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INFLUENCE OF MICROALGAE ON BACTERIA, YEASTS, AND MOLDS OF SIGNIFICANCE IN AGRICULTURE AND FOOD PRODUCTION

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Introduction

Algae are known to be a potential source of biologically active secondary metabolites. The objective of this research was to investigate the influence of various microalgae and their extracts on beneficial and detrimental microorganisms (bacteria, yeasts and molds) in an effort to find evidence for stimulation or inhibition.

Part 1

In the first phase of this study, aqueous and methanolic extracts of microalgal species, such as *Anabaena variabilis*, *Nostoc commune*, *Spirulina china*, *Tolypothrix tenuis*, and *Chlorella vulgaris*, were tested for stimulatory or inhibitory activity on bacteria (*Bacillus thuringiensis* NCAIM B.01292, *Bacillus subtilis* NCAIM B.01095), yeasts (*Candida parapsilosis* NCAIM Y.01011, *Saccharomyces cerevisiae* NCAIM Y.00151, *Zygosaccharomyces bailii* NCAIM Y.00734), and phytopathogenic (*Botrytis cinerea* NCAIM F.00744, *Fusarium oxysporum* NCAIM F.00728) and spoilage (*Aspergillus wentii* NCAIM F.00167, *Penicillium expansum* NCAIM F.00601, *Rhizopus stolonifer* NCAIM F.00654) filamentous fungi, also known as molds.

As for the preparation of algal extracts, the microalgae were grown under controlled laboratory conditions. The final suspensions were centrifuged to obtain a supernatant-free biomass. The biomass was freeze-dried and stored at –20°C until the start of bioassays. Just before the bioassays, the samples were resuspended in distilled water (200 g/20 L), ultra-sonicated for 5 minutes and diluted. The in vitro bioassays were based on the agar diffusion method. Specific culture media (bacteria: CASO agar, yeasts: GYP agar, molds: PD agar) were inoculated with a standardized quantity of suspension containing 1.5×10^8 CFU/mL of bacteria or 1.0×10^7 CFU/mL of yeasts or 1.0×10^5 CFU/mL of molds (conidia). Wells of 8 mm diameter were punched in the agar plates (20 mL in standard glass Petri dishes) and filled with approximately 90 µL of microalgal extracts at concentrations of 2.5 g/L, 5 g/L and 10 g/L apiece. The inhibition or stimulation zones were measured after incubation at 37°C (bacteria) or 26°C (fungi).

The results indicated that *C. parapsilosis* was largely sensitive to the inhibitory substances found in some of the *Chlorella* and *Spirulina* extracts screened (data not shown). It is clearly evident from **Figs 1 and 2** that the aqueous extracts of *A. variabilis* and *T. tenuis* inhibited the growth of *B. cinerea* and *Z. bailii* to a great extent. It is worth noting that *Z. bailii* is a major spoilage organism in soft drinks and foods, due to its capability of tolerating sorbate concentrations of up to 4% and a water activity of 0.85 or below.

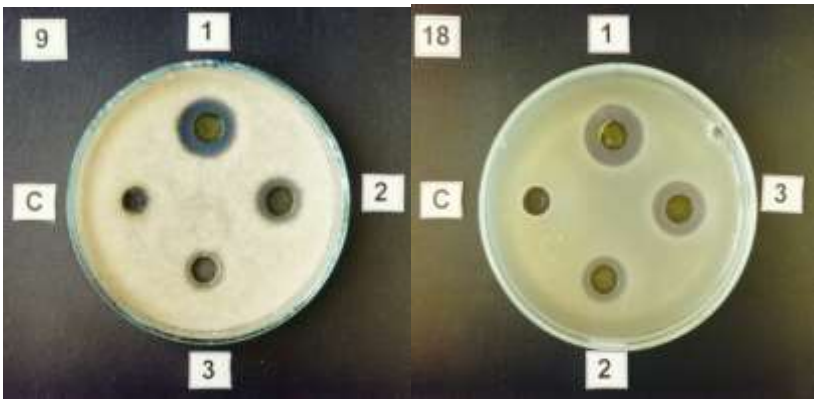

Figure 1 Inhibitory properties of the aqueous extract of *Anabaena variabilis* on *Botrytis cinerea* (left) and *Zygosaccharomyces bailii* (right).
C: control, 1: 10 g/L *Anabaena* extract, 2: 2.5 g/L *Anabaena* extract, 3: 5 g/L *Anabaena* extract.

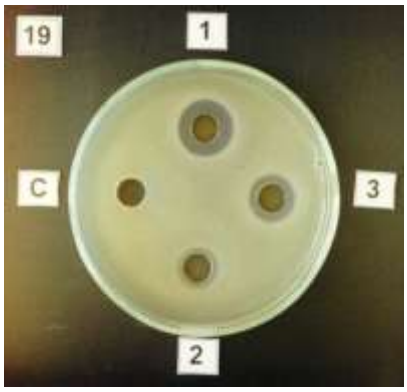

Figure 2 Inhibitory properties of the aqueous extract of *Tolypothrix tenuis* on *Zygosaccharomyces bailii*.
C: control, 1: 10 g/L *Tolypothrix* extract, 2: 2.5 g/L *Tolypothrix* extract, 3: 5 g/L *Tolypothrix* extract.

The influence of an *Anabaena* (83/95) biomass at two levels (1 g/L and 3 g/L) on growth rate of *B. thuringiensis* during fermentation was also monitored. The results obtained are illustrated in **Fig. 3**.

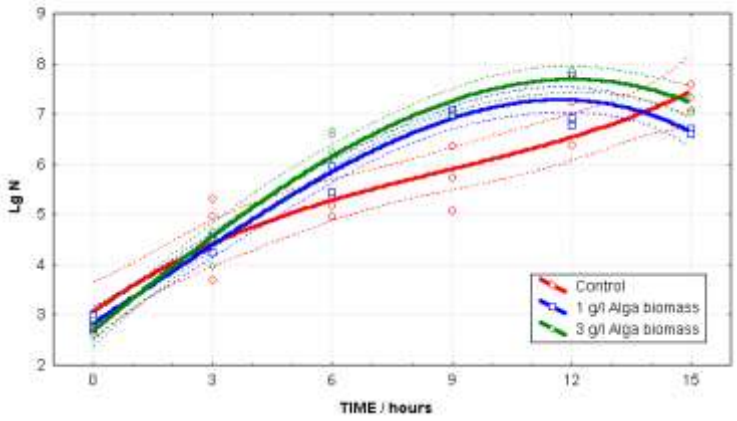

Figure 3 Influence of an *Anabaena* 83/95 biomass on growth rate of *Bacillus thuringiensis*

The growth of *B. thuringiensis* was stimulated considerably by *Anabaena* and *Spirulina* biomasses and their extracts. *Bacillus thuringiensis* is a Gram-positive, aerobic or facultatively anaerobic entomopathogenic bacterium found in soil and on plant surfaces.

During sporulation, it can be distinguished from other *Bacillus* species by their ability to produce crystalline protein inclusions that have toxic activity against different invertebrates, especially insects. Therefore, the use of certain cyanobacterial extracts in the manufacture of this biocontrol agent may have advantages in terms of production efficiency.

Part 2

In the second part of our research work, we aimed to find out whether growth and acid production of commercial dairy starter cultures could be stimulated by addition of a *Spirulina platensis* biomass, and to identify the substances responsible for the effects observed. The influence of 3 g/L *S. platensis* Hau biomass enriched with trace elements on the rate of acid development by and growth rate of pure and synchronized mixed cultures of *Streptococcus thermophilus* CH-1, *Lactobacillus delbrueckii* subsp. *bulgaricus* CH-2, *Lb. acidophilus* La-5, *Bifidobacterium lactis* Bb-12, *Lactococcus lactis* subsp. *lactis* 89.07 and *Lc. lactis* subsp. *cremoris* 89.07 were evaluated in milk. The components of the cyanobacterial biomass responsible for the stimulation caused were also identified in laboratory simulations wherein trace elements, vitamins and nitrogenous compounds were tested.

It was concluded that addition of the *Spirulina* biomass resulted in increasing the rate of acid development by all six individual strains significantly ($P < 0.05$), although to varying degrees. The stimulation observed was especially pronounced in the case of two probiotic strains, *Lb. acidophilus* La-5 and *B. lactis* Bb-12, as shown in **Figs 4 and 5**.

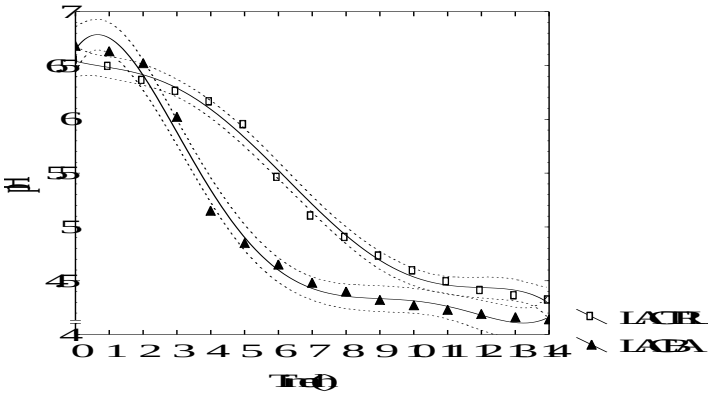

Figure 4 Effect of 3 g/L *Spirulina platensis* biomass on acid production of *Lactobacillus acidophilus* La-5 in milk

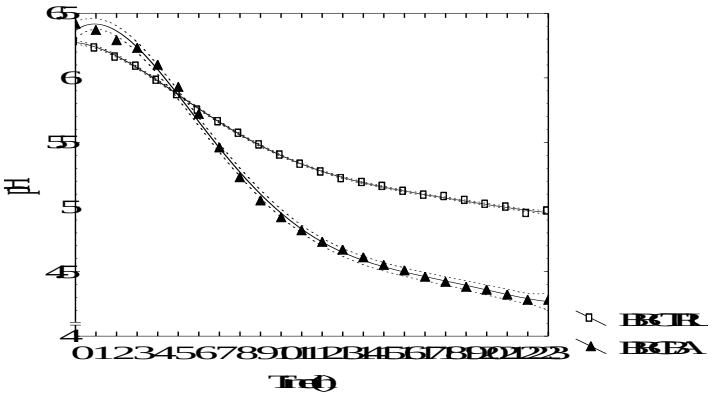

Figure 5 Effect of 3 g/L *Spirulina platensis* biomass on acid production of *Bifidobacterium lactis* Bb-12 in milk

It was demonstrated that stimulation of all the aforementioned strains by the *Spirulina* biomass was, for the most part, due to peptone, adenine and hypoxanthine; and the *S. platensis* biomass, being rich in trace elements, vitamins, sulfur-containing amino acids and unsaturated fatty acids, also had a highly beneficial effect on the nutritional value of milk, thus providing a new opportunity for manufacture of functional dairy foods.

Thereafter, storage experiments were performed to monitor the changes in survival of the microflora of *Spirulina*-enriched and control yogurts and probiotic fermented milks kept at 4°C or 15°C for 5 to 6 weeks. Characteristic viable cell counts of over 10^8 CFU/g were found both in control and *Spirulina*-fortified yogurts, regardless of storage temperature. However, the viable cell counts were significantly higher ($P < 0.05$) in the *Spirulina*-containing samples than in controls at 4°C (**Fig. 6**).

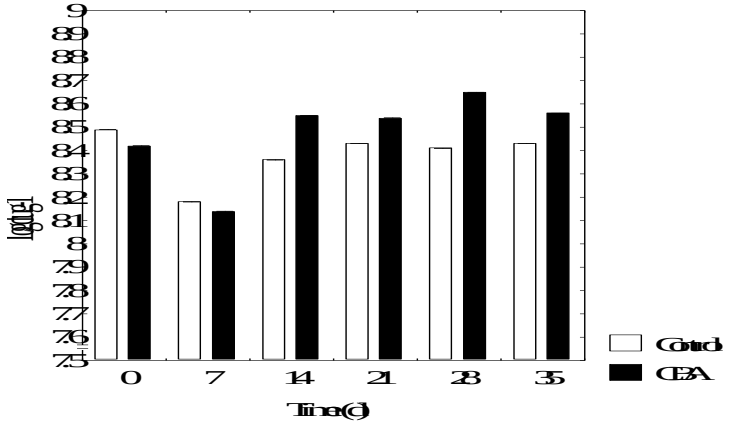

Figure 6 Changes in the characteristic viable cell counts of control and *Spirulina*-enriched (CBA) yogurts during storage at 4°C

The yeast and mold counts, both in the *Spirulina*-supplemented and control samples, were as high as 10^1 CFU/g on day 6 and 10^5 CFU/g on day 15 of the storage period at 15°C; whereas the *Spirulina* yogurt, after 1 month of storage at 4°C, had a significantly lower ($P < 0.05$) yeast and mold count than did the control yogurt (**Fig. 7**).

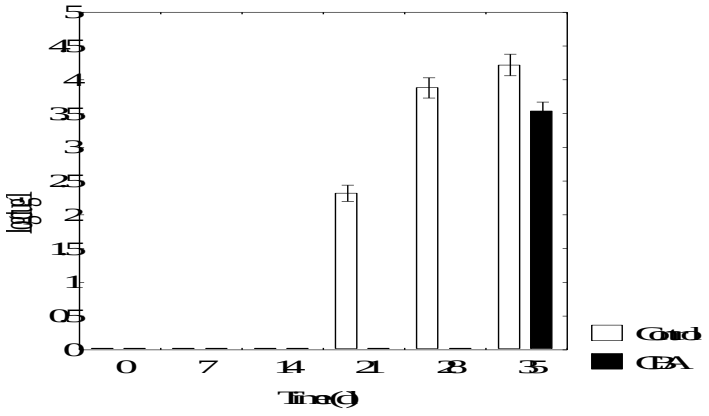

Figure 7 Changes in yeast and mold counts in control and *Spirulina*-enriched (CBA) yogurts during storage at 4°C

As for the probiotic fermented milk, the results obtained showed that the *S. platensis* biomass positively influenced the survival of starter bacteria regardless of storage temperature. The presence of *Lb. acidophilus* gave values within the range of 10^7 CFU/mL at each sampling time and the *Strep. thermophilus* counts also exceeded the critical level by far,

reaching values higher than 10^9 CFU/mL in most of the cases. As one would expect, bifidobacteria were highly susceptible to acid injury. Their counts fell more sharply than did those of lactobacilli and streptococci, however, the addition of *Spirulina* biomass was of beneficial effect on their viability.

All things considered, by inhibiting the growth of contaminating yeasts and molds and by maintaining the count of characteristic microorganisms at a high level during storage, the *S. platensis* biomass extended the shelf life of fermented milks at low storage temperatures.

Part 3

Finally, in the third stage of this research project, the effect of spray-dried *Spirulina platensis* and *Chlorella vulgaris* biomasses, added at a rate of 3 g/L, on acid production and growth of *Lactobacillus plantarum* and *Enterococcus faecium* strains used for feed fermentation purposes was evaluated in milks with total solids contents ranging from 12% to 30%. The acid development by and growth rate of *Lb. plantarum* and *E. faecium* were found to be stimulated significantly ($P < 0.05$) by *S. platensis* and *C. vulgaris*, respectively, in all culture media formulations used (data not shown). The dry matter content of milks did not influence remarkably the growth and acidification properties of the starter organisms tested. In conclusion, the powdered *Chlorella* and *Spirulina* biomasses rich in biologically active compounds proved to be potentially suitable for use in cost-effective production of milk-based functional fermented feeds.