Effect of carbon source on carotenoid production by *Rhodotorula* sp.

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SUMMARY

The aim of the study was to determine the influence of some carbon sources on the growth and the carotenoid accumulation in the case of several *Rhodotorula sp.* strains. It was emphasized that the tested carbon sources positively influenced the growth of the yeasts cells, as well as the concentration of total carotenoid. The new isolate strain (Rd3) prove to have the highest growth rate and carotenoid concentration on glycerol medium, compared to Rd1 and Rd2 strains, for which optimum variant was sucrose medium.

Keywords: *Rhodotorula sp.*, carbon source, carotenoids

INTRODUCTION

Carotenoids represent a group of valuable molecules not only because they act as vitamin A precursors but also for their pharmaceutical, chemical, coloring, antioxidant properties, possible tumor-inhibiting activity and involvement in the visual attraction of animals such as flower pollinators or mating partners (Johnson and Schroeder, 1995). Several microorganisms, including bacteria, algae, molds and yeasts of the genera *Rhodotorula, Rhodosporidium, Sporobolomyces* and *Phaffia* are able to produce carotenoids naturally (Frengova and Beshkova, 2009).

Carotenoids are of importance in animals and humans, including enhancement of the immune response, conversion to vitamin A and the scavenging of oxygen radicals (Kiokias and Gordon, 2004). Animals cannot synthesize carotenoids and these pigments must therefore be added to the feeds of farm animal, including aqua-cultured salmon (Frankis, 2000). Humans are exposed to carotenoids through their diet, compounds that are present in vegetables and fruits as well as in animal products rich in carotenoids. These products might be additionally enriched in these components by specific feed additives.

Despite the availability of a variety of natural and synthetic carotenoids, there is always an interest in microbial sources (Ausich,

1997). Compared with the extraction from vegetables or chemical synthesis, the microbial production of carotenoids is very important, mainly because of the problems of seasonal and geographic variability in the production and marketing of several of the colorants of plant origin, and because of the economic advantages of microbial processes using natural low-cost substrates as carbohydrate sources (Frengova and Beshkova, 2009).

The synthesis of different natural important carotenoids by several yeasts species belonging to the genera *Rhodotorula* and *Phaffia*, has led to consider these microorganisms as potential pigment sources. Yeasts are more convenient than algae or molds for large-scale production in fermenters, due to their unicellular nature and high growth rate.

The objective of the present study was to investigate the influence of different carbon sources (glucose, sucrose, glycerol and fructose) on yeasts growth as well as on the carotenoids pigments biosynthesis.

MATERIAL AND METHODS

The biological material used in experiments was represented by three *Rhodotorula sp.* strains, designated as Rd1, Rd2 and Rd3. Two of them, strains Rd1 and Rd2, are from collection of Microbiology laboratory of Polytechnic University of Bucharest. The strain Rd3 is new isolated from Romanian grapes and it was identified as *Rhodotorula sp.*

The medium composition, designated as MS3, was previously obtained by research work (Ungureanu et al., 2010), with the following formula: carbon source to be tested, 1,5 g/L yeast extract, 5 g/L NH_4NO_3, 1 g/L KH_2PO_4, 0,4 g/L $MgSO_4 \cdot 7H_2O$ and 0,4 g/L NaCl. Trace elements are assumed to be taken from tap water.

The experiments were performed in 500 ml conical flasks, each containing 200 ml culture medium, on a rotary shaker (Heidolph Unimax 1010) at 150 rpm, for 7 days, at 28^oC, in order to formation of the carotenoids mixture, in the stationary phase of growth curve. The pH was not adjusted during the process.

A suspension of yeasts cells in sterile water was used for the inoculum preparation. Inoculum was analyzed in terms of number of cells/ml. The parameters analyzed during the cultivation were Optical Density (OD) at λ = 600 nm, evolution of pH and dried biomass concentration.

After cells separation by centrifugation, three freeze-thaw cycles were performed. The pigments extraction procedure was done after method of Peterson et al. (1954): acetone extraction of the total pigments mixture, including water soluble types, followed by n-hexane extraction to separate the total carotenoids content. The total carotenoids concentration was determined using a spectrophotometer

UV-VIS. For each extract corresponding to the mentioned 2 stages extraction in specific solvents – acetone and n-hexane – absorption spectra were obtained in 380 – 800 nm domain and the peaks were determined.

RESULTS AND DISCUSSION

On MS3 agar-medium the *Rhodotorula sp.* cells were colored from coral to pink, smooth, sometimes reticulate and rough. Microscopic aspect emphasized shapes ranging from spherical to elongate of the yeasts cells.

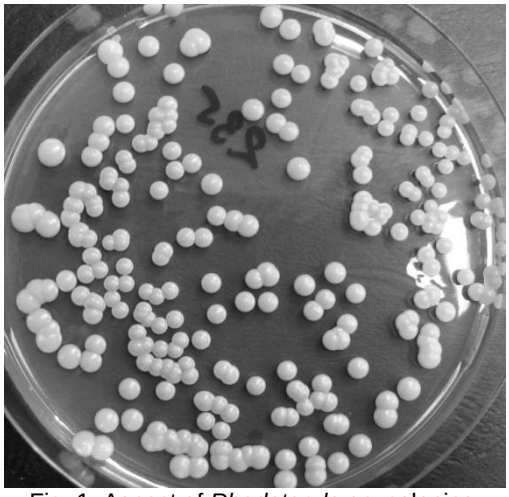

Fig. 1. Aspect of *Rhodotorula sp.* colonies.

The objective of the experiment was to study the influence of different carbon sources on yeasts growth as well as on the carotenoids pigments biosynthesis. Thus, as carbon sources there were used glucose, sucrose, glycerol and fructose.

In all cases, no matter the carbon source, it was observed an important growth of yeasts cells, for all three strains, during the exponential phase (48-72 h). Accumulation of pink-orange pigmented cells began to be obvious after two – three days. Figure 2 emphasize that on medium with glucose, Rd2 strain had the highest growth compared to Rd1 strain and the new isolate Rd3. Also it was observed that different concentrations of glucose used in our experiments, as carbon source in the culture medium, did not influence the initial result regarding the accumulation of cells and pigment biosynthesis (data not shown).

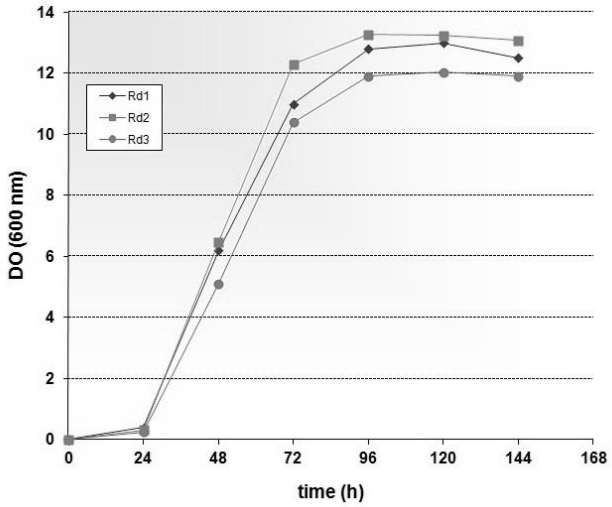

Fig. 2. The growth of *Rhodotorula sp.* strains on MS3 medium with glucose, as carbon source.

In similar experiments, Ferrao and Garg (2011) have shown that all the carbohydrates tested (glucose, sorbitol, xylose, sucrose and starch) served as good substrates and glucose was best for growth of their *Rhodotorula sp.* strain. When the carbon source content was highly increased (C:N ratio 20:1) it was observed an inhibitory effect on growth and β-carotene accumulation.

Also, glucose was reported to be a better carbon source for carotenoid production in earlier studies with *Rhodotorula glutinis* (Nam and Rhee 1991, Bhosale, 2001) and *Phaffia rhodozyma* (Fang and Cheng 1993). Easily utilizable sugars, like glucose, promote rapid cell growth and since the pigment production is directly proportional to the growth, presumably results into the higher production of carotenoids.

The pH-value of growth medium influences not only biosynthesis activity of culture, but also culture growth rate. In our experiments, it was observed that the optimum initial pH is 6.0 – 6.5. Also, the pH values decreased to acid domain (1.0 – 2.0) during the carotenoids formation process. Figure 3 emphasizes the relation between the pH values of the culture medium and the ratio OD final/ OD final maxim, being observed that the strain Rd2 had the best correlation.

These results confirm the general behavior of yeasts in their preference for slightly acidic pH (near 6.0) observed in previous experiments. Thus, at extreme pH, delayed lag phase and low specific growth rate were observed. Maximum carotenoid content was obtained at pH 7.0, while decline in cellular production was observed at both sides of neutral pH (Bhosale, 2001). Aksu and Tugba Eren (2005), Choudhari

and Singhal (2008) and Maldonade et al. (2008) also showed that as the pH increased, specific growth and carotenoid production rates increased and reached to a maximum level at pH 6,8 - 7,0 with a drop off at higher pH values. Moreover, the optimal pH also yielded the highest values of biomass cells and carotenoid.

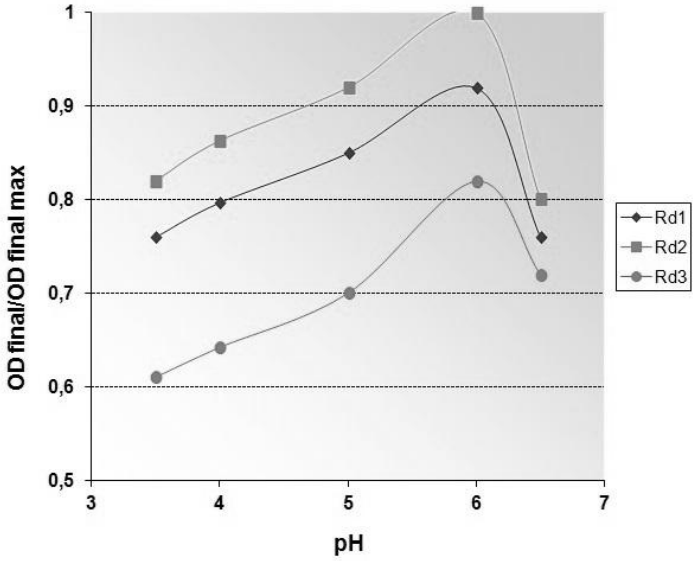

Fig. 3. The influence of pH values of the culture medium on the *Rhodotorula sp.* strains growth.

The different carbon sources have an important influence on the cell growth and also on the carotenoids biosynthesis. All four carbon substrates (glucose, sucrose, glycerol and fructose) prove to have a stimulating effect on these yeasts growth, although there are differences between the strains. Thus, regarding both the evolution and the carotenoids formation when glycerol was used in the medium, it was observed that the new isolate *Rhodotorula* strain Rd3 had the highest growth and total carotenoids content. These data were not obtained on the other medium formulas. In the same time, on the glycerol medium formula, Rd2 and Rd1 strains prove to have the lowest values regarding the cells growth and carotenoids accumulation, compared to those obtained on glucose, sucrose or fructose.

In similar experiments, Ferrao and Garg (2011), Ferdes et al. (2011), Frengova and Beshkova (2009) showed that glucose, glycerol, xylose, cellobiose and sorbitol were found to support biomass accumulation equally. Glucose appears to be the best carbon source for biomass and β-carotene production followed by glycerol. With higher carbon to

nitrogen ratio (20:1), glycerol was the best carbon source followed by sucrose.

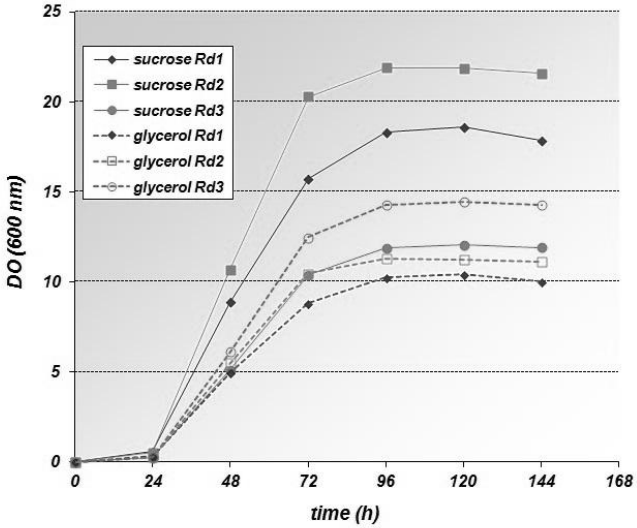

Fig. 4. Comparison between sucrose- and glycerol-medium formulas.

Contrary, whey, sucrose, glycerol, lactose and molasses were found to be poor substrates with respect to both cell growth as well as β -carotene production for other carotene producer (*Blakeslea trispora)*, while glucose and fructose were good carbon source for production of β -carotene (Choudhari and Singhal, 2008).

Glycerol may provide the major energy source for cell metabolism as well as the carbon element for biosynthesis of biomolecules. However, excessive glycerol was found to repress the synthesis of the carotenoids. This was also observed in the carotenoids production of *Phaffia rhodozyma* and not only for *Rhodotorula sp.* (Saenge et al., 2011).

In order to quantify the total carotenoids pigments concentration, it was determined the cell dry biomass and the ratio dry biomass/culture liter was calculated (data not shown).

The total carotenoids extraction was performed in two stages, using specific solvents: acetone and n-hexane. For each extract sample, specific absorption spectra were obtained, in 380 – 800 nm domain, the individual peaks being determined.

The final results indicate that the highest values for total carotenoids concentration were obtained on sucrose medium (initial concentration 30 g l^{-1}) for both Rd1 and Rd2 strains. On the other hand, on glycerol

medium formula, the highest value for total carotenoids concentration was obtained for Rd3 strain.

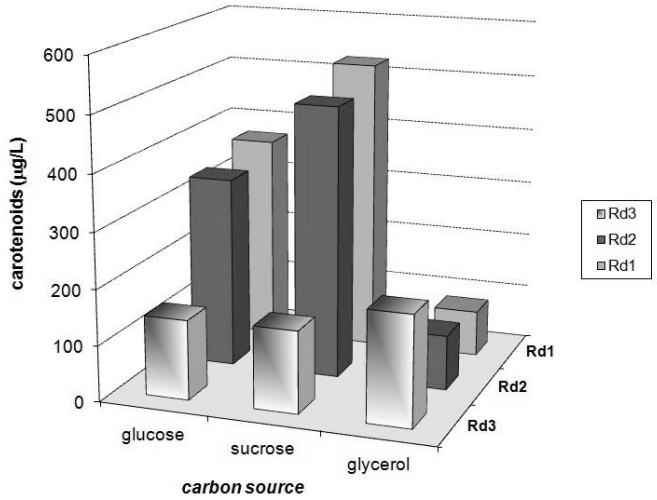

Fig. 5. The correlation between the carbon sources and the total carotenoids concentration.

Results are similar with those of Bhosale (2001), when sucrose yielded a comparable cell mass (g/l) and carotenoid production (mg/g). Also, experiments of Aksu and Tugba Eren (2007) and Latha et al. (2005) showed that the carotenoids formation rate and carotenoids production was the highest in sucrose medium and reached a maximum level at 20 g l^{-1} initial sucrose concentration.

Contrary to these results, Ferdes et al. (2011) observed that, for the tested *Rhodotorula* strain, the highest pigments yield it has been obtained in the case of fructose medium, followed by the concentrations accumulated when glucose and sucrose were used.

Also, for *Blakeslea trispora*, glucose and fructose were good for production of β-carotene, but no production was found with sucrose (Choudhari and Singhal, 2008).

CONCLUSIONS

Culture medium proves to have a significant influence on the yeasts biomass accumulation and carotenoids production.

For all carbon sources tested, the *Rhodotorula sp.* strains used in experiments emphasized an optimum growth.

The highest pigments concentration was obtained in the case of sucrose medium formula for two of the *Rhodotorula sp.* strains (Rd1 and Rd2), 526 µg/L respectively 488 µg/L. For the third yeast strain (Rd3), the highest carotenoids accumulation was obtained on glycerol medium (198 µg/L).

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