THE INFLUENCE OF SOME LIPOSOLUBLE VITAMINS ON ALKALOID BIOSYNTHESIS OF *CLAVICEPS PURPUREA IN VITRO*

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Abstract: The addition of some liposoluble vitamins in the submerged growth medium has a positive impact on the alkaloid biosynthesis. The changes are induced by the genetic characteristics of the *Claviceps purpurea* strains and the level of the growth factor used.

INTRODUCTION

Under natural conditions, the nutritive needs of the species of the *Claviceps* genus are fulfilled by the nutrient supply provided by the host plant to the parasite through the phloemic sap. During the growth and development of the sclerotia in the ovary of the *Graminea* flowers, changes occur, not only quantitative, but also and especially qualitative ones allowing the biochemical and morphological differentiation of the fungal structures capable of alkaloid biosynthesis (Taber, 1985). The presence of growth factors in the ovary of the host plant flowers (Neamtu, 1981), as well as the intersection of the metabolic biosynthesis pathways of a number of vitamins and ergoline alkaloids are the main arguments which led to a complex investigation of the nutritive requirements of the *Claviceps purpurea* strains grown in vitro and in vivo; the study also examines the related growth factors and their influence on the morphogenesis and alkaloid biosynthesis. To this purpose, we aimed at investigating the impact of some liposoluble vitamins on the dynamics of the alkaloid biosynthesis process in various *Claviceps purpurea* strains grown under submerged conditions.

MATERIAL AND METHODS

We used *Claviceps purpurea* strains exhibiting different biosynthetic potential. This is characterized by the total quantity of biosynthesized alkaloids and the ratio of the peptide alkaloids.

Sclerotia fragments were grown on agar medium (Strnadova et al., 1974). The colonies were inoculated in Erlenmeyer flasks. The culture medium contains sugar, citric acid, magnesium sulphate, monopotassium phosphate, sodium chloride, and ammonia to pH 5.2 (control culture) (Wack et al., 1983) and the vitamins A or D (ergosterol). The submerged, shaken cultures were grown at 24°C for 12 days.

The alkaloids were assayed in the sclerotia, mycelium and the supernatant of the submerged cultures at different stages of growth by Rumpel method (1954), while the ratio of different types of ergot alkaloids was determined by thin layer chromatography (Brevet 1977).

RESULTS AND DISCUSSIONS

The bio-productive potential of the strains used for the submerged cultures is different. In the medium without vitamin addition (the control) (Figure 1) the alkaloid biosynthesis is characterized by dynamics and intensity, by the ratio between the endo- and exocellular alkaloids. Of the examined strains, only those representative for each alkaloid type were selected. It was noted that throughout almost the entire cultivation period the ergotamine strains have a slow rhythm of accumulating alkaloids, the process growing in intensity towards the end. Alkaloid accumulation in predominantly

ergocristine strains (SP 803) is more intense in the first part of the period, decreasing towards its end.

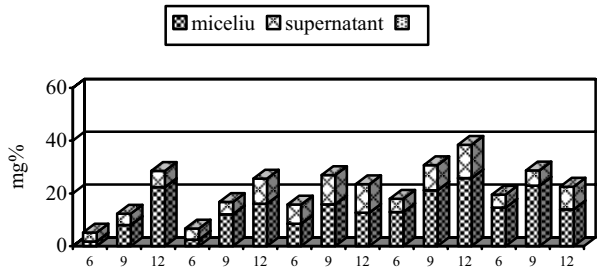

Figure 1. Quantity of total alkaloids present in the mycelium and the supernatant of the *Claviceps purpurea* submerged control cultures, strains of different alkaloid type, at 6, 9 and 12 days.

The strains capable to produce different alkaloids (STP 804, STP 805) have the best alkaloid yield; in these strains the biosynthesis process starts sooner and is more intense than in the other strains.

The alkaloids are present mainly in the mycelium. The highly alkaloid producing strains release higher quantities of alkaloids in the medium.

Vitamin A addition improves the biosynthetic properties of some strains, the positive trend being recorded in most cases in the second half of the cultivation period (Figure 2). A significant accumulation of alkaloids is noted in the ergotamine type. In the ergotoxine strains, regardless of their initial biosynthesis potential, maintenance and/or progressive increase of the quantity of alkaloids in the last days of cultivation is noticed

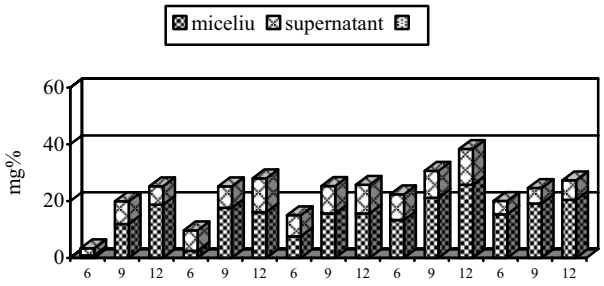

Figure 2. Quantity of total alkaloids present in the mycelium and the supernatant of the *Claviceps purpurea* submerged cultures with vitamin A, strains of different alkaloid type, at 6, 9 and 12 days

The quantity of alkaloids released in the culture medium is different from the control cultures. In most of the strains, the quantity of alkaloids excreted in the medium remains high throughout the cultivation period.

Alkaloid biosynthesis reaches the highest levels in the presence of ergosterol (Figure 3). In the strain STP 804, classified as high producing, the quantity of total alkaloids is

40% higher than in the control culture, the maximum level being recorded after 9 days of submerged cultivation. For the ergotamine type, the use of ergosterol is less stimulating.

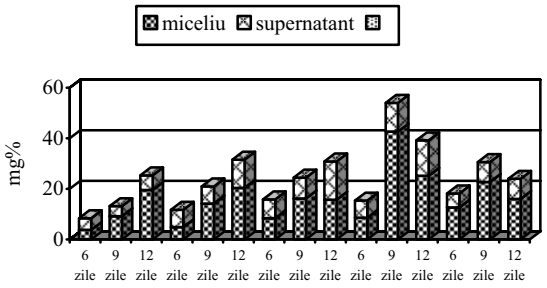

Figure 3. Quantity of total alkaloids present in the mycelium and the supernatant of the *Claviceps purpurea* submerged cultures with ergosterol, strains of different alkaloid type, at 6, 9 and 12 days

The ergosterol induces the release of alkaloids in the culture medium in the ergotoxine strains, the highest quantity of alkaloids being recorded towards the end of the cultivation period.

The biosynthetic characteristics of the strains (of sclerotia) used for the submerged cultures manifest differently in vitro. The differences among them increase under submerged cultivation conditions probably due to the change in the heterokaryotic status; thus, among the different nuclei of the heterokaryon of these strains a ratio more or less favorable to alkaloid biosynthesis establishes.

Vitamin A and ergosterol, in certain concentrations, stimulate the process of alkaloid biosynthesis and the alkaloid release in the culture medium. We may assume that one of the ways in which the quantity of alkaloids increases is the preferential use of the common precursors of the liposoluble vitamins and alkaloids to biosynthesize the secondary metabolites when vitamins, the final product of one of the pathways, are present in the medium.

CONCLUSIONS

The submerged cultures of *Claviceps purpurea* are influenced differently by the presence of vitamins, according to their concentration and to the genetic characteristics of the strains. The ergosterol has a more efficient impact on alkaloid biosynthesis than Vitamin A.

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