymes may help to transform bigger to smaller compounds which may be absorbed by brush border. The brush border is the structure formed by microvilli increasing the cellular surface area responsible for secretion, absorption, adhesion and transduction of signals. Within the gastrointestinal tract brush border is crucial for digestion and nutrient absorption. It has been shown that oral administration of probiotic strain of *Sb* enhanced the activities of the brush border ectomembrane enzymes (ex. sucrase, maltase, trehalase, lactase, aminopeptidase, alkaline phosphatase), carriers (sodium glucose cotransporter-1) receptors of immunoglobulins (the secretory component) or secretory immunoglobulin A [44-48]. *Sb* cells contain substantial amounts of polyamines (spermidine and spermine) which are known to affect cell maturation, enzyme expression and membrane transport, thus polyamines were suggested as mediators in the intestinal trophic response [45]. **Trophic effect** Fig 2E has been recently reviewed by Buts [33, 43]. It was postulated that *Sb* upgraded intestinal function by at least three mechanisms:

- The endoluminal secretion of various compounds by yeast
- The secretion of polyamines triggering transduction trophic signals and resulting in enhanced synthesis of brush border membrane proteins (enzymes and carriers).

Clinical studies have shown that oral administration of *Sb* is effective in treatment of inflammatory bowel diseases and control of irritable bowel syndrome. There are several possible mechanisms of **anti-inflammatory effect** (Fig 2G) recently reviewed by Pothoulakis [49], Vandenplas [50] or Vohra [51]. The activity may be exerted through released compounds which modifies epithelial cell and mucosal immune system signaling pathways. One mechanism of anti-inflammatory effect could be exerted by producing by *Sb* a heat stable low molecular weight (<1 kDa) soluble factor [52]. The mechanism is based on blocking activation of nuclear factor-kappa B (NF-◎B) and mitogen activated protein kinase (MAPK). As a result, pro-inflammatory compounds such as interleukin 8 (IL-8), tumor necrosis factor alpha (TNF-◎) and interferon gamma (IFN- ◎) are down regulated. *Sb* and *Sb* secreted-protein(s) inhibit

production of pro-inflammatory cytokines by interfering with the global mediator of inflammation nuclear factor ◎B, and modulating the activity of the mitogen-activated protein kinases ERK1/2 [53] and p38 [54]. *Sb* activates expression of peroxisome proliferator-activated receptor-gamma (PPAR-γ) that protects the digestive track from inflammation. *Sb* also suppresses 'bacteria overgrowth' and host cell adherence as described before.

Another mechanism mutually related to inflammation and synergistically acting with antimicrobial and anti-inflammatory effect [55] is **immunomodulation** Fig 2F. Sb has been shown to increase secretion of immunoglobulin A [48]. Immunomodulation could be exerted by *Sb* interactions with mucosal dendritic cells. Dendritic cells discriminate commensal microorganisms from potential pathogens and take part in maintaining the balance between tolerance and active immunity. They respond to intestinal inflammation and thus are potential target in inflammatory bowel disease [56]. Dendritic cells produce regulatory cytokines and induce T cells. *Sb* inhibits dendritic cell-induced activation of naïve T cells [57] and may interfere with IBD pathogenesis by trapping T cells in mesenteric lymph nodes [58].

Bacterial infections leading to inflammatory bowel diseases results in intestinal epithelial cell damage. Thus, remission of these diseases requires both the cessation of inflammation and the **cell restitution** Fig. 2H within the damaged epithelium, which is effected by enterocyte migration. It has been recently shown that *Sb* accelerate enterocyte migration by secretion of motogenic factors that enhance cell restitution through the dynamic regulation of $\alpha 2\beta 1$ integrin activity [59].

4. Effect of *Sb* on the virulence factors of *Candida albicans*

While there is quickly increasing information on the influence of *Sb* on the bacterial origin diseases the interaction between *Sb* and *Candida albicans* is much less studied filed. *C. albicans* is a dimorphic fungus growing commensally in the gastrointestinal tract of healthy humans. Switching between morphotypes is a striking feature enabling the growth as budding yeast or as filamentous forms. It also enables in formation of complicate biofilm structures [60]. The transition between morphotypes contributes to the overall virulence and constitutes potential target for development of antifungal drugs.

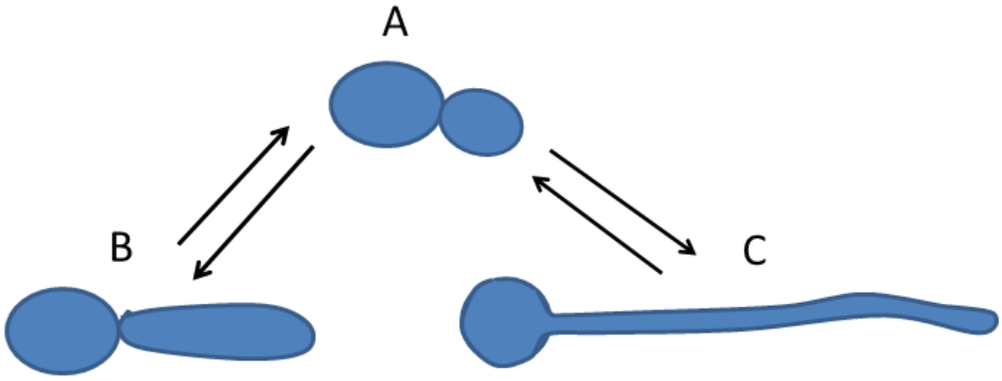

Figure 3. Phenotypic switching of *C. albicans*. (A) budding yeast, (B) pseudohyphal growth, (C) hyphal growth.

Pathogenicity of *C. albicans*, like all pathogens, is conditioned by their virulence. All the features that improve microbial colonization of host cells, multiplication and spread within organism or toxins production, which in turn leads to the development of the disease are called virulence factors. The virulence of *C. albicans* include: the ability to adhesion, biofilm formation and production of coatings, as well as morphological transformation, phenotypic switching and secretion of proteases, phospholipases and endotoxin [61]. Morphogenesis in *C. albicans* can be impaired by various small molecules such as farnesol, fatty acids, sugars, rapamycin, geldanamycin, histone deacetylase inhibitors, and cell cycle inhibitors recently reviewed by Shareck [62]. Affecting metabolism of the C. albicans may also have indirect effect as for example synergism with the antifungal drugs. Indeed metabolic state of the cell greatly affects activity of the PDR pump activity [63].

It has been shown that both live *Sb* cells and the extract from *Sb* culture filtrate diminish *C. albicans* adhesion to and subsequent biofilm formation [38]. Thus, independently of the trophic relationships, for example, elimination from the medium of carbon source (sugars) or polyunsaturated fatty acids [64], *Sb* releases to the medium active compounds. These compounds in dose dependent manners are able to inhibit switching from budding yeast to hyphae growth. The extract prepared from *Sb* culture filtrate was showed to contain 2-phenylethanol, caproic, caprylic and capric acid. The highest activity reducing candidal virulence factors was capric acid (C10:0), which is responsible for inhibition of hyphae formation. It also reduced candidal adhesion and biofilm formation, though three times less than the extract. Thus *Sb* release to the medium other factors, not yet identified, suppressing *C. albicans* adherence [37]. Capric acid acts through the activation of cAMP pathways and Hog1 kinase cascade, reducing the expression of genes of *C. albicans* virulence. Capric acid reduces *CSH1*, *INO1*, *HWP1* transcripts. *CSH1* encodes a protein related to the hydrophobicity surface of the fungal cell wall and is involved in adhesion. *INO1* encodes an enzyme involved in the biosynthesis of inositol, which is a precursor components on the surface of the cell wall of *C. albicans* involved in the virulence. *HWP1* (Hyphal Wall Protein) encodes protein present in hyphae and pseudogyphae and involved in adhesion and biofilm formation. Besides inhibition of *C. albicans* adhesion to epithelial cell lines, *Sb* living cells and compounds released to the medium, reduced cytokine-mediated inflammatory host response. In fact the IL-8 gene expression was suppressed in *C. albicans*-infected epithelial cells by the compounds released to the medium by *Sb* [65].

It is clear that *Sb* secretes many compounds and some of them may act as quorum sensing and modulate growth of other microorganisms including other eukaryotes such as *C. albicans*. Besides identified compounds and their activity it is clear that there are still other biologically active compounds produced by *Sb* which remain to be discovered [65].

5. Conclusions and future perspectives

A century after publication of the Metchnikov's theory there is no more doubt concerning potential positive influence of selected strains of living microorganisms in the ingested food on human health. Nevertheless, the discussion has been even more turbulent and the topic

is “hot”, as seen from increasing number of scientific publications. In contrast to most of the registered drugs which are single, pure compounds, *Sb* has been shown to be beneficial through various mechanism. Thus, due to very complex and various interactions it is exiting research area with a lot of things to discover, but it is also extremely laborious, costly and time consuming. There is a number of organisms in traditional fermented food that has been shown to be potentially beneficial for human health. However, probiotic properties are strain specific and very often not well characterized. Properties of strains from the same species may be very different, thus for human health benefits potential probiotic strain should be very well characterized. It is clear that microflora of the human body is very complex and it is important to maintain appropriate homeostasis, which may be unbalanced by use of antibiotics. This can be prevented or regained by use of appropriate probiotics. However, due to the complexity of the possible interactions and various mechanisms of actions it is very difficult to register and commercialize a new probiotic. It is a great challenge to resolve this bottleneck in the future.

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