STUDY OF THE INFLUENCE OF AQUEOUS EXTRACTS FROM *ASCLEPIAS SYRIACA* ON THE DEVELOPMENT OF SPECIES OF *RHODOTORULA SP.*

STUDIUL INFLUENTEI EXTRACTELOR APOASE DIN *ASCLEPIAS SYRIACA* ASUPRA DEZVOLTARII UNOR SPECII DE *RHODOTORULA SP.*

HAINAL Anca Roxana, IGNAT Ioana, VOLF Irina, POPA V. I.
"Gheorghe Asachi" Technical University Iasi, Romania

Abstract. *The paper presents results obtained in the cultivation of two different strains of Rhodotorula spp. in a medium containing an aqueous extract of different concentrations in dry matter and polyphenolic compounds separated from Asclepias syriaca. The extracts were characterized in terms of content of polyphenols and sugars using various analytical techniques. Development of yeasts was monitorized by determining the amount of wet biomass during the experiment for nine days. After recovery of biomass, culture broth was characterized by total polyphenols concentration by the Folin-Ciocalteu method and pH evolution. The results obtained have shown the consumption of polyphenols by yeast, which influenced the growth in the biomass of the two different strains compared with the control.*
Key words: *Asclepias syriaca, Rhodotorula* spp., wet biomass.

Rezumat: *În lucrare sunt prezentate rezultate obţinute în procesul de cultivare a două tulpini diferite de Rhodotorula într-un mediu ce conţine un extract apos de diferite concentraţii în substanţă uscată şi compuşi polifenolici separat din Asclepias syriaca. Extractele au fost caracterizate din punct de vedere al conţinutului de polifenoli şi al zaharurilor folosind diferite tehnici analitice. Dezvoltarea drojdiilor a fost monitorizată prin determinarea cantităţii de biomasă umedă în cursul desfăşurării experimentului timp de nouă zile. După recuperarea biomasei, în lichidul de cultură s-au urmărit concentraţia în polifenoli totali prin metoda Folin-Ciocâlteu şi evoluţia pH-ului. Rezultatele obţinute au evidenţiat un consum al polifenolilor de către drojdii, consum care a influenţat creşterea în biomasă a celor doua tulpini diferite comparativ cu martorul.*
Cuvinte cheie: *Asclepias syriaca, Rhodotorula* sp., biomasa umeda

INTRODUCTION

Asclepias syriaca is a plant native of North America, with large, opposite, elliptical leaves, which contains a large amount of latex toxic to animals. The plant is also called the bees flower, being cultivated as ornamental and melliferous plants; in the wild it grows in the Ostrovul of Moldova Veche (http://www.eukarya.ro/enciclopedie/). The plant has been much studied because of many types of chemical compounds and each possesses its large number of uses. Initially the plant was investigated as a source of rubber, but small amounts of *cis*-

isoprene polymers with low molecular weight justify its application only as a component of chewing gum.

Along with rubber, latex extracted from the plant contains a resinous component, their ratio depending on the morphological and old plant parts (rubber / resin: first year: 0.2-0.7/8.2, year II: 0.2/0.7, year III: 0/16.3) (Rusan v. et al., 1984).

Asclepias syriaca plant is little demanding with respect to ground and exposure, easily multiplies and can be cultivated in our country on soils with normal chemical composition. Although, *Asclepias syriaca* possesses a high potential of chemicals the literature signals only sporadic, isolated and unidirectional (Corlateanu E. et al., 1982) separation and use.

As following extraction with hot water, we can extract inorganic salts, oligosaccharides, sugars and polyphenols (Chow P. et al., 2008), which can be used successfully as a carbon source in fermentative processes. Polyphenols include several classes of compounds like: phenols, phenolic acids, flavonoids, anthocyanins, and more complex structures such as tannins and lignins. Polyphenols are secondary metabolites normally produced by plants or in response to stress conditions, such as infections, the action of UV radiation doses and other factors. The recovery of compunds with nutritive and antioxidant potential of plant biomass is an economic problem, relevant to food and pharmaceutical industry. Currently there is little information regarding the use of polyphenolic compounds in fermentation yeasts. However, some research shows that there are species of yeasts that have the potential to fragment the polyphenolic compounds and to use as a carbon source (Dănăilă M. et al., 2007). In other cases oxidized polyphenols may have inhibitory effect on the growth and development of certain microbial strains. The mechanism of polyphenols toxicity can be explained by inhibiting hydrolytic enzymes, or other transport mechanisms such as blocking the protein, non-specific interactions with carbohydrates, etc. (Popa V.I. et al., 2007). In this context, the purpose of this study is to determine the influence of aqueous extracts of *Asclepias syriaca* on the growth and development of two strains of yeast *Rhodotorula* sp. compared, along with how polyphenols compunds of culture medium are consumed.

MATERIAL AND METHOD

Two strains of yeast *Rhodotorula sp*. denoted by R1 and R2 which were selected by Biotechnology Applied in Food Industry – Integrated Center for Research and Education – Bioaliment, "Dunarea de Jos" University, Galati were used. The preculture was done on culture medium with the following composition: 10 g/L glucose, 5g/L peptone, 3 g/L malt extract, 3 g/L yeast extract. The experiment was conducted on platform thermostatic mixers for 48h at 27ºC and 120 rpm. The cells were recovered by centrifugation at 5000 rpm for 15 minutes, washed twice with distilled water and inoculated on culture medium containing the following mineral and organic chemical composition: 15 g/L glucose, 2.5 g/L yeast extract, 3 g/L sodium acetate, 1 g/L $(NH_4)_2SO_4$, 1 g/L KH_2PO_4, 0.1 g/L $CaCl_2$, 0.25 g/L $MgSO_4 \cdot 7H_2O$, 0015 g/L $ZnSO_4$, 0015 g/L $CuSO_4 \cdot 5H_2O$. The culture medium was prepared in aqueous extract obtained from 0.5 and 5 g of *Asclepias syriaca* dry material, brought to 1L. The

culture medium was distributed in 250 mL Erlenmayer flasks with a volume of 100mL, after which inoculation took place. Determination of the number of inoculated cells was performed by reading optical density at 620 nm. An absorbency of 0.5 is equivalent to 10^7 cells in 1mL inoculum (Buzzini P., 2001). Each flask was inoculated with 4×10^7 CFU (CFU = colony forming unit).

Three cultures were perfomed, a reference culture that does not contain polyphenols extract, a culture containing polyphenolic extract from 0.5 g/L dry plant and a culture containing a polyphenol extract from 5 g/L dry plant. Each culture was developed for 9 days and at every 24 hours one sample was investigated. Cultures were coded as follows: R1 - culture on medium containing only recipe compunds, R1AS0.5 - culture on medium obtained with components of above the aqueous extract from 0.5 g/L dry plant brought to 1L, and R1AS5 - culture medium prepared using an aqueous extraction of 5 g/L dry plant material (Hainal A.R. et al., 2009), R2 - the culture medium that contains only ingredients of recipe, R2AS0 5 - culture on the medium containing in addition to the recipe extracts from 0.5 g/L dry plant material, and R2AS5 - culture on medium containing aqueous extracts of 5 g/L dry plant material with the basic components of the culture medium.

After every 24 hours, cells were recovered by centrifugation at 4000 rpm for 15 min and washed twice with distilled water. Wet cell mass was determined by weighing, and expressed in g/L culture medium, the pH was not regulated during culture, being determined after cells recovery by centrifugation. Also, the culture medium was characterized from the point of the concentration of total polyphenols by Folin-Ciocalteu method (Popa V.I. et al., 2007).

RESULTS AND DISCUSSIONS

Aqueous extracts were characterized and data are presented in table 1. As following tests carried out, we did identify simple and complex sugars.

Table 1

The characteristics of *Asclepias syriaca* aqueous extracts

Sample	Total polyphenol concentration, mg/L gallic acid	Content in dry substance, g/L extract	Content in organic matter, g/L extract	Ash content, g/L extract	Ash content on dry weight basis,%
AS 5	58.0	1.084	1.004	0.08	7.0
AS 0.5	6.2	0.1084	0.1004	0.0008	0.7

AS 5 - aqueous extract frrom 5 g dry plant material brought to 1L;
AS 0.5 - aqueous extract frrom 0.5 g dry plant material brought to 1L;

From table 1 we can notice a huge difference in terms of total polyphenol concentration; the difference will influence the behavior of two different strains of *Rhodotorula sp.* during the process. Thus, in the case of R1 strain (fig. 1) we may notice a positive influence on the growth of yeast when it is used extract from 5 g dry material, compared with the reference culture. When an extract from 0.5 g is used we distinguish an inhibition on the development of the micro-organism compared with the control culture, which characterizes a better efficiency of the cultivation process when a culture is carried out in the extract from 5 g of dried plant material. In terms of the two strains, the situation is different in the case of R2.

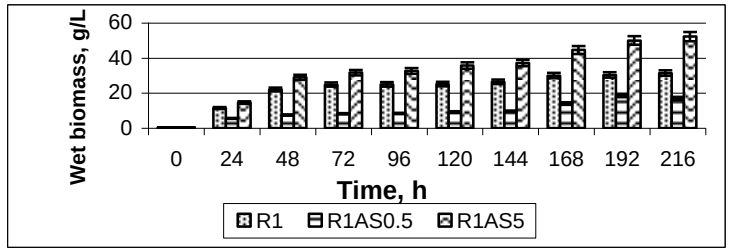


Fig. 1. Variation of amount of wet biomass depending on time cultivation for strain R1

One can also see the stimulating effect on the culture process when is using extracts from 5 g of plant material and the phenomenon of inhibition for media prepared with extract from 0.5 g plant material, compared with blank. This time, however, there is no a pronounced inhibition effect in comparison with reference culture (fig. 2).

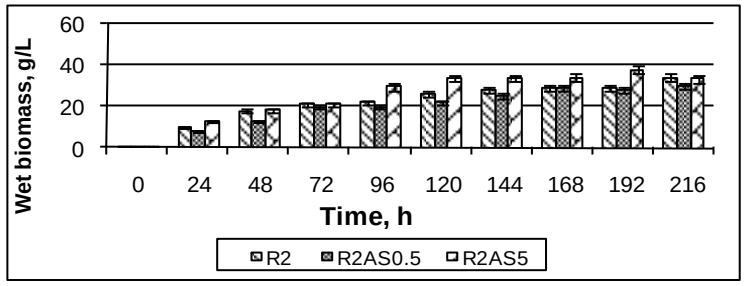


Fig. 2. Variation in the amount of wet biomass during the cultivation of strain R2

A comparison of the behavior of the two strains is more obvious in Figure 3, which could explain by different metabolic pathways of the studied micro-organisms.

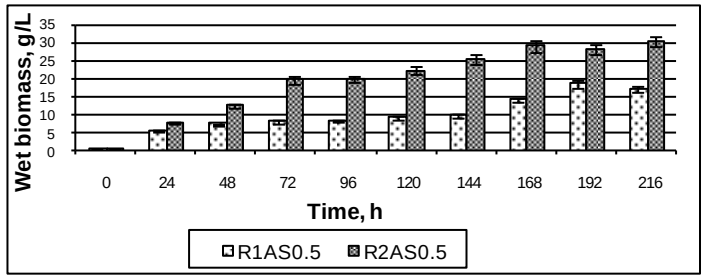


Fig. 3. Comparison of the biomass yield of the two strains grown on aqueous extract from 0.5 g/L dry plant material

In figure 4 we can see a comparison between the evolutions of the two strains of *Rhodotorula sp*., grown in medium containing 5 g extract of plant

material. In this case we can clearly observe a much better yield in biomass of strain R1 compared to that provided by R2 strain throughout the culture process.

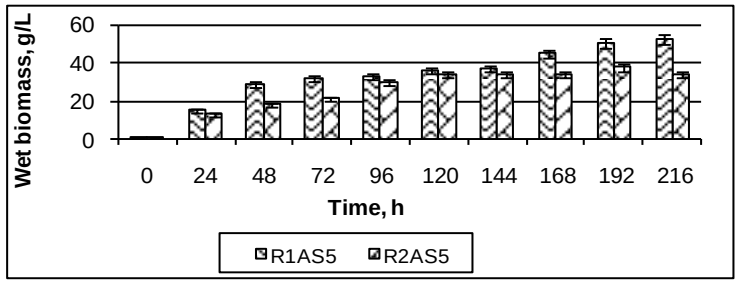
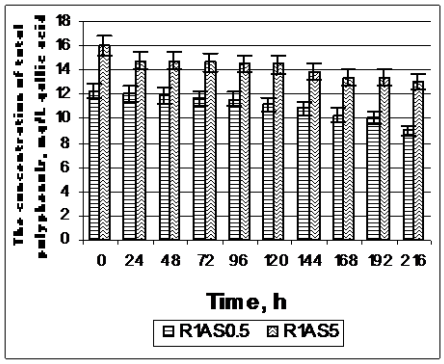
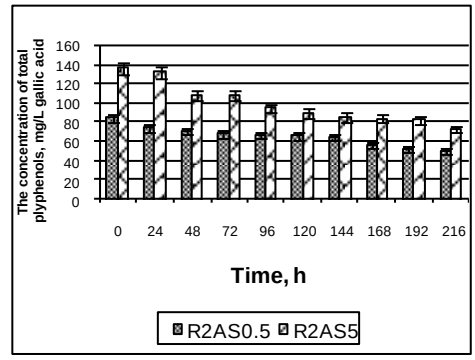


Fig. 4. Comparison of the biomass yield of the two strains grown on aqueous extract from 5 g/L dry plant material

The evolution of total concentration of polyphenolic compounds throughout the experiment can be followed in figure 5. The data obtained during the process show that the content in polyphenols is decreased because they are used by yeasts. The two strains are different from each other because the rate of consumption of polyphenols is slowly reduced in the case of strain R1 (fig.5 a) and decreases for the strain R2 within 48 hours to be progressively reduced by the end of the process.





a) **b)**

Fig. 5. Variation of total polyphenol concentration from culture medium:
a - strain R1; **b** - strain R1

Regarding the change of pH during the culture process it can say that: the value of this parameter decreases sharply, both for the reference culture and of that in the presence of the polyphenolic extract performed for a period of 72 hours, then jump to maintaining an upward trend until the end of the experiment (fig. 6).Decreased pH values by weak acid could be explained by the fact that the process of monosaccharides glycolysis acidic compounds are formed (acid-enzyme complex reaction-intermediate, 3-fosfogliceric acid, pyruvate).

Further increase of pH after 72 hours of experiments could be determined by the formation of different phosphorylated compounds in various stages of glycolysis, compounds that induce a weak basic character and alter the pH to values around 7.

Also, changes in pH could be determined by the acidic products which are intermediary compounds in the degradation and metabolism of polyphenols.

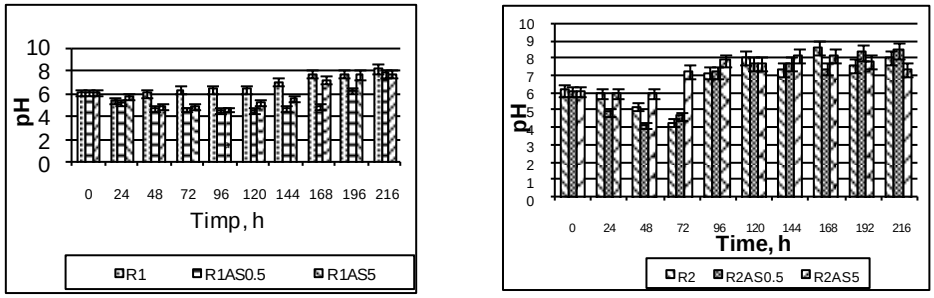


Fig. 6. Changes in pH during cultivation; A- strain R1; B- strain R1

CONCLUSIONS

1. Yeast strain *Rhodotorula sp.* denoted by R1 biosynthesizes biomass yields much better in a medium containing 5 g extract from dried plant material, in comparison with the reference culture. At lower concentrations, polyphenols exhibit a strong inhibitory effect. This occurs less pronounced when strain R2was cultivated. Polyphenolic compounds present in extracts are used as carbon source for the two different microorganisms and metabolic pathways are specific to each of them.

2. Therefore, aqueous extracts from *Asclepias syriaca* may be used in culture media compositions of the two yeast strains studied whose components may be metabolized as carbon sources. The metabolic pathways seem to be specific to the two organisms, aspect which will by later analyzed.

REFERENCES

1. **Buzzini P., 2001** - *Batch and fed-batch carotenoid production by Rhodotorula glutinis-Debaryomyces castellii co-cultures in corn syrup*. J. Appl. Microbiol., Vol. 90, pg. 843-847;
2. **Corlateanu E., Ailincai N., Moise A., 1982 –** *Cercetari privind planta celulozica Asclepias syriaca*. Celuloza si Hartie, nr 4, pg. 201-204;
3. **Chow P., Nakayama F. S., Blahnik B., Youngquist J. A., Coffelt T. A., 2008** - *Chemical constituents and physical properties of guayule wood and bark*. Industrial crops and products 2 8, pg. 303–308;
4. **Dănăilă M., Popa V.I., Volf I., 2007** - *Influence of polyphenols on the biosynthesis of carotenoids by yeasts*. Proceedings of the 8 th ILI Forum, Rome, May, 10-12, p. 105-108;
5. **Hainal A. R., Volf I., Popa V. I., 2009** - *Study regarding the influence of Asclepias syriaca aqueous extracts in the development of a strain of yeast (Rhodotorula sp.)*. Buletinul Institutului Politehnic din Iasi, Tomul LV(LIX), Fasc. 4, pg. 95-101;
6. **Popa V. I., Anghel N., Dănăilă M., 2007** - *Natural polyphenolic compounds. Structure, bioavailability and microbial interactions*. Proceedings of the 8 th ILI Forum, Rome, May, 10-12, p. 97-100;
7. **Rusan V., Cascaval C. N., Rosu D., Popa V., 1984** – *Valorificarea complexa si integral a plantei laticifere Asclepias syriaca L. I. Consideratii asupra potentialului biologic si chimic*. Institutul agronomic "Ion Ionescu de la Brad" Iasi, Lucrari stiintifice, Vol 28, pg. 107-111, seria agronomie, ISSN 0379-8364;
8. http://www.eukarya.ro/enciclopedie/regnul-plantae/asclepias-syriaca-ceara-albinei