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Investigations on Applied Microbiology Regarding Carbon Capture, and Fermentative Production of the Ingredients in Sweeteners, Agricultural Biosurfactants and Cosmetics

Edina Eszterbauer[1], Bernadett Kiss[1], Jesse Sakiyo[1], Pál Tóth[1], Áron Németh[1*]

[1] Department of Applied Biotechnology and Food Science, Faculty of Chemical Technology and Biotechnology, Budapest University of Technology and Economics, Műegyetem rkp. 3., H-1111 Budapest, Hungary
[*] Corresponding author, e-mail: naron@f-labor.mkt.bme.hu

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Abstract

Regarding the celebration of the Faculty of Chemical Technology and Biotechnology 150 years, we want to highlight our recent results in the Fermentation Pilot Plant Laboratory (F-labor) which have a focus on applied microbiology. In all of our research activities, we first select an appropriate microorganism for the desired tasks, then optimize its cultivation and product formation, finally investigate process scale up and economics. Here we want to shortly summarize and introduce 4 pillars of our recent research topics: 1. carbon capture by microalga cultures, 2. complex utilization of *Yarrowia* yeasts with main focus on erythtritol production, 3. bacterial formation of biosurfactants and their use against plant pathogenic fungi on the field of agrobiotechnology and 4. development of different fermented cosmetic ingredients with major focus on *Lactobacilli*. Regarding microalga cultivation among many methodical developments we could build successfully a 850 L scale tank reactor for *Spirulina* cultivation. In term of *Yarrowia* non-conventional yeast complex biotechnology utilization was demonstrated from erythritol to cosmetic applications. Agricultural biosurfactants were successfully applied against *Alternaria alternata* plant pathogenic fungi. Finally *Lactobacilli based* cosmetic ingredients were proved to be potential skin-moistening agents.

Keywords

microalgae, erythritol, *Yarrowia*, *Bacillus* biosurfactants, *Alternaria*, skin moisturizing by *Lactobacilli* fermentation broth

1 Introduction

1.1 Microalgal carbon capture

Our research group investigate the cultivation of microalgae since 2010. The use of algae in food and feed, as well as a soil conditioner, goes back long time ago. Due to their valuable content and minimal nutritional requirements, they are receiving more and more attention. In addition to providing food, more and more research is devoted to their utilization as an alternative energy source and to the capture of carbon dioxide emitted by industry.

During our tests, we mainly work with freshwater microalgae, *Chlorella* species, but we also study *Spirullina* and *Nanochloropsis* species. In our research, we examine microalgal cultivations on different scales, from microplates (ca. 1.5 ml) with our developed illumination system to volumes of almost 1000 liter photobioreactor (Fig. 1). Despite the fact of successful scale-up experiments with *Chlorella* and *Spirullina* species, most of our results were run in the smaller scales i.e., in microtiter plates like different screening works and in small photobioreactors for carbon-dioxide supplement.

For high throughput microalga screening our research group developed a 24-well microtiter plate illuminating system [1, 2] of which findings were confirmed in 1–3 L

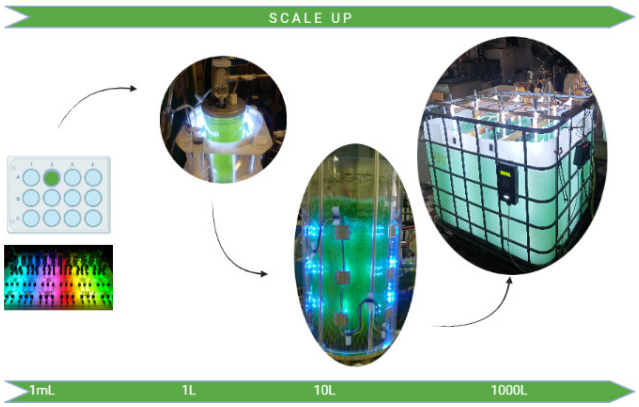

Fig. 1 Scale up of autotrophic microalga cultivations

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stirred tank photobioreactor (STPR) indicating the usefulness of produced algal biomass for energy generation by hydrothermal gasification.

In our microplate tests, we investigated the illumination of the culture with different colors and intensities [1, 2].

We determined the optimal lighting conditions for *Chlorella* and *Nannochloropsis* strains with basic colors (red, green, blue) and mixed colors (blue-green, yellow, purple, white) with RGB LEDs. In addition, the used device is also suitable for other "usual" screening tests, such as nutrient medium selection. For such purposes we examined waste effluents of various technologies (incl. wastewater plant, biogas power plant), in order to convert the technology released CO_2 into value-added products such antioxidants, unsaturated fatty acids, plant hormones or proteins.

In larger volume photobioreactors, we also investigated the effects of illumination, regarding the wavelength, color or intensity of the illumination [2]. Furthermore, we investigated the effect of aeration and the mixing of carbon dioxide (1–5%) on the change in biomass content and found, that as low as only 1% of CO_2 supplementation can increase the biomass yield by 24% compared to 5%, which phenomenon is generally explained by growth inhibition via media acidification and pigment reduction. The samples were analyzed by FT-IR spectroscopy [3], meanwhile also online living cell measuring capacitance sensor was adapted and used first by our research group for microalgae [4].

Recently we examined our largest scale bioreactor for *Spirulina* algae species. 8/16 h photo/dark period changes are controlled, constant air flow is provided and temperature is also controlled at 30 °C by Julabo ED labor thermostat. Axenic growth is supported by the used media and its pH of 9. A typical growth curve is presented on Fig. 2, indicating a very long lag phase because of very low inoculation ratio (0.3%).

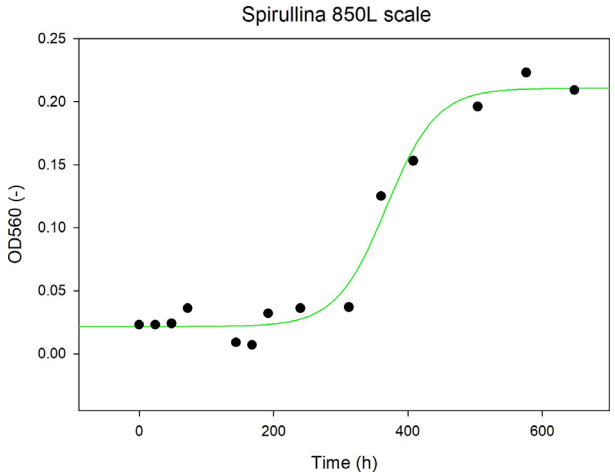

Fig. 2 Microalga growth curve in 850 L scale

2 Alternative sweetener development

Yarrowia lipolytica is one of the best-studied yeast strains that has been attention in basic research and applied biotechnology over the last decades and in the field of applied biotechnology. The growth of *Y. lipolytica* and the quantity and quality of the various metabolites it secretes are influenced by various environmental factors. One of the most important is the amount of oxygen, which plays an important role in the growth of the microorganism [5]. *Y. lipolytica* is able to assimilate and ferment efficiently both hydrophobic (fatty acids, triglycerides and alkanes) and hydrophilic substrates (glucose, glycerol, alcohol and acetic acid) [6]. The growth of many yeast species is limited to a certain pH optimum, and their growth rate decreases when the pH is not optimal. However, *Y. lipolytica* is very different in this respect, being able to adapt to a wide range of pH conditions [7]. In terms of its industrial use, it is most widely used in the food industry, and is known for its positive and negative effects as well: it causes a brownish lesion on the surface of cheese, which does not affect its taste, but its appearance makes it unattractive to consumers and therefore can cause economic loss. The enzyme tyrosinase, produced by the yeast, causes browning by oxidising tyrosine [8]. The pyomelanin pigment is responsible for this discolouration [9, 10]. Using glucose carbon source, erythritol is naturally synthesised by the pentose phosphate pathway in *Y. lipolytica*. However, glycerol can be considered as a more beneficial carbon source, since it is not competing with human food resources, may be less expensive as a frequently arisen byproduct, thus its application support the progress towards the circular economy, and may induce through its high osmotic preasure the erythritol formation. The Rymowicz research group has made great progress in this polyalcohol production by using glycerol as a substrate in combination with *Y. lipolytica* [6].

Erythritol ($C_4H_{10}O_4$) is a naturally occurring 4-carbon sugar alcohol or polyol that is widely distributed. Erythritol is 60–70% as sweet as table sucrose, yet has almost zero calories (0.2 kcal/g), does not affect blood glucose levels, does not cause tooth decay [11], has antioxidant activity, binds free radicals, has no glycaemic index and increases the absorption of fructose [12].

During the research work 3 strains of *Yarrowia* species (*Y. lipolytica* NCAIM 00597, *Y. lipolytica* NCAIM 00594, *Yarrowia divulgata* NCAIM 1485) were examined

on two carbon sources, i.e., glycerol and glucose, for the purpose of erythritol fermentation in microtiter plate. Glycerol was proved to be more usable for all tested *Yarrowia* strains. *Y. divulgata* was found as the most productive, reaching 44.38 g/L erythritol in the media supplemented with Na-citrate during microplate fermentation. In case of *Y. lipolytica* strain 594 the highest amount (10.95 g/L) was reached on the 14th day of fermentation without media-supplementation but containing 100 g/L glycerol in combination with 50 g/L glucose supplementation. Finally, in the case of strain *Y. lipolytica* 597, the highest amount of erythritol was 14.04 g/L in a media that had contained sodium nitrate. The tested supplementations could increase erythritol concentration from 24.7 to 44.38 g/L, erythritol yield from 33.15 to 37.86% and productivity from 0.049 to 0.102 g/(L×h) [13].

In our recent experiments, we investigated the effect of oxygen at controlled oxygen levels (10%, 20%, 30%, 40%) for *Y. divulgata* on the formation of erythritol, on the emulsification index of the fermentation foam and on the skin conditioning effect of the cell lysate in a new Hungarian developed jFermi [14] bioreactor (Fig. 3). The results showed that the best oxygen level was 20%, which produced 19.66 g/L of erythritol, its fermentation foam had an emulsification index of 92% and the cell lysate increased skin hydration by 33.3% determined with Multidermascope DS800 according to Tóth and Németh [15].

Among the reported *Yarrowia* results, the achieved erythritol concentrations above are not really high, but *Y. divulgata* may reach the recently used strains after optimization. Despite the erythritol alone, the complex utilization of the broth for biodetergent isolation from the fermentation foam and cosmetic application of the cells may enhance feasibility and can generate high interests.

3 Fermentation and application of agricultural biosurfactants

Bacillus species have been shown to generate a variety of antimicrobial and plant growth-promoting chemicals, including antibiotics and enzymes. These compounds can boost plant growth, increase nutrient absorption, and protect plants against diseases. Our investigation on the production of biosurfactants mainly done by three bacterial strains, *Bacillus subtilis, Geobacillus stearothermophilu*s, and *Bacillus licheniformis*, and their ability to inhibit the plant pathogenic fungus *Alternaria alternata*, is one focus of our research. The fermentation process for *Bacillus licheniformis* was optimized, and the impacts of several

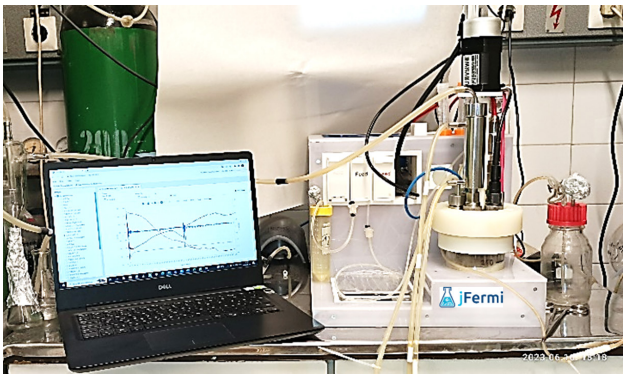

Fig. 3 jFermi fermentor with *Y. divulgata* cultivation

parameters on the production of biosurfactants were investigated. The analysis used a central composite design (CCD) model. The optimized medium (pH = 8 and glucose concentration = 38 g/L) decreased the surface tension to 60 mN/m and increased the product yield to 2.7 g/L [16]. The data also showed a significant drop in surface tension (ST) between 20 and 30 hours after fermentation began, with ST values ranging from 75.5 to 68.3 mN/m. The collected foam, having a ST of 60 mN/m, suggested that the biosurfactants generated were predominantly present in the foam phase [16]. The study also examined the effects of glucose concentration and pH on biomass growth and surface tension. Optimal biomass growth (as measured by optical density, OD) was attained at a glucose content of 40 g/L and an initial pH of around 7, resulting in an OD value of 4.5. Fermentation at pH 9 resulted in the lowest surface tension, whereas greater glucose concentrations yielded maximum product yield [17, 18].

Comparing the three examined bacterial strains, *B. licheniformis* grew the fastest and produced the most biosurfactant, producing the highest output. Compared to *B. subtilis, G. stearothermophilus* and *B. licheniformis* produced more biosurfactants with 15% and 5.2% more crude product, respectively. In comparison, earlier research, reported yields ranging from 0.5 g/L to 1.8 g/L [19, 20]. We also evaluated the correlation coefficients between conductivity and pH and the relationship between permittivity and OD_{600} to determine their potential as monitoring indicators for biosurfactant production on a large scale utilizing an inline system. Significant associations were found between these variables, suggesting that conductivity and pH might be used as reliable markers of biosurfactant production. The significant correlation between permittivity and OD_{600} adds to their use as monitoring measures, giving valuable insights for process optimization and control in biosurfactant production. Furthermore, it emphasizes the

efficiency of lipopeptide-type biosurfactants as bio-fungicides over polymeric-type emulsifiers [4, 21].

During *in vitro*, all three bacterial strains efficiently suppressed the plant pathogenic fungus *A. alternata*. However, in in vivo plant tests, the broth supernatant of *G. stearothermophilus* demonstrated less efficiency in lowering disease incidence and severity when compared to *B. subtilis* and *B. licheniformis*. When applied to plants in vivo, biosurfactant-producing bacteria, namely *B. subtilis, G. stearothermophilus*, and *B. licheniformis*, reduced *A. alternata* disease severity by 30%, 5%, and 25% in the plant leaves, respectively (Fig. 4). Furthermore, treatment with bacterial supernatant resulted in moderate to high chlorophyll a and b concentrations in the plants, indicating increased photosynthetic activity. According to these findings, *B. licheniformis* is the most efficient bacteria for biosurfactant generation and plant treatment [17, 21].

Using *B. licheniformis* and *B. subtilis* as bio-fungicides offers a sustainable agricultural alternative. To increase our understanding of biosurfactant-based bio-fungicides, future studies will focus on increasing plant sample numbers since we have had only limited plant growing capacity until yet, and on applying advanced disease severity analysis methodologies, as well as on exploring the relationship between biosurfactant treatment and plant production.

(a) (b)

Fig. 4 *In vivo* biofungicid plant tests; (a) *A. alternaria* infected beans (yellow leaves) infected and treated (green leaves); (b) healthy control beans (non-infected, non treated)

4 Fermented cosmetic ingredients

Our research group has been investigating different *Lactobacilli* including probiotic strains as well for several decades. Recently, this research was extended to the aim of cosmetic usage both fermentation broth and harvested cells.

Given that three out of the five healthiest meals on earth are fermented, fermentation has garnered attention from customers, product developers, and researchers worldwide. Even in the cosmetics industry, fermented cosmetics have gained popularity, creating a market that emphasizes the positive association of healthy fermented substances with environmental friendliness and skin health. Additionally, there is a growing focus on exploring the applications of various naturally occurring organo-mineral rocks.

In our research, we investigated the effects of alginite, a volcanic-origin rock, on the biomass production of probiotic strains and its potential use in cosmetics. This unique raw material has shown benefits in terms of gastrointestinal health and has exhibited positive effects on probiotic strains in animal models [22, 23]. In our study, we discovered that alginite also enhances the specific growth rate and dry mass of *Lactobacillus paracasei* (NCAIM B.01525) [15]. We observed similar effects on the other four probiotic strains as we did on *L. paracasei*.

Furthermore, we examined the fermented filtrates with and without alginite for cosmetic purposes, such as their moisturizing effect on the skin (Fig. 5), antioxidant activity (measured using 1,1-diphenyl-2-picrylhydrazyl (DPPH)), and skin whitening properties (measured through mushroom tyrosinase activity). The results indicated that alginite generally does not have a positive added effect on skin moisturization, and in fact, it reduces the antioxidant activity of the fermented filtrate. However, we encountered unexpected results regarding mushroom tyrosinase inhibition. The alginite-based filtrates showed an increase in this activity, while the filtrates without alginite inhibited the enzyme, suggesting that these ferment filtrates contribute to skin browning [24].

Currently, we are investigating the ferment lysates for cosmetic purposes using the same methods mentioned earlier, with one exception: we are assessing the antioxidant activity based on the CUPric Reducing Antioxidant Capacity (CUPRAC) method instead of DPPH.

5 Summary

In our researchgroup called Fermentation Pilot Plant Laboratory of the 150 years old Faculty we focus on application and development of microbial fermentation-based

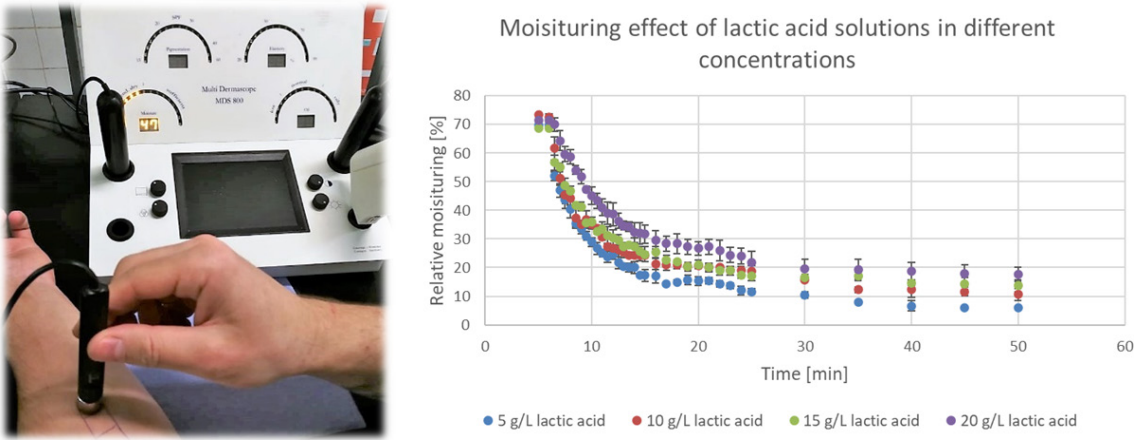

Fig. 5 Short term skin-moisturing tests with Dermatoscope for lactic acid solutions as controls for *Lactobacilli* broth

processes and products. Here we gave a short overview of our 4 ongoing research filed including microalgae, yeasts, *Bacilli* and *Lactobacilli*. The presented developments clearly shows the capability of our research group in generating innovative solutions for many different applications of different microorganism in accordance with the high scientific and technology standards at our faculty.

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