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Effects of the polyphenolic extract over bacterial proteins biosynthesis on methanol

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Abstract

The paper investigates the possible effects of the polyphenolic extract from vine in the case of biosynthesis process of the Methylomonas sp.M.14.1 that is a producer of the bacterial proteins on methanol.

The paper looks into aspects related to the quantity of polyphenolic extract that can be added to the culture medium, in such a way that the biomass production should increase and the cell accumulation dynamics and biomass chemical composition should register high qualitative and quantitative modifications.

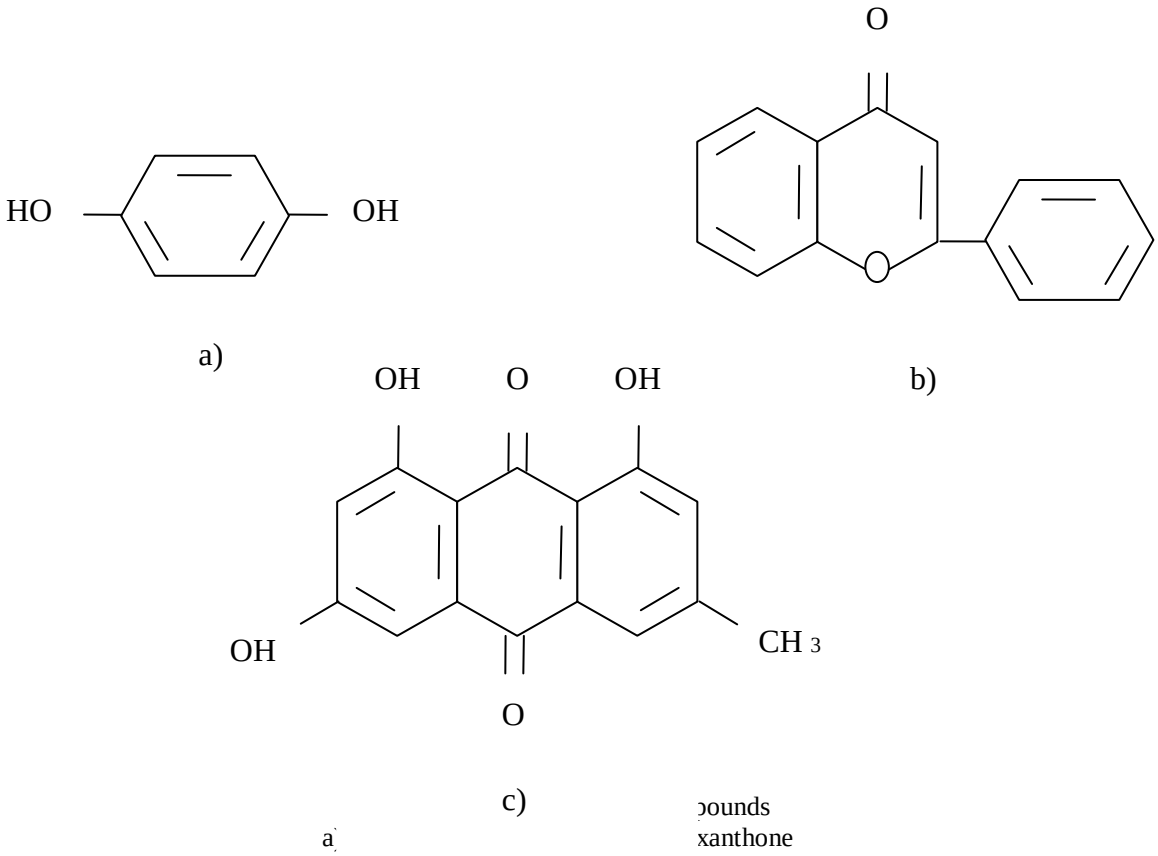
Keywords: bacterial proteins (SCP), polyphenolic extract, biomass.

Introduction

Due to their biological properties and multiple practical applications, polyphenolic compounds have raised in recent years a high interest in biotechnology [4,5,6,9]. Their biostimulating, biotransformative and biocatalytic effects are studied by a lot of authors [3,4,5,8,9].

Microorganisms and especially yeasts, as well as, some bacteria that are producers of microbial proteins (SCP) respond to the addition of polyphenolic extract by a high increase in the biomass. The biomass produced by those organisms has superior attributes both from a quantitative and qualitative point of view [3,9,10,11].

Polyphenolical compounds are biological substances synthesized by plants such as vine [2,5,16]. Phenolical compounds can be extracted from vine both from stalk as well as from grape peel [2,7]. From a molecular point of view, polyphenolical compounds are complex substances formed by one or more benzene cycles, free or condensed or from benzene cycles and heterocyclic where there are grafted groups: -OH; -C=O; -COOH; -OCH3 (Figure 1 a, b, c).



a) pounds
xanthone

The majority of polyphenolical compounds, can be catabolized by microorganisms, either by aerobic or by anaerobic means irrespective of their chemical nature [4,9,11].

Following the aerobic means, the catabolization of the polyphenols is achieved by the participation of the two essential enzymes: monooxygenize and diooxygenize [4,9]. The polyphenolical compounds are transformed under the action of enzymes in the catabolic intermediates which are generally used by microorganisms as substituted dihydrical phenolies. The aromatic nucleus diminishes the permanency under the action of microbial enzymes which favors catabolization.

Our research investigates the possible effects of the polyphenolic extract from vine in the case of increasing biomass production and the *Methylomonas sp.M.14.1* bacterium, capacity to synthesize (SCP) having superior bacterial proteins properties both from a quantitative and a qualitative point of view.

Method and material

It was used as a (SCP) protein producer microorganism on methanol the *Methylomonas sp.M.14.1* bacterium. It reaches cultivation under laboratory conditions according to the operating method showed in our last paper [1].

We obtained the polyphenolic extract from stalk vine and it reached maturity. It carried out the extract in ethylic alcohol according to the technology devised by Irina Tudose [7]. The polyphenolic extract was used as alcoholic solution.

Determination of the biomass by the centrifugation of the culture liquid at 4000 rpm has also been achieved. It was expressed in g/l. Dried biomass was obtained by drying the moist biomass at 105ºC. It has been expressed in g %.

The gross protein was appreciated by the determination of the total nitrogen using the Keldhal method [12]. The lipid content has been appreciated by means of the extraction method. The content in lipids has been appreciated by means of the extraction method with etherethilic at Soxlet [13]. The content in glucides has been determined by means of the O-toluidina method [13]. The ash has been determined by calcinations of the moistured biomass at 600°C over two hours. It was expressed in g%. The amino acids have been determined by one amino acids analyzer JLK-6 AS. Japan, by using the standards of the Perce Chemical Co Rock Ford II USA company.

Results and discussion

The production of the (SCP) proteins on methanol having different microbial stem has been researched by a lot of authors [1,14,15].

The usage of the some "raising stimulators" of the proteic concentrated biomass production is less known, at least in the case of the methylotrophe bacterium which is a producer of (SCP) microbial proteins. Our research has demonstrated that polyphenolic extract addition has important effects over microbial biosynthesis process, determining high increase in biomass production.

In our first research, we have investigated the manner in which the addition of polyphenolic extract on a methanol medium determines the increase in biomass production. Results of the research are shown in Table 1.

Table 1. The biomass production of the *Methylomonas sp.M.14.1* methylothrophe bacterium cultivated on methanol medium and on methanol and polyphenolic extract addition medium10 ml/l.

Methanol medium		Methanol and polyphenolic extract addition medium	
Moist biomass (g/l)	Dried biomass (g%)	Moist biomass (g/l)	Dried biomass (g%)
26.04	16.43	30.06	21.03
Growth (%)	-	15.43	21.87

We can conclude from Table 1 that biomass production increases by 15.43% in a moist state while in a dried state the increase is of 21.87%. The experimental results clearly show that the polyphenolic extract added to the methanol culture medium in a quantity of 10ml/l has stimulating effects over biomass production growth in the case of the *Methylomonas sp.M.14.1* methylothrophe bacterium. These growths can be explained both by more efficient utilization of the methanol as a carbon and energy source and as a carbon and energy supplementary source of the polyphenolic extract. Because of their molecular structure, phenols from the phenolical extract probably offer the *Methylomonas sp.M.14.1* bacterium an important supplement of carbon units that can be used in the structure of the cellular protein molecules. Furthermore, the metabolizing of the polyphenols offers the bacterial cell a supplementary energy that contributes to the acceleration of the biosynthesis process and implicitly to the increase in biomass production.

In a previous stage of our research, I stated the optimal quantity of polyphenolic extract that can be added to the culture medium with methanol in such a way that biomass production would register maximum growth .The results of this research are shown in Table 2.

We can conclude from Table 2 that biomass production varies in comparison with the quantity of the polyphenolic extract added to the culture medium with methanol and it is of 10ml/l.

Table 2. The biomass production of the *Methylomonas sp.M.14.1* bacterium cultivated on methanol medium and having polyphenolic extract in variable quantities.

Experimental variant	Polyphenolic addition (ml/l)	Moist biomass (g/l)	Dried biomass (g %)
1	0.5	21.63	15.08
2	2	23.96	16.04
3	4	24.08	16.38
4	6	24.30	16.48
5	8	28.60	19.03
6	10	29.88	20.98
7	12	27.81	19.06
8	14	23.16	17.36

For this quantity, moist and dried biomass production reaches maximum values: 29.88,g/l, and 20.98 g% respectively. Bigger or smaller polyphenolic extract quantities have diminished or limited biosynthetic stimulating effects. This shows that the *Methylomonas sp.M.14.1* bacterium has metabolic possibilities limited to catabolization but capable of anabolization of the polyphenolic compounds.

Another aspect of our research refers to the establishing of the dynamics of accumulating the biomass produced by *Methylomonas sp.M.14.1* bacterium cultivated in a methanol medium and the addition of polyphenolic extract 10ml/l. The research results are shown in Table 3 and Figure 2.

Following the results shown in Table 3, we can conclude that the biomass accumulation at *Methylomonas sp.M.14.1* bacterium cultivated on methanol medium and addition of polyphenolic extract 10ml/l is typical for any microbial culture. Having a good accumulation rate, the *Methylomonas* bacterium increases exponentially up to 24 hours while the cellular production is at maximum: 29.61g/l, and 21.36 g% respectively, after that the biomass accumulation comes into decline, waning gradually in such a way that at 32 hours of cultivation its value is of 22.11g/l, and 18.04g % respectively. Diminishing shows the entrance of the culture cells into the aging stage which makes their biosynthetic likely to diminish, a fact confirmed by the small quantity of biomass: 22.11 g/l, and 18.04g% respectively.

Table 3. Biomass accumulation at *Methylomonas sp.M.14.1* bacterium cultivated on methanol medium and addition of polyphenolic extract 10ml/l.

Cultivation time (h)	Moist biomass (g/l)	Dried biomass (g%)
4	5.30	3.40

8	9.68	4.83
12	16.64	8.31
16	23.07	12.33
20	24.69	18.73
24	29.61	21.36
28	26.24	20.77
32	22.11	18.04

It subjected dried biomass to chemical analyses for giving emphasis to the basic substances and the polyphenolic extract effects over the bacterial proteins biosynthesis produced by *Methylomonas sp.M.14.1* bacterium have been appreciated.

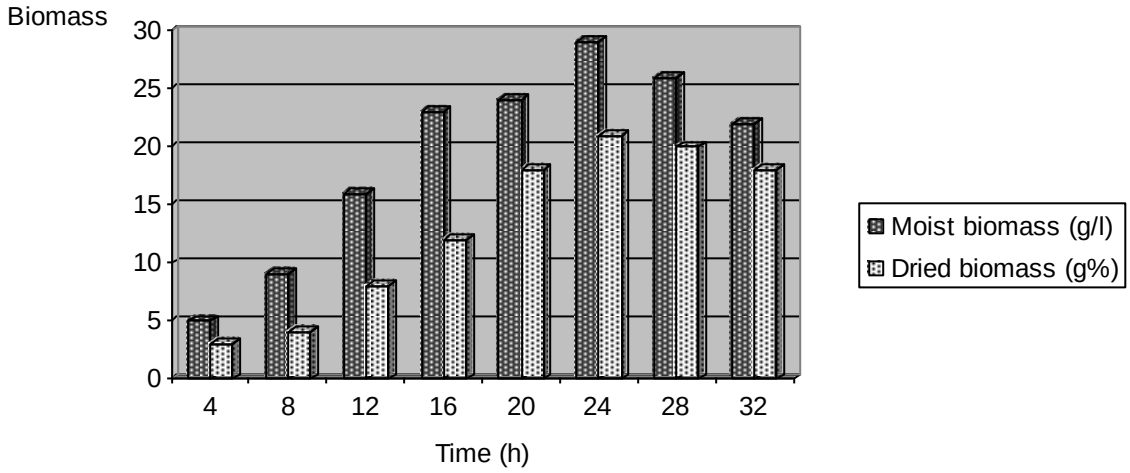


Figure 2. Biomass accumulation dynamics *Methylomonas sp.M.14.1* bacterium

We determined the content of gross proteins, lipids, glucids, ash and amino acids. The research results are shown in Table 4.

Table 4. Chemical composition the *Methylomonas sp.M.14.1* bacterium biomass cultivated on methanol medium and addition of polyphenolic extract.

Sample	Total protein (%)	Lipides (%)	Glucides (%)	Ash (%)	Amynoacides (%)
Biomass on methanol	78.12	1.23	7.20	10.30	67.34
Biomass on methanol medium and addition of polyphenolic extract	86.31	1.36	7.63	10.86	73.37
Growth %	10.48	10.57	3.09	5.43	8.95

One can notice in Table 4 that all cellular molecular compounds register quantitative growths that confirm the fact that the polyphenolic extract contributes to the running of the biosynthetic process. Taking into consideration the fact that the polyphenolic extract accelerates the biosynthesis processes, it can play a role as provider of molecular units needed to both cellular syntheses as well as the role of energetic source. The increase of the content in gross proteins by 10.48%, of lipids by 10.57 %, of glucides by 3.09% and amino acids by

8.95 % clearly shows that in the presence of polyphenolic extract, the *Methylomonas sp.M.14.1* bacterium accelerates cellular oxidation-reduction processes by efficiently using the methanol but it transforms the compounds from the extract in molecular units needed for cellular synthesis of substances and energy. One can appreciate the fact that in a quantity of 10ml/l the polyphenolic extract can be a component of the culture medium for the *Methylomonas sp. M.14.1* bacterium producer of (SCP) protein on methanol.

Conclusions

Polyphenolic extract added to the culture medium of the *Methylomonas sp.M.14.1* bacterium stimulates growth in biomass production. It registers a moist biomass production growth of 15.43% and of 21.87% of dried biomass.

The optimal quantity of polyphenolic extract added of the culture medium is of 10ml/l.

The polyphenolic extract determines quantitative growths of biomass molecular compounds. The gross protein increases with 10.48 %, lipids with 10.57 %, glucides with 3.09 %, ash with 5.43 and amino acids with 8.95%.

The polyphenolic extract can be an excellent compound in the culture medium for *Methylomonas sp.M.14.1* bacterium, producer of (SCP) protein.

References

1. I. BONTAŞ, *Researches over some methylotrophe bacteria regarding the obtaining of the (SCP) microbial proteins on methanol*. PhD thesis ”Alexandru Ioan Cuza “ University, of Iasi,1986.
2. I. TUDOSE, *Polyphenolic compounds accumulation dynamics in Vitia vinifera and some aspects to concerning bioactive properties,* PhD thesis Technical University “Gh.Asachi” of Iasi, 2002.
3. KONRAND T. HOWITZ , *Small molecule activators of sirtuins extend Saccharomyces cerevisiae lifespan,* Nature, 2003.
4. MCHAEL WILLITSA, MAYTE DE LUCA, JOHN STENSA, GERSON GRADERA, *Phytochemistry*, **65,** 31-41 (2004).
5. LINDA BISSON, SUSAN EBELER, *Biological effects of phenolic compounds on Saccharomyces cerevisae,* American Vineyard Foundation, *http://www.avf.org/endowment.html*.
6. MARA BRUNATI, FLAVIA MARINELLI, CRISTINA BETTOLINI, RAFFAELLA GONDOFFI, DANIELE DAFFONCHIO, F. MOLINARI, *Enzyme and Microbial Technol.*, **34,** 3-9 (2004).
7. I. TUDOSE, *Methods of investigation and separation the polyphenolic compounds of Vitia vinifera and biological active properties,* Technical University “Gh.Asachi” Iasi, 1999, pp.3.
8. XUE PENG X., N. MISAWA , S. HARAYAMA, *Appl.and Envirom.Microbiol.*, **69,** 3, 1417-1427 (2003).
9. R.E. PARALES, N.C. BRUCE, A. SCHMID, *Appl. and Envirom.Microbiol.,* **68,** 10, 4699-4709 (2002).
10. BONG KYUN KIM, PYOUNG KYU PARK, HEE JEONG CHAE, EUI YONG KIM, *Korean J.Chem.Eng.*, **21,** 3, 689-692, (2004).
11. J. ROSAZZA, L. DOSTAL, *The Journal of Biological Chemistry*, **268,** 32, 23954-23958, (1993).
12. I.F. DUMITRU, *Practical Working of Biochemistry,* Ed. Did and Ped.,Buc., 1977, pp. 76.

13. GH. NUTA, C. BUSNEAG, *Biochemical Investigation,* Ed. Did.and Ped.,Buc., 1977, pp. 49, 69, 224.
14. L. EBBINGHAUS, M. ERICSSON, M. LIDBAM, *Adv.Biotechnol.,* V. **II,** Ed. Pergamon Press, 1981.
15. A.I. LASKIN, *Single Cell Protein,* Ann. Reports on Fermentation Processes, V.**I,** Ed. D.Perlman, Acad. Press New York, 1977, pp.151.
16. C. FLEURY, B. MIGNOTTE, L.J. VAYSSIERE, *Biochem.,* **84,** 131-141 (2002).