Evaluation of salt and light stress on carotenoids production by *Micrococcus luteus*.

Vitória Durães Chlusewicz[a].

[a] Bioprocess Engineering and Biotechnology, Federal Technological University of Paraná, Toledo, Paraná, Brazil, chlusewicz@alunos.utfpr.edu.br.

Abstract. Some bacteria can produce carotenoids as secondary metabolites, and they may have a photooxidative protection function, and previous studies point out the potential of *Micrococcus luteus* for the accumulation of sarcinaxanthin, a 50-carbon carotenoid. These compounds may have antimicrobial and antioxidant properties, protection against UV radiation, and health benefits. Given the various possible applications, the present work aimed to evaluate the influence of salt and light stress on the production of carotenoids from *M. luteus*. The experiments were performed in 125 mL Erlenmeyer flasks with 50 mL of soy tryptone broth with sucrose and potassium nitrate at 35 °C, pH 7.00 and 150 rpm, with sufficient inoculum volume for optical density of 0.1. The experimental conditions were no salt without light, no salt with light, salt without light, salt with light, and salt with blue light. The latter condition was evaluated by packing the flasks with blue cellophane paper. For the no-light condition, the flasks were covered with kraft paper. For the light supply, three 20 W led lamps were attached to the surface of the orbital shaker. The samples were removed at different times up to 40 h and centrifuged, the supernatant was separated to measure the pH, the pellets were resuspended in 10 mL of methanol for carotenoid extraction, and then resuspended in water for biomass determination. According to the results, there was a decrease in the pH of all experiments evaluated, which showed that the salt and light did not influence the pH. Similar behaviour was observed in biomass production, as the different experiments presented a similar growth curve. Regarding pigment production, it was verified that the presence of salt did not interfere, but the highest productivities occurred in the conditions with light. At blue light condition, it was observed an increase in productivity, corresponding to a value of 13.78 % in relation to the visible light condition. It is concluded from that the bacterium *M. luteus* has a great carotenoid producing potential when submitted to light stress conditions, but salt did not influence such production.

Keywords. Fermentation, Biopigment, Sarcinaxanthin, Influence.

1. Introduction

Carotenoids are pigments derived from terpenoids that can have yellowish to reddish tones and are found in plants, algae, fungi and bacteria. In fungi and bacteria, they are secondary metabolites that have a photooxidative protection function. These compounds have become increasingly prominent due to their antimicrobial, antioxidant and UV radiation protection properties, as well as their health benefits in terms of their potential to prevent cardiovascular and Alzheimer's diseases. [1]

Several microorganisms in nature are capable of producing carotenoids, as yeasts, algae or bacteria. Among bacteria, previous studies point to the potential of *Micrococcus luteus*, a Gram-positive bacterium belonging to the Micrococcaceae family in the Actinomycetales order. They are cocci without motility and do not form spores. Some studies on this microorganism have shown its potential for accumulating sarcinaxanthin, a 50-carbon carotenoid. [2,3]

In this sense, with a view to the applications of carotenoids in various areas of the food, pharmaceutical and cosmetics sectors, as well as the potential of the bacterium *Micrococcus luteus* to produce these pigments, the present work aimed to evaluate the associated and disassociated light and salt stress in this bioprocess.

2. Research Methods

The experiments were conducted at the fermentation laboratory of the Federal Technological University of Paraná (UTFPR), Toledo Campus.

2.1 Cultivation conditions

To carry out the experiments, the microorganism was grown in 125 mL Erlenmeyer flasks with 50 mL of medium, the composition and conditions of which are shown in Table 1. After preparing the medium, the microorganism was added to a volume of inoculum that corresponded to an optical density of 0.1 at the initial time at a wavelength of 600 nm. [4]

Tab. 1 – Cultivation conditions .

Conditions	
Culture medium (g L^{-1})	Soy tryptone broth (30)
Carbon source (g L^{-1})	Sucrose (17,5)
Nitrogen source (g L^{-1})	KNO_3 (20)
Temperature (°C)	35
pH	7,00
Shaking (rpm)	150

To carry out the condition with light, three 20 W LED lamps were attached to the orbital shaker (Fig. 1), constantly emitting light with a wavelength in the visible light range (400 to 700 nm), with a total intensity of 4500 lumens [5] during the fermentation period.

Fig 2 – Cultivatons conditions with and without ligth, and with blue light.

For the no-light condition, the flasks were covered with kraft paper and sealed with tape so that no light could pass through. And to analyze the influence of light in the blue light wavelength range, approximately 450 nm, the Erlenmeyer flasks were covered with blue cellophane paper.

2.2 Analysis

After inoculation, samples of each condition were taken at different time intervals (h): 0, 8, 16, 24, 32 and 40. [6] At the end of each cultivation time, the samples were transferred to 50 ml Falcon tubes and centrifuged at 5,000 rpm. The supernatants were separated for pH determination.

The pellets formed were separated, resuspended in 10 ml of cold methanol and the samples were ultrasonicated for 10 minutes. The samples were then left to stand for 30 minutes at - 16 °C and then centrifuged at 5,000 rpm at 4 °C for 10 minutes. After the pigments were extracted, they were read in a spectrophotometer at a wavelength of 450 nm, determining the absorbance of each extract, using methanol as a blank.

The pellets formed were set aside for determining biomass production, so a solution was made with the pellet in a 50 mL flask with distilled water. The samples were read in a spectrophotometer at a wavelength of 600 nm, using distilled water as a blank.

3. Results and Discussion

To identify the experiments, acronyms were used: medium without salt (S-), with salt (S+), without light (L-), with light (L+) and with blue light (LA+).

3.1 pH and Biomass production

At the start of fermentation, the pH of the culture media was adjusted to 7.0 and, as time went by, there was a drop in these values, for all the conditions evaluated, to between 5.0 and 5.5, as shown in Figure 2. In general, it can be seen that salt and light did not cause a significant difference between the experiments in relation to the different treatments evaluated, since the values observed were very close.

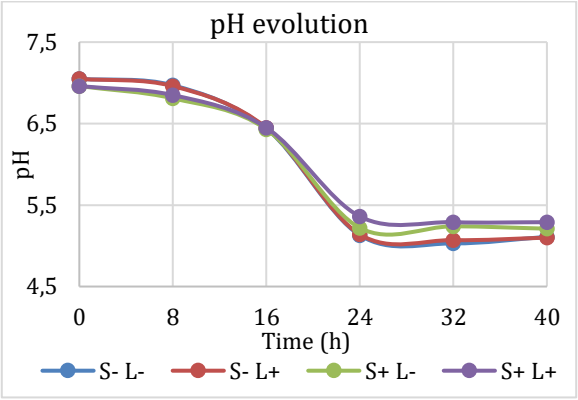

Fig. 2 – ph evolution during the fermentation.

In the work by Akbar *et al.* (2014)[7], there was no significant acidification of the nutritional medium, only a slight decrease in pH to values close to 5.00 observed after 24 h of incubation, a result similar to the present work. Also in their study, the *M. luteus* bacterium was unable to grow in a highly acidic (pH ≈ 3.0) or alkaline (pH ≈ 8.0) environment, with growth only being observed in a medium with a pH ≈ 5.0. In the work by Benjamin et al. (2016), maximum growth was found at a pH value of 7.0 after 24 hours of incubation in a medium containing

nutrient broth. This same pH value corresponded to maximum pigment production, but in 36 h of incubation.

As with the pH of the media, the different treatments apparently had no significant influence on biomass production either, as all the treatments showed a similar growth curve. According to Figure 3, it can be seen that the S-L+ condition showed the highest growth between 8 and 32 h, but at 40 h it showed the lowest concentration, possibly due to the depletion of substrates and nutrients at the end of the process. The S+L+ condition showed the lowest concentration at times 16 and 24 h, but showed the highest concentration at the end of the process, similar to the S-L condition. Finally, it can also be seen that both salt and light did not prove to be inhibitory to microbial growth at the concentration and luminosity used in this work.

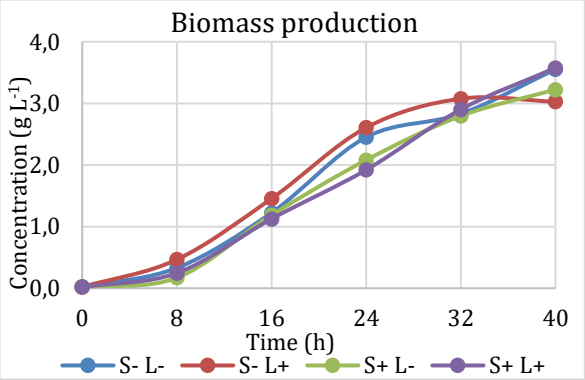

Fig. 3 – Biomass production during the fermentation.

In relation to salt stress on the formation of *M. luteus* biomass, Akbar *et al.* (2014) observed that this bacterium can grow in bile salt (2%) and NaCl 4%, and no growth was observed in increasing salt concentrations (6.0% and 8.0%). In the research carried out by Fernandez-Sanchez *et al.* (2021)[8], different salt concentrations were also evaluated, but it was observed that the bacterial mass decreased as the concentration of NaCl increased, with maximum biomass production occurring in the presence of 1 g L^{-1} of NaCl (0.1%) in 48 h of cultivation, while in higher concentrations of NaCl, the bacteria take longer to reach this level of mass.

With regard to light, Al-Wandawi (2014), based on his results, observed that white light was not essential for the growth of *M. luteus*, a fact that corroborated the results of the present work, since light also did not result in a difference in biomass production. The same author also suggests that the incubation period should not exceed 36 hours, while Benjamin *et al.* (2016) reported that maximum growth occurred in 30 h of incubation.

3.2 Carotenoids production

The S-L- condition was the one with the lowest carotenoid productivity, followed by the S+L- condition, as shown in figure 4. The S+L+ condition provided higher values, resulting in maximum production of the biopigment by the end of fermentation. Once again, salt did not interfere, as the lowest productions occurred in the conditions without light (S-L- and S+L-), which were 53.62 % and 55.11 % lower, respectively, compared to the conditions with light (S-L+ and S+L+). The blue light condition showed an increase of 13.78 % compared to white light. Although the blue light condition improved production compared to the visible light condition, it was still a small increase.

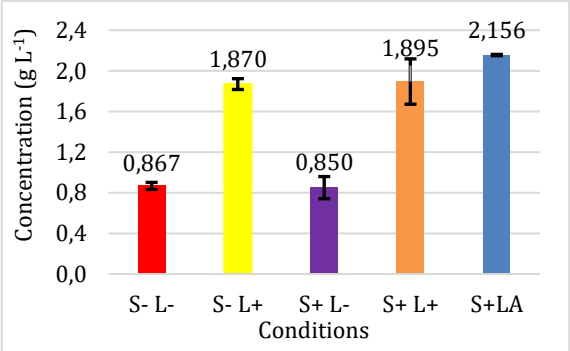

Fig. 4 – ph evolution during the fermentation

Schmidt (2016), in his work, found that maximum pigment production occurred at 48 hours of fermentation, corresponding to the end of the stationary phase and the beginning of the decline phase, followed by a considerable decrease in pigment production.

With regard to carotenoid production, this work resulted in a much higher value than those reported in the literature. This is possibly due to the fact that the conditions studied positively influenced the increase in pigment concentration inside the cell, given that biomass production was low.

Al-wandawi (2014) evaluated the influence of light on biomass production and obtained results that showed that light did not interfere with biomass production, which is consistent with the results obtained in this research, unlike carotenoid production. In carotenoid production, as already observed, light was very influential and increased the concentration of the pigment.

The study of the influence of light on the production of carotenoids by *M. luteus* is scarce, but there are several studies with algae, in which it is reported that the alga *Haematococcus pluvialis* can accumulate approximately 3% (m/m) of astaxanthin when exposed to light. [9] However, according to this author, when exposed to blue light, there is an increase in astaxanthin biosynthesis, which resulted in an improvement of approximately 7 % (m/m) in its content.

4. Conclusion

Based on this preliminary study, it was concluded that the *Micrococcus luteus* bacterium showed great carotenoid production potential and was strongly influenced by the presence of light. It was also noted that the condition created to simulate blue light

resulted in a small increase in carotenoid production, making further studies on the influence of the wavelength of light necessary.

This study made it possible to determine the optimum conditions for obtaining carotenoids, to quantify the production of cells and product, and to evaluate the influence of the incidence of light in the blue spectrum (±450 nm) in relation to the visible spectrum (400-700 nm).

The high productivity under the optimized conditions showed that *M. luteus* is a natural and alternative source of carotenoids, making it possible to produce them on an industrial scale for use in various sectors of industry. This study is innovative in the sense that it evaluates and contributes to the understanding of carotenoid production, as well as assessing the interfering factors that led to the higher yield of this bioprocess.

5. Acknowledgement

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