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IMPACT OF FUROATE AND SALICYLATE COORDINATION COMPLEXES ON THE LIPID FRACTIONAL COMPOSITION OF *Actinomadura* sp. 37

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Abstract: The aim of this study was to investigate the influence of furoate and salicylate coordination complexes on *Actinomadura* sp. 37, a promising producer of secondary metabolites such as lipids. Biomass accumulation, total lipid content, and lipid fractional composition were analyzed following cultivation of the strain in Gause medium supplemented with various concentrations of furoate (F) and salicylate (S) complexes. The results regarding biomass accumulation and lipogenesis activity of the *Actinomadura* sp. 37 strain showed no positive impact on biomass accumulation. For both complexes, the decrease ranged between 2 and 48 %. However, in terms of lipogenesis, the strain exhibited stimulatory activity only in variant 4 (Gause + 10 % F), where the lipid content was 2.5 times higher compared to the control sample. Based on the fractional composition, the highest amount of mono- and diglycerides was obtained during cultivation on medium 6 (G + 5 % S), which was 52 % higher than in the control sample. Triglycerides and sterols reached the maximum amount in case 5 (G + 1 % S), more by 66 % and 95 %, respectively. The increase in the most important lipid fractions is attributed to the presence of a carboxyl group in the coordination complexes used. The enriched microbial biomass obtained can potentially be used in animal husbandry as a dietary supplement for farm animals in the future.

Keywords: *actinobacteria, biomass, lipids, coordination complexes, monoglycerides, diglycerides, triglycerides, sterols*

INTRODUCTION

Throughout their life cycle, actinobacteria produce a wide range of biologically active substances that are utilized in various fields, including pharmaceuticals, cosmetology, the food industry, and agriculture. Of particular interest are their metabolic products, such as vitamins, antibiotics, amino acids, enzymes, hormones, and lipids [1 – 4].

Data obtained from genome sequencing research highlighted a diversity of genes, enzymes, and genetic organization of components responsible for lipid synthesis in actinobacteria. Fatty acid biosynthesis is highly energy-intensive for cells, so regulating the rate of fatty acid synthesis to maintain membrane lipid homeostasis is the main point for bacterial survival. Fatty acid biosynthesis depends on regulatory elements that control these pathways [5].

Representatives of the genus *Actinomadura* are an effective and economical source of lipids. They are capable of synthesizing various lipid fractions, including mono- and diglycerides, triglycerides, phospholipids, sterols, sterol esters, and waxes. According to numerous literature sources, each of these fractions plays a distinct biological role [3, 6].

It is well known that a key requirement in the animal husbandry industry is the use of safe feed supplements. The inclusion of lipids in animal diets positively influences growth rates and enhances metabolic function. Recent studies have primarily focused on the effects of high-quality lipid supplementation on growth performance, digestion, and metabolism in young animals. However, there is considerable variability in the composition and quality of lipid sources available for animal feed [7]. Moreover, recent findings indicate that the consumption of balanced lipids can reduce the risk of chronic diseases and improve overall health [8].

For these reasons, actinobacteria of the genus *Actinomadura* may be considered promising lipid producers for application in animal nutrition [9].

The quantity and fractional composition of lipids are directly influenced by the carbon source used, which is the primary additive in microbial cultivation media [10]. Therefore, optimizing the nutrient medium is essential for enhancing the production of specific lipid fractions. Coordination complexes of 2-furoic acid and salicylate can be proposed as experimental carbon sources.

The effect of trinuclear complexes of 2-furancarboxylic acid on the biochemical composition of the cyanobacterium *Spirulina platensis* biomass was investigated. The results demonstrated that Fe (III) coordination compounds can serve as regulators of the biochemical composition of this cyanobacterium. A positive influence was observed on the content of amino acids, peptides, and carbohydrates [11].

Some actinobacteria are capable of producing salicylic acid; however, it is not the primary secondary metabolite synthesized [12]. In coordination chemistry, salicylic acid is of particular interest due to its carboxyl group, which plays a key role in interactions with biologically important metal ions [13].

Taking into consideration the specificity of *Actinomadura* strain as a lipid source, and also the importance of lipids as constituents of cell membrane, the action of coordination complexes of furoate and salicylate on the biomass yield, as well as quantitative and fractional composition of lipids were studied.

MATERIALS AND METHODS

Material

The *Actinomadura* sp. 37 strain kept in the National Collection of Non-pathogenic Microorganisms (Institute of Microbiology and Biotechnology of Technical University of Moldova) was used in this study. The strain is of interest because it is a producer of biologically active substances, including lipids. The strain was kept on agar medium Gause (soluble starch – 20.0 g·L^{-1}, KNO_3 – 1.0 g·L^{-1}, K_2HPO_4 – 0.5 g·L^{-1}, $MgSO_4$ – 0.5 g·L^{-1}, NaCl – 0.5 g·L^{-1}, $FeSO_4$ – 0.01 g·L^{-1}, agar – 20.0 g·L^{-1}, *p*H = 7.2 – 7.4).

Biomass production

The *inoculum* was cultivated in liquid mineral medium Dulaney (glucose – 20.0 g·L^{-1}, $(NH_4)_2HPO_4$ – 7.5 g·L^{-1}, NaCl – 5 g·L^{-1}, K_2HPO_4 – 2.0 g·L^{-1}, $MgSO_4 \cdot 7H_2O$ – 1.0 g·L^{-1}, $CaCl_2$ – 0.4 g·L^{-1}, $ZnSO_4 \cdot 7H_2O$ – 0.01 g·L^{-1}, $FeSO_4 \cdot 7H_2O$ – 0.01 g·L^{-1}, *p*H = 7.0), in Erlenmeyer flasks with 100 mL of medium for 3 days at 28 ºC on orbital shaker (200 rpm).
To obtain the biomass, inoculum in an amount of 8 % was added to the flasks (V = 1000 mL) with liquid complex medium Gause of 200 mL and cultivated for 5 days at 28 °C, on a shaker (200 rpm).
Additionally, medium Gause was supplemented with furoate (F) and salicylate (S) complexes, in concentrations: 1 % / v; 5 % / v; 10 % / v. The coordination compounds were obtained in the Laboratory of Bioinorganic Chemistry and Nanocomposite, Institute of Chemistry, State University of Moldova.
For the determination of the quantity of biomass, it has been separated from culture supernatant on a centrifuge (5000 rpm during 20 min).

Extraction and quantitative determination of lipids from biomass

The quantity of absolute dry biomass (ADB) was determined by weight method.
The intracellular lipids were extracted from biomass by Folch method modified in the laboratory:
- moist biomass was diluted with system of solvents chloroform and ethanol (ratio 1 : 5 *v*/*v*) on orbital shaker for 20 min;
- another portion of chloroform was added to the system for obtaining chloroform and ethanol in ratio 2 : 1 (*v*/*v*) and agitation for 20 min;
- obtaining of dissolved lipids in system chloroform and ethanol from biomass *via* filtration;
- lipid fractions dissolved in chloroform from ethanol fractions were isolated with separating funnel with distilled water (5-fold) and filtrated through anhydrous Na_2SO_4;
- lipid fractions were separated on rotary evaporator from chloroform;
- the quantity of total lipids was determined by weight method, expressed as % / quantity ADB [14].

Fractional characterization of total lipids

Fractional composition of the total lipids was determined by thin layer chromatography with "Sorbfil" plates (100 x 150 mm) in the solvent mixture hexane - diethyl ether - glacial acetic acid system (73:25:5), the quantity of each lipid fraction being recorded by densitometry method.

Statistical analysis

Data are displayed as mean ±SEM. The statistical significance was evaluated using a one-way ANOVA with post hoc Tukey test. A $p \leq 0.05$ value was considered statistically significant. Data were analyzed using PAST 3.26 statistical software [15].

RESULTS AND DISCUSSIONS

Quantity of biomass and total lipids content

The results regarding the biomass accumulation and lipogenesis activity of the *Actinomadura* sp. 37 strain grown in Gause liquid medium are shown in Table 1. The medium was supplemented with furoate and salicylate complexes at different concentrations. As a result of the furoate (F) action on the growth of strain, it was observed that there was no positive impact on the accumulation of biomass (it decreased by 21 - 48 %). In case of salicylate (S), the decrease was not so high (by 2 - 33 %).

Table 1. *Quantity of biomass and total lipids content of Actinomadura sp. 37 strain after cultivation in Gause medium supplemented with coordination complexes of furoate (F) and salicylate (S)*

Sample	ADB [g·L^{-1}]	Total lipids [% / ADB]
1 Control (Gause)	1.65	1.75
2 (Gause + 1% F)	1.02**	1.27**
3 (Gause + 5% F)	1.31	1.06*
4 (Gause + 10% F)	0.70**	4.42
5 (Gause + 1% S)	1.63	1.20
6 (Gause + 5% S)	1.55	1.16
7 (Gause + 10% S)	1.11*	1.26**

*$p \leq 0.05$, **$p \leq 0.01$

The influence of coordination complexes on the lipogenesis process of the *Actinomadura* sp. 37 strain showed a stimulating activity, only in variant 4 (Gause + 10 % F), the content of lipids constituted 4.42 %, which is by 2.5 times more in comparison with the control sample. In other cases, a higher quantity than in control sample were not registered.

This study highlighted the inversely proportional dependence of biomass accumulation and lipid synthesis on studied strain during cultivation in the presence of coordination complexes. If compounds have a stimulatory effect on lipogenesis of the strain, the inhibition of biomass accumulation is observed and vice versa.

The influence of furoate and salicylate complexes on the quantity of lipid fractions

The results of the action of furoate and salicylate complexes on the modification of the fractional composition of lipids from the biomass of *Actinomadura* sp. 37 strain during cultivation in liquid medium Gause are presented and analyzed below. The quantity of the main lipid fractions from the biomass are shown in Table 2, which have undergone modifications under the influence of both coordination complexes.

Table 2. *The influence of coordination complexes of furoate (F) and salicylate (S) on the quantitative ratio of lipid fractions of Actinomadura sp. 37 strain (% / total lipids)*

Samples	Mono- and di-glycerides	Tri-glycerides	Phospholipids	Sterols	Sterol esters
1 Control	11.46±0.58	8.25±0.68	26.40±1.31	7.03±0.26	16.03±0.27
2 (G + 1 % F)	13.83±1.20	10.50±0.72*	21.43±1.11	9.95±0.75*	12.03±0.34
3 (G + 5 % F)	12.22±0.35*	11.44±0.35	25.42±0.21**	12.51±0.53*	13.68±0.65*
4 (G + 10 % F)	14.13±0.39	11.14±0.23	20.34±0.51	11.67±0.19	14.24±0.18
5 (G + 1 % S)	15.11±0.10	13.73±0.32**	17.16±0.37	13.73±0.32	15.03±0.34
6 (G + 5 % S)	17.46±0.07	13.06±0.34	19.03±0.33*	12.69±0.34*	15.52±0.45
7 (G + 10 % S)	14.30±0.14*	13.07±0.14	21.41±0.14	12.83±0.14	14.71±0.22

*p ≤ 0.05, **p ≤ 0.01

Phospholipid fraction content in the experimental samples decreased in comparison with the control sample in the presence of both coordination complexes. The range of decrease in all cases of experiment was 3.71 - 35.0 %. The same situation was noted for the sterol esters and waxes. In case of sterol esters, the decrease varied between 3.18 - 24.95 %. The quantity of waxes in all experimental cases varied within the range of 22.24 - 28.98 %, while in the control sample it was 30.83 % (decreased by 6.0 - 27.86 %).

In the case of mono- and di- glycerides, triglycerides, and sterols, a tendency of increase is observed. The largest amount of mono- and di- glycerides can be obtained during cultivation on medium 6 (G + 5 % S), by 52 % more than in the control sample. Triglycerides and sterols reached the maximum amount in case 5 (G + 1 % S), by 66 % and 95 % more than in the control sample, respectively.

Actinobacteria are the richest known sources of bioactive substances [16]. Their mycelium has an important adaptive property, determined by the ability to synthesize a large number of secondary metabolites, including lipids [17].

Lipids play a great role in metabolic processes of different organisms. Therefore, it is necessary to pay attention to such functional lipids as omega-3 and omega-6 polyunsaturated fatty acids, conjugated linoleic acids, medium chain triglycerides, and sterols [1, 8, 18]. It should be also mentioned that membrane lipids are the first to react to adverse factors from environment to provide maximal protection. That process is characterized by changes of temperature and pressure, as well as altering the fatty acid and sterol composition of the lipid bilayer [18].

Mono- and diglycerides are widely used in food industry as additives [19]. Hydrophobic in nature, these non-ionic emulsifiers are not soluble in water, but completely soluble in oil. They stabilize without emulsions, forming inverted micelles. They are also

characterized by complex phase-forming properties in the presence of water, oil and other co-solvents; additionally, they are able to be crystallized [20]. The increased quantity of this fraction in biomass of the *Actinomadura* sp. 37 strain is the evidence of high energetic nutritional source [7].
Triglycerides are insoluble in water, so they must be transported along with proteins during metabolic processes [21]. They serve as the main form of preserving and transporting fatty acids within cells and in plasma [22].
Phospholipids play an important role in live cells. The membrane constituted of phospholipids acts as a regulatory membrane. Phospholipids affect changes in fluidity of membranes and help in adapting to physical changes, primarily, temperature. Phospholipids are the main part of the lipid molecules present in the membrane. They are composed of saturated and unsaturated fatty acids. Changes in individual fatty acids of the cell membrane can lead to changes in functions and properties of the membrane [23, 24]. In spite of decrease, the quantity in biomass is enough for influencing metabolic processes in cell membrane of fed animals.
Sterols are of no less importance in the process of preserving microorganisms – as this plays an important role in fundamental biological processes, such as signal transduction, reorganization of the cytoskeleton, asymmetric growth, etc. These components are considered to be key molecules in maintaining cell membranes in the state of fluidity appropriate to their function. The role of sterols in achieving cell protection has been demonstrated in numerous studies, elucidating their action of increasing membrane cohesion to maintain them in a dynamic state less sensitive to thermal shocks [25].
Sterol esters are less studied than other lipid fractions. But, like as phospholipids, they play a protective role for cells [26]. They contribute to the specific characteristics of the oils and fats [27]. Finally, waxes have a very complex structure, include long-chain fatty acids, primary and secondary alcohols, hydrocarbons, sterol esters, sterols etc. [28, 29]. Their quantity also decreased, but has a different variation which does not depend on concentration of coordination complexes added into cultivation medium.
By taking into account all mentioned above, the obtained biomass in experimental cases containing an increased quantity of mono- and di-, triglycerides, and sterols can be applied in future experiments dealt with improve feed for animals [30 – 32].

CONCLUSIONS

Actinomadura sp. 37 is a lipid-producing strain that synthesizes valuable lipid fractions suitable for use as feed additives in animal husbandry. Although strains of the genus *Actinomadura* require stable cultivation conditions - specifically, a *p*H of 7.0 and a temperature of 26-28 °C - they do not depend on expensive substrates. A simple nutrient medium containing a complex of mineral salts and basic sources of carbon and nitrogen is sufficient for their growth.
The addition of furoate and salicylate coordination complexes to the cultivation medium led to an increase not only in the total lipid content but also in specific lipid fractions. The highest total lipid yield was observed in variant 4 (Gause + 10 % F), where lipid content was 2.5 times greater than that of the control sample. Regarding lipid fractions, the maximum quantity of mono- and diglycerides was recorded in variant 6 (Gause + 5 % S), exceeding the control by over 50 %. Triglycerides and sterols reached their peak

levels in variant 5 (Gause + 1 % S), showing increases of 66 % and 95 %, respectively, compared to the control.
It is noteworthy that the positive effect on the accumulation of total lipids was obtained at a 10 % F concentration, which could play the role of a stress factor. While, salicylate selectively enhances specific lipid fractions. This effect is likely due to the presence of carboxyl groups in the coordination complexes, which play a role in fatty acid synthesis.

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