DYNAMICS OF ALKALOID BIOSYNTHESIS IN CORRELATION WITH LIPID BIOSYNTHESIS IN SUBMERGED CULTIVATED STRAINS OF *CLAVICEPS PURPUREA*

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Abstract: Lipid metabolism is associated with alkaloid biosynthesis due to acetyl-CoA common precursor. For this reason, in the present paper the dynamics of total alkaloid content is investigated both in mycelia and supernatants of some submerged cultivated strains of *Claviceps purpurea*, as well as the dynamics of the brute lipid amount in the mycelia of the studied strains. The comparative analysis of the results concerning the total alkaloid content in the mycelia of analyzed strains and in the supernatants of culture media generally shows that the profile of the variation curves is comparable for the analyzed strains and the value amplitudes are dependent of them. At certain ages, as a main trend we found that the lipid accumulation in the mycelia of investigated strains takes place in a parallel manner with alkaloid biosynthesis and the crude lipid level is kept stable or diminishes after alkaloid biosynthesis begins.

INTRODUCTION

Ergot alkaloids constitute a large and complex family of nitrogen containing fungal metabolites. Recently, new therapeutical applications of these alkaloids have been identified such as schizophrenia treatment or new uses based on antibacterial, cytostatic, immunomodulatory, or hypolipidemic effects of these compounds (Mukherjee and Menge, 2000). Their lack of specificity for individual monoamine receptors often leads to unpredictable and undesirable effects like in the case of LSD ((Panaccione *et al*., 2006). The effort to discover new bioactive molecules of ergot alkaloids by different approaches is a continuous process. So, chemical syntheses of ergot alkaloids or of their analogues are considering, as well as bioconversions or direct biosynthesis by *Claviceps purpurea* fungus supplying with specific precursors or other nutrients (Kobel and Sanglier, 1986, Puc *et al*., 1987; Kozokowski *et al*., 1988; Perellino *et al*., 1993; Kozokowski *et al.*, 1993). The chemical composition of the species of *Claviceps* genus is extremely complex (Surdu *et al.*, 2005). A significant number of *Claviceps* sp. strains produce high levels of exocellular polysaccharides (Flieger *et al*., 2003) with important role in the maintaining of medium optimal viscosity and of a convenient aeration, this physico-chemical parameters being involved in alkaloid biosynthesis regulation.

Today the production of ergot alkaloids is mainly performed by strain submerged cultivation and utilization of modern tools like physiological control and genetic engineering.

In this research, *Claviceps purpurea* strains obtained by somatic hybridization and subsequently subjected to caffeine and ethidium bromide treatments have been investigated in order to analyse the dynamics of alkaloid and crude lipids biosynthesis and to establish the optimal moment for the collection of biological material in view of evaluation of its cytostatic potential.

MATERIAL AND METHODS

The biological material is constituted by lyophilized fungal biomass and by supernatants collected after the culture media centrifugation of submerged cultivated *Claviceps purpurea* strains. The strains, conventionally coded as T1-3, T2-1 and T13-1, have been obtained by somatic hybridization and then subjected to caffeine (5mM) and ethidium bromide treatment (5mM). The strain submerged cultivation was performed in 500 ml Erlenmeyer flasks containing the SN-101 liquid medium, at la 24^0 C and 200 rpm/min. Total alkaloid content and crude lipid content have been determined during 14 days of submerged fermentation. The biological material was collected at every 48h, starting with the fourth day.

For determination of total alkaloid content the method described by Rumpel (1955) was used. The method principle is based on biological material extraction with methanolic solution of tartaric acid and reading spectrophotometric determination of alkaloids with van Urk reagent. For calculus of the results, a calibration curve was constructed with ergotamine tartrate in tartric acid. Depending on investigated material, the results are expressed in mg/100ml supernatant and mg/100g lyophilised mycelium.

The crude lipid content was quantified by Soxhlet gravimetric method. The lipids are extracted at heat, by repeated washing (percolation), with specific organic solvents (ethylic ether, petroleum ether, chloroform, dichloroethan), under reflux in a special glassware and they are gravimetrically determined (Artenie and Tănase, 1981).

RESULTS AND DISCUSSIONS

The analysis of total alkaloid content (TAC) in the mycelia of the three investigated strains evidences an ascendant trend of the values determined in the biosynthesis dynamics (fig. 1-3), except T13-1 strain in which the diminution of total alkaloid content was evidenced after the fourth collection of biological material (fig. 3). For the interval up to the fourth collection of the biological material, the highest values of alkaloid content have been registered for T13-1 strain, followed for de T1-3 and respectively T2-1 strains.

Concerning total alkaloid content in supernatants of culture media, a general increasing trend of the values of this parameter was registered up to the middle of investigated period, except the T2-1 strain in which the alkaloid accumulation in supernatant is similar to the curve profile representing the dynamics of alkaloid biosynthesis in mycelium. For de T1-3 and T2-1 strains, after the third collection of the biological material (age of 8 days), the total alkaloid content decreases in supernatant in a more pronounced manner in T13-1 strain.

The comparative analysis of the graphically represented results concerning total alkaloid content in mycelia and in supernatants generally shows that curve profile is comparable in T1-3 and T2-1 strains, the values registered both in mycelium and supernatant being higher for T1-3 strain. Concerning T13-1 strain which has maximum values of total alkaloid content, we observed a decline of alkaloid biosynthesis at the age of 12 days.

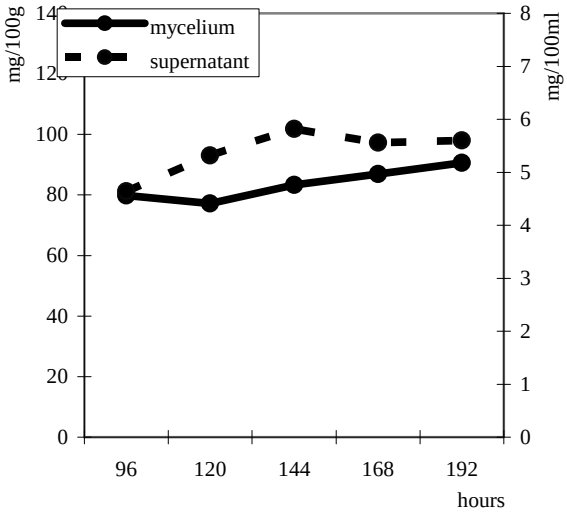

Figure 1 – Dynamics of total alkaloid content in the mycelium and supernatant of T1-3 submerged cultivated strain of *Claviceps purpurea*

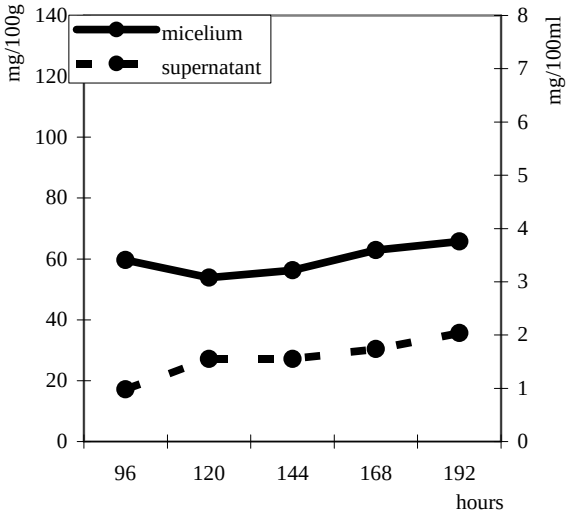

Figure 2 – Dynamics of total alkaloid content in the mycelium and supernatant of T2-1 submerged cultivated strain of *Claviceps purpurea*

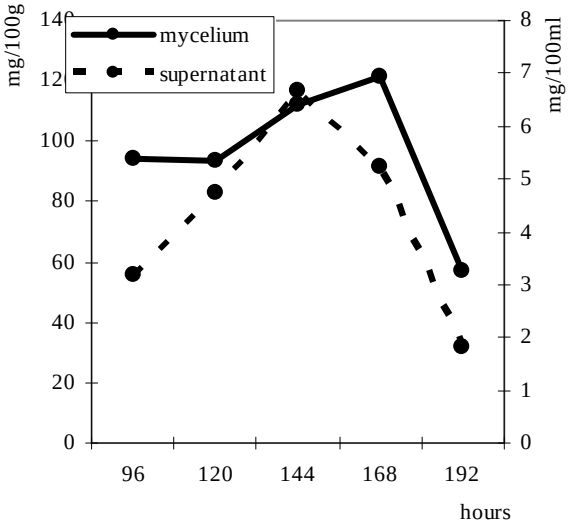

Figure 3 – Dynamics of total alkaloid content in the mycelium and supernatant of T13-1 submerged cultivated strain of *Claviceps purpurea*

The results regarding the dynamics of the content of crude lipids are comparable for the three investigated strains (fig. 4-6). So, the minimum value of total crude lipid content is

registered in 4 days old cultures, while the maximum value is noted in the middle point of cultivation period. For T13-1 strain, the value of this quantitative biochemical parameter shows a small increase after a stopping of biosynthesis process (fig. 6). The highest values for total crude lipid content are registered for T13-1 strain, but the maximum amplitude is present in 8 days old culture of T2-1 strain.

Lipid metabolism is associated with alkaloid biosynthesis due to acetyl-CoA common precursor, reason for which we graphically represented the dynamics of lipid biosynthesis in comparison with the dynamics of alkaloid biosynthesis in the mycelia of the investigated *Claviceps purpurea* strains. At certain ages, as a main trend, we noted that the lipid accumulation in the mycelia of the investigated strains takes place in a parallel manner with alkaloid biosynthesis or that the amount of total crude lipids maintains at the same level or diminishes after the beginning of alkaloid biosynthesis.

The presence of the lipid inclusions is characteristic for the alkaloid-producing strains (Surdu *et al*., 2005) and the level of total crude lipids is dependent of the alkaloid type of the investigated strain (Olteanu *et al*., 2007).

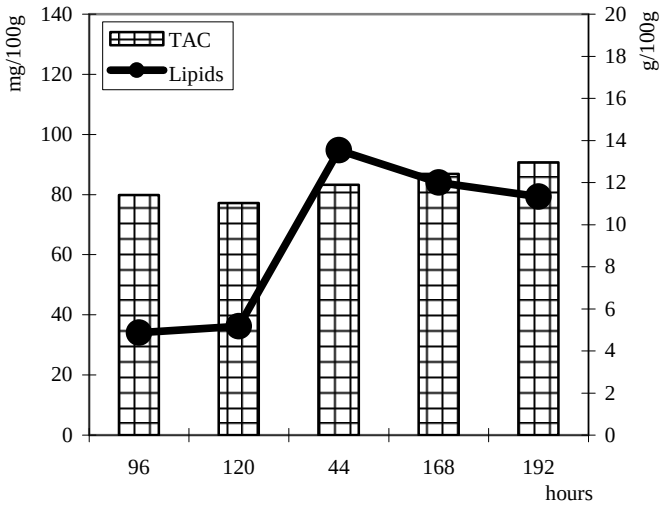

Figure 4 – Dynamics of total alkaloid content and of crude lipid amount in the mycelium of T1-3 submerged cultivated strain of *Claviceps purpurea*

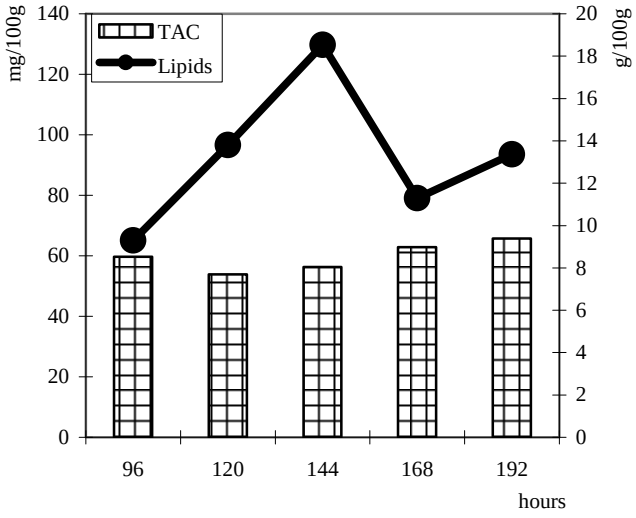

Figure 5 – Dynamics of total alkaloid content and of crude lipid amount in the mycelium of T2-1 submerged cultivated strain of *Claviceps purpurea*

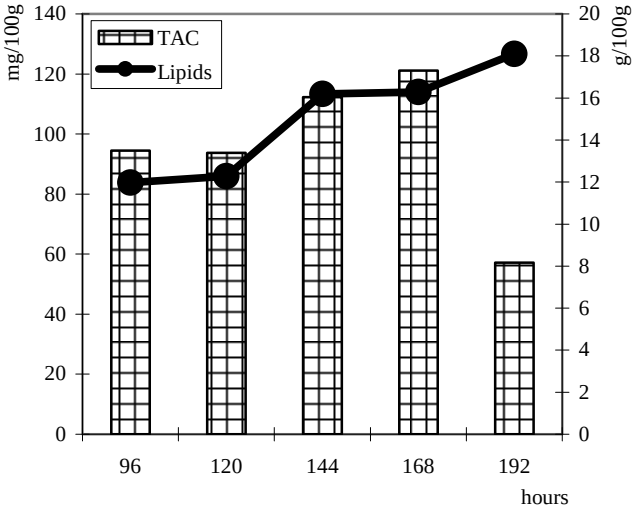

Figure 6 – Dynamics of total alkaloid content and of crude lipid amount in the mycelium of T13-1 submerged cultivated strain of *Claviceps purpurea*

A similar behaviour was encountered after the comparative analysis of total crude lipid content in mycelia and total alkaloid contend evaluated in the supernatants. So, the shape of

curves indicates for T1-3 strain an increasing trend for lipid biosynthesis up to the age of 8 days and a decrease up to the experiment end, process following the dynamics of determined alkaloids (fig. 7). The same general tendency is graphically represented for T2-1 strain (fig. 8). Concerning T13-1 strain, if up to the culture age of 8 days the lipid biosynthesis follows the variation curve of total alkaloid content determined in the supernatant of culture medium of this strain, after this moment the diminution of alkaloid content is accompanied by a stagnation followed by a small increase of lipid content in mycelium (fig. 9).

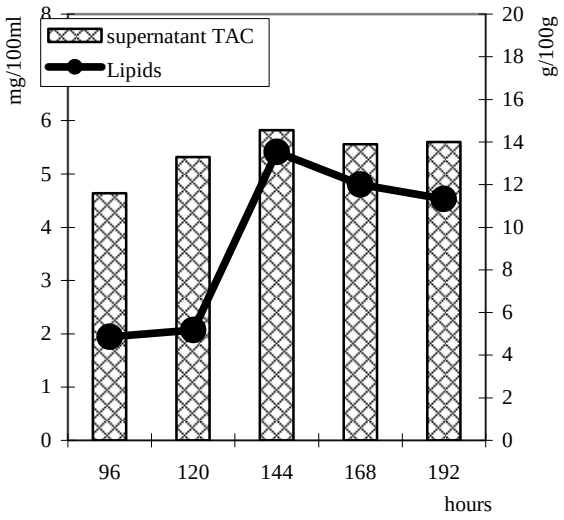

Figure 7 – Dynamics of total alkaloid content in supernatant and of crude lipid amount in the mycelium of T1-3 submerged cultivated strain of *Claviceps purpurea*

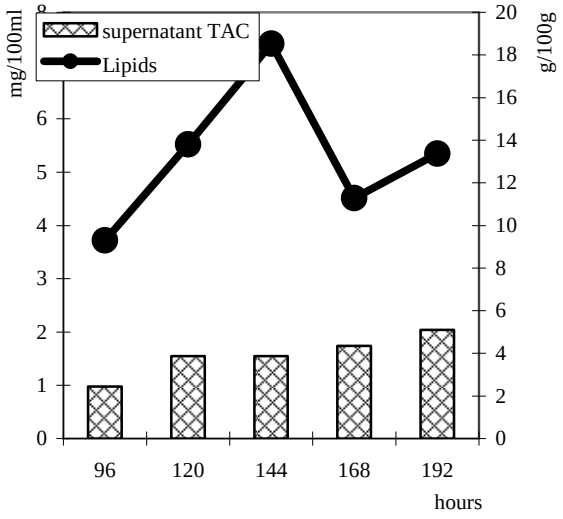

Figure 8 – Dynamics of total alkaloid content in supernatant and of crude lipid amount in the mycelium of T2-1 submerged cultivated strain of *Claviceps purpurea*

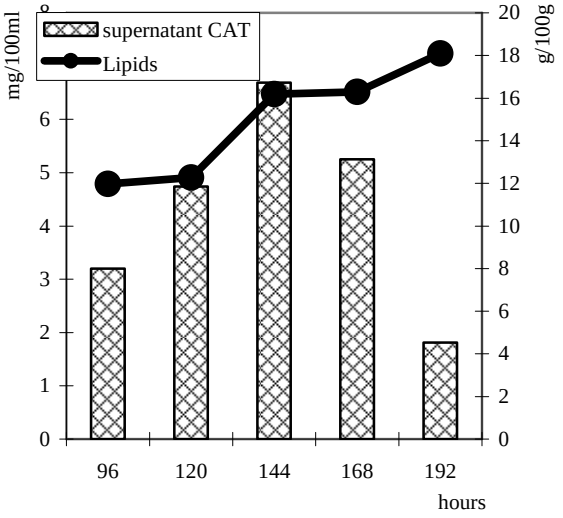

Figure 9 – Dynamics of total alkaloid content in supernatant and of crude lipid amount in the mycelium of T13-1 submerged cultivated strain of *Claviceps purpurea*

CONCLUSIONS

Analysis of the obtained results evidences that the variation of total alkaloid content is direct correlated with the age of the investigated fungal strains.

The total alkaloid content of the supernatants of culture media shows an increasing trend in young cultures, up to the middle of period established by our experimental model.

The maximum content of crude lipids in fungal biomass is registered in the eighth day of the culture.

Our results suggest the tendency according to which the alkaloid biosynthesis is accompanied by lipid biosynthesis.

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