# Intensification of gibberellin synthesis by surfactant producers *Acinetobacter calcoaceticus* IMV B-7241 and *Rhodococcus erythropolis* IMV Ac-5017

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## Abstract

**Introduction**. The surfactant-producing strains *Acinetobacter calcoaceticus* IMV B-7241 and *Rhodococcus erythropolis* IMV Ac-5017 can synthesize gibberellin phytohormones, although generally at low levels. One approach to enhance gibberellin production is the introduction of biosynthetic precursors into the cultivation medium, which may stimulate metabolic flux toward hormone formation.

**Materials and methods.** Bacteria were cultivated in liquid media supplemented with 100–500 mg/L erythritol, which was introduced either during the lag phase or at the onset of the stationary growth phase. Phytohormone concentrations were quantified using high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS). Extracellular surfactants were extracted with a methanol–chloroform mixture, and the activity of key enzymes involved in surfactant and gibberellin biosynthesis was measured in cell-free extracts using spectrophotometric assays.

**Results and discussion**. In the presence of 300–500 mg/L erythritol as an exogenous precursor, and regardless of the carbon source used in the culture medium (refined oil, ethanol, or biodiesel production waste), the concentration of biologically active gibberellins $GA_3$ and $GA_4$ synthesized by *Acinetobacter calcoaceticus* and *Rhodococcus erythropolis* increased significantly by approximately 1.5- to 16-fold relative to cultures grown without erythritol. This pronounced enhancement indicates that erythritol effectively increases metabolic flux through the gibberellin biosynthetic pathway.

Under such conditions, a 1.4–1.7-time increase in the C-methyl-D-erythritol-4-phosphatecytidylyl transferase activity, a key enzyme of these phytohormones biosynthesis in the methyl-erythritol-4-phosphate pathway, was observed in the cells of both strains. The presence of erythritol in the cultivation medium of strains IMV B-7241 and Ac-5017 didn`t affect the surfactant synthesis.

**Conclusions**. The data obtained provide a basis for the development of an efficient, integrated technology for the co-synthesis of surface-active compounds and phytohormones, with potential applications in plant production.

## Introduction

Recently, there has been a growing interest among biotechnology researchers in so-called integrated microbial technologies (Linda et al., 2024; Katagi et al., 2024; de Siqueira et al., 2024). Their appeal lies in the fact that several valuable metabolites can be obtained during the implementation of a single technological process, which allows significantly reducing the cost of target products, increasing their competitiveness in the market and the overall efficiency of biosynthesis.

Previous studies (Pirog et al., 2019) demonstrated that the surfactant-producing strains *Acinetobacter calcoaceticus* IMV B-7241 and *Rhodococcus erythropolis* IMV Ac-5017 are capable of synthesising phytohormonal compounds, including auxins, cytokinins, and gibberellins, on various substrates such as sunflower oil and biodiesel production waste. In addition, the positive effects of the strains' exometabolites on crop yield have been reported (Piatetska and Pirog, 2023). However, the concentrations of phytohormones produced by strains IMV B-7241 and IMV Ac-5017 were relatively low, substantially limiting the effectiveness of the complex preparation for crop production.

It has been shown that the synthesis of auxin-type phytohormones can be enhanced by supplementing the culture medium of *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017 with exogenous tryptophan, a key precursor of auxin biosynthesis (Pirog et al., 2020). Based on these findings, it was hypothesised that a similar strategy, namely, the addition of an exogenous precursor of the target product biosynthesis, could also be effective in stimulating gibberellin synthesis.

In eukaryotes, including industrial producers of gibberellins, these phytohormones are synthesised via pathways involving mevalonic acid (Salazar-Cerezo et al., 2018). Until the late 1990s, the mevalonate pathway was considered the only mechanism for the biosynthesis of isoprenoid precursors, including gibberellins. However, in the 1990s, the methyl-erythritol-4-phosphate (MEP) pathway for isoprenoid biosynthesis was discovered, which functions in bacteria, green algae, and higher plants (Rohmer et al., 1993).

The MEP pathway begins with the condensation reaction of pyruvate and D-glyceraldehyde-3-phosphate to form 1-deoxy-D-xylulose-5-phosphate, which is subsequently converted to 2-C-methyl-D-erythritol-4-phosphate (Rohmer et al., 1993). Since gluconeogenesis reactions are required for the synthesis of MEP pathway intermediates during cultivation on non-carbohydrate carbon sources, it has been suggested that the addition of exogenous erythritol, a possible precursor for gibberellin biosynthesis, to the medium may be accompanied by an increase in the synthesis of these phytohormones.

In this context, the aim of this study was to identify the key enzymes involved in the methylerythritol 4-phosphate pathway in *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017, and to evaluate the effect of exogenous erythritol on the synthesis of gibberellins and surfactants during cultivation of these strains on non-carbohydrate substrates.

## Materials and methods

### Research objects

The research objects were strains of oil-oxidising bacteria isolated from oil-contaminated soil, identified as *Acinetobacter calcoaceticus* K-9 and *Rhodococcus erythropolis* KU-8 and registered in the Depository of Microorganisms of the D.K. Zabolotny Institute of Microbiology and Virology of the NAS of Ukraine under the numbers IMV B-7241 and IMV Ac-5017, respectively.

**Cultivation conditions**

Cultivation of *R. erythropolis* IMB Ac-5017 was carried out in a liquid mineral medium (g/L): $NaNO_3$ – 1.3, NaCl – 1.0, $Na_2HPO_4 \cdot 12H_2O$ – 0.6, $KH_2PO_4$ – 0.14, $MgSO_4 \cdot 7H_2O$ – 0.1, $FeSO_4 \cdot 7H_2O$ – 0.001, pH 6.8–7.0. The source of carbon and energy was ethanol, as well as refined oil at a concentration of 2% (volume fraction) (Pirog et al., 2022a, b).

*A. calcoaceticus* IMB B-7241 was cultivated in a medium with the following composition (g/L): $(NH_2)_2CO$ – 0.35, $MgSO_4 \cdot 7H_2O$ – 0.1, NaCl – 1.0, $Na_2HPO_4$ – 0.6, $KH_2PO_4$ – 0.14, pH 6.8–7.0. The medium was supplemented with yeast autolysate – 0.5% (by volume) and a solution of trace elements – 0.1% (by volume). A solution of trace elements with the following composition (g/100 ml): $ZnSO_4 \cdot 7H_2O$ – 1.1, $MnSO_4 \cdot H_2O$ – 0.6, $FeSO_4 \cdot 7H_2O$ – 0.1, $CuSO_4 \cdot 5H_2O$ – 0.004, $CoSO_4 \cdot 7H_2O$ – 0.03, $H_3BO_3$ – 0.006, KI – 0.0001, EDTA – 0.5. pH 6.8–7.0. Biodiesel production waste (biofuel plant, Zaporizhzhia region) and refined sunflower oil at a concentration of 2.0% (volume fraction) were used as a carbon source (Pirog et al, 2022).

Erythritol was added as a 1% solution at the beginning of the process or at the end of the exponential growth phase at a concentration of 100−500 mg/L.

As an inoculum, a culture in the exponential growth phase was used, grown in a medium of the above composition with 0.5% (volume fraction) of the corresponding substrate. The amount of inoculum ($10^4$–$10^5$ cells/ml) was 5–10% of the volume of the nutrient medium. Bacteria were cultivated in 750 ml flasks with 100 ml of medium on a rocker (320 rpm) at 28–30 ºC for 168 h.

**Determination of surfactants and phytohormones concentration**

After the *R. erythropolis* IMB Ac-5017 and *A. calcoaceticus* IMB B-7241 cultivation in a medium with refined oil, the residual oil was removed from the culture liquid by three-fold extraction with hexane (ratio 1:1). The biomass was separated by centrifugation (5000 g) for 25 min.

The surfactant isolation was carried out using modified method of Bligh and Dyer (1959), after extraction with a chloroform and methanol mixture (2:1) from the supernatant of the culture liquid as described in our work (Pirog et al., 2024). The concentration of extracellular surfactants (g/l) was determined by the gravimetric method.

Extracellular phytohormones, gibberellins, were isolated from the supernatant of the culture liquid by extraction with ethyl acetate at a pH of 2.5. The obtained extracts were evaporated in a vacuum at 40–45 °C. The dry residue was dissolved in 80% ethanol and transferred to microtubes. The obtained extracts were stored at a temperature of 24 °C. Preliminary purification and concentration of phytohormonal extracts (accumulating thin-layer chromatography) were carried out on plates with silica gel brand «Silufol UV254» (Chemapol, Czech Republic) in a mixture of solvents introduced sequentially: chloroform, 12.5% aqueous ammonia, and ethyl acetate: acetic acid (20:1). The qualitative and quantitative composition of gibberellins was analysed by high-performance liquid chromatography using an Agilent 1200 liquid chromatograph equipped with G6120A mass detector (Agilent Technologies, USA).

The separation of aliquots of extracts was carried out on an analytical column (Zorbax Eclipse Plus C18, 4.6 mm × 250 mm, 5 μm) (Agilent Technologies, USA). The column thermostat temperature was maintained at 30 °C, and the injection volume was 20 μL. Elution was carried out in the system acetonitrile – water+acetic acid in a gradient mode: 0 min: acetonitrile/0.1% solution of acetic acid in deionized water (30/70) – 20 min:

acetonitrile/0.1% solution of acetic acid (70/30) – 30 min: acetonitrile/0.1% acetic acid solution (100/0) at a constant flow rate of 0.5 ml per minute. The duration of the column equilibration after analysis (post-run) was 15 min. Standards of gibberellins $GA_3$ and $GA_4$ (Sigma-Aldrich, Germany) were used for identification.

Gibberellins were detected using a diode array detector with signal recording at wavelength 210 nm. The molecular weight was determined using a single-quadrupole mass spectrometric detector. Ionisation was performed in the combined mode (electrospray and chemical ionisation at atmospheric pressure), resulting in the formation of negative ions. Ion detection was carried out in the SCAN and SIM (selected ion monitoring) modes, with a mass-to-charge (m/z) range of 200-500. $GA_3$ and $GA_4$ were identified by comparing the retention times, molecular mass values of ions, and spectral characteristics of the obtained peaks. The quantitative content of $GA_3$ and $GA_4$ was determined by the method of external calibration using the SIM mode for ions 345 and 331 m/z (monitoring of the 345 and 331 m/z values according to the time table).

HPLC/MS analysis of gibberellin extracts of *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017 was performed at the Center for the Collective Use of Chromatography and Mass Spectrometry of the National Academy of Sciences of Ukraine at M.G. Kholodny Institute of Botany of the NAS of Ukraine.

**Enzymatic analyses**

Trehalose phosphate synthase (EC 2.4.1.15) activity was analysed by the formation of uridine diphosphate, which was determined spectrophotometrically by the oxidation of NADH at 340 nm in coupled reactions with pyruvate kinase and lactate dehydrogenase.

The activity of 2-C-methyl-D-erythritol-4-phosphate cytidylyltransferase (EC 2.7.7.60), one of the key enzymes of the MEP pathway of gibberellin biosynthesis, was determined by the rate of lactate formation, which was determined by the oxidation of NADH at 340 nm in a conjugate reaction with pyruvate kinase and lactate dehydrogenase (Kuzuyama et al., 2000).

**Statistical processing**

All experiments were conducted in triplicate or more, and the results were expressed as mean±standard deviation.

## Results and discussion

**Activity of the methyl-erythritol-4-phosphate pathway key enzyme in *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017 isoprenoid biosynthesis**

The enzyme 2-C-methyl-D-erythritol-4-phosphate cytidylyltransferase catalyses the formation of 4-(cytidine5'-diphospho)-2-C-methyl-D-erythritol from cytidine triphosphate (CTP) and 2-C-methyl-D-erythritol-4-phosphate, a key intermediate in the methyl-erythritol-4-phosphate pathway of isoprenoid biosynthesis (Diamanti et al., 2022; Kuzuyama et al., 2000). The presence of 2-C-methyl-D-erythritol-4-phosphate cytidylyltransferase activity in *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017 cells grown on different substrates (Table 1) may indicate the functioning of the MEP pathway in these bacteria.

**Table 1**

**Activity of the methyl-erythritol-4-phosphate pathway key enzyme during the growth of *Acinetobacter calcoaceticus* IMB B-7241 and *Rhodococcus erythropolis* IMB Ac-5017 on different substrates**

| Strain | Growth substrate | 2-C-methyl-D-erythritol-4-phosphate-cytidylyltransferase activity, nmol/min·mg protein |
|---|---|---|
| *Acinetobacter calcoaceticus* | Refined sunflower oil | 5263±263 |
| | Biodiesel production waste | 667±33 |
| *Rhodococcus erythropolis* | Refined sunflower oil | 278±13 |
| | Ethanol | 103±5 |

The data presented in Table 1 served as the basis for further studies to determine the effect of erythritol on the gibberellins synthesis in surfactant producers.

**Gibberellin synthesis in erythritol-supplemented cultures of *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017**

Today, it has been proven that the ability to synthesise phytohormones of gibberellin nature is inherent in many living organisms. Various species of plants, bacteria, fungi and yeast produce more than 130 forms of gibberellins (Hernández Rodríguez et al., 2024). At the same time, only some of them, in particular $GA_1$, $GA_3$, $GA_4$ and $GA_7$, are characterised by high biological activity, while other forms are physiologically inactive and serve as intermediates in the biosynthesis of active gibberellins. Our previous studies (Leonova et al., 2020) showed that A. calcoaceticus IMV B-7241 and R. erythropolis IMV Ac-5017 produce $GA_3$ and $GA_4$.

In experiments on the effect of erythritol on the gibberellin synthesis, surfactant producers were grown in a medium with refined oil, biodiesel production waste, and ethanol.

Our previous studies (Pirog et al., 2020) have established a positive effect of exogenous tryptophan (a precursor of the phytohormone auxins biosynthesis) on the auxins formation during the cultivation of A. calcoaceticus IMV B-7241 on biodiesel production waste and R. erythropolis IMV Ac-5017 on ethanol. Thus, there is a potential opportunity to increase the synthesis of both auxins and gibberellins on these substrates with the simultaneous introduction of tryptophan and erythritol into the medium. This, in turn, will significantly enhance the efficiency of integrated technologies for surfactant and phytohormone production, as well as the practical application of these exometabolites in plant production.

The erythritol concentration choice (100-500 mg/L) and the moment of its introduction into the medium (lag phase and the beginning of the stationary phase) was because the concentration of exogenous precursors is usually 10–20% of the concentration of the main growth substrate, and phytohormones are secondary metabolites, the synthesis of which begins in the stationary phase of growth (Cen et al., 2020).

Table 2 shows the biologically active gibberellin synthesis in the presence of erythritol in the culture medium of A. calcoaceticus IMV B-7241.

The data presented in Table 2 show that during the cultivation of strain IMV B-7241 on biodiesel production waste, the concentration of $GA_3$ increased with an increase in the precursor content in the medium.

**Table 2**

**Effect of erythritol on the biologically active gibberellins synthesis by *Acinetobacter calcoaceticus* IMB B-7241**

| Substrate | Erythritol concentration, mg/L | Erythritol addition (growth phase) | Gibberellin concentration, % of control | |
|---|---|---|---|---|
| | | | $GA_3$ | $GA_4$ |
| Refined sunflower oil | 100 | lag phase | 116 | 79 |
| | | beginning of stationary phase | 187 | 84 |
| | 200 | lag phase | 406 | 80 |
| | | beginning of stationary phase | 909 | 87 |
| | 300 | lag phase | 911 | 178 |
| | | beginning of stationary phase | 1590 | 182 |
| | 400 | lag phase | 938 | 165 |
| | | beginning of stationary phase | 1500 | 178 |
| | 500 | lag phase | 476 | 115 |
| | | beginning of stationary phase | 586 | 115 |
| Biodiesel production waste | 100 | lag phase | 21 | 87 |
| | | beginning of stationary phase | 55 | 96 |
| | 200 | lag phase | 25 | 101 |
| | | beginning of stationary phase | 64 | 115 |
| | 300 | lag phase | 33 | 159 |
| | | beginning of stationary phase | 97 | 170 |
| | 400 | lag phase | 43 | 174 |
| | | beginning of stationary phase | 429 | 169 |
| | 500 | lag phase | 60 | 156 |
| | | beginning of stationary phase | 984 | 158 |

**Note:** Control – concentration of gibberellins in medium without erythritol.

The amount of $GA_4$ practically didn`t depend on the moment of erythritol introduction, unlike $GA_3$, the concentration of which was higher in the case of the precursor introduction at the beginning of the stationary growth phase. The optimal concentration of erythritol for the $GA_3$ formation was a concentration of 500 mg/L (an increase in synthesis of almost 10 times compared to that without the precursor). An increase in $GA_4$ synthesis by 156–174% was observed at erythritol concentrations from 300 to 500 mg/L. Interestingly, regardless of the application time, lower concentrations (100–300 mg/L) of erythritol had a more positive effect on $GA_4$ formation (increase in synthesis of 87–170%) than $GA_3$ (increase in synthesis of 21–97%). At the same time, when applied in the stationary phase of growth at higher concentrations (400–500 mg/L), the precursor had a more positive effect on the $GA_3$ formation (increase in the amount by 429–984%) (Table 2).

During the cultivation of *A. calcoaceticus* IMV B-7241 on refined oil, the $GA_3$ and $GA_4$ amounts increased with increasing erythritol concentration from 100 to 400 mg/L. In comparison, at 500 mg/L of the precursor, the synthesis of both gibberellins decreased (see Table 2). The synthesis of $GA_4$ (as well as on biodiesel production waste) did not depend on the moment of adding erythritol to the medium with refined oil. At the same time, the concentration of $GA_3$ was higher when the precursor was added in the stationary phase of growth compared to its addition at the beginning of the strain IMV B-7241 cultivation process. The optimal erythritol content in the medium for the formation of $GA_3$ was 300–400 mg/L (an increase in synthesis by 911–938 and 1500–1590% when the precursor was added in the lag and stationary phases, respectively) (Table 2).

It should be noted that the effect of adding erythritol to a medium with refined oil or biodiesel production waste on the formation of $GA_4$ was practically the same: in the presence of the precursor (depending on its concentration), an increase in the synthesis of this phytohormone by 79–182% was observed compared to the indicators without erythritol. Other patterns were found for the $GA_3$ formation in the presence of the precursor in a medium with various substrates: the introduction of different concentrations of erythritol into a medium with refined oil and biodiesel production waste was accompanied by an increase in the synthesis of $GA_3$ by 116-1590 and 21-984%, respectively (Table 2).

In our opinion, the substrate-dependent effect of the precursor on the $GA_3$ synthesis can be explained as follows. The order of gibberellin synthesis from 7-hydroxykaurenic acid is as follows (Kamiya, 2025):

gibberellin $GA_{14}$ → gibberellin $GA_4$ → gibberellin $GA_7$ → gibberellin $GA_1$ → gibberellin $GA_3$.

Since $GA_4$ is an intermediate metabolite in the synthesis of $GA_3$, it is quite likely that the composition of biodiesel production waste contains components that inhibit the activity of enzymes responsible for the transformation of $GA_4$ into $GA_3$. Such inhibitors of enzyme activity can be monovalent cations (potassium and or sodium), or alcohols (ethanol, methanol).

Table 3 presents data on the erythritol effect in the cultivation medium of *R. erythropolis* IMV Ac-5017 on the synthesis of biologically active gibberellins.

**Table 3**

**Effect of erythritol on the biologically active gibberellin synthesis by *Rhodococcus erythropolis* IMB Ac-501**

| Substrate | Erythritol concentration, mg/L | Erythritol application time (growth phase) | Gibberellin concentration, % of control | |
|---|---|---|---|---|
| | | | $GA_3$ | $GA_4$ |
| Refined sunflower oil | 100 | lag phase | 125 | 131 |
| | | beginning of stationary phase | 105 | 113 |
| | 200 | lag phase | 161 | 156 |
| | | beginning of stationary phase | 135 | 129 |
| | 300 | lag phase | 262 | 234 |
| | | beginning of stationary phase | 158 | 130 |
| | 400 | lag phase | 380 | 361 |
| | | beginning of stationary phase | 211 | 151 |
| | 500 | lag phase | 177 | 109 |
| | | beginning of stationary phase | 150 | 58 |
| Biodiesel production waste | 100 | lag phase | 121 | 116 |
| | | beginning of stationary phase | 132 | 129 |
| | 200 | lag phase | 130 | 125 |
| | | beginning of stationary phase | 143 | 138 |
| | 300 | lag phase | 152 | 136 |
| | | beginning of stationary phase | 186 | 157 |
| | 400 | lag phase | 171 | 164 |
| | | beginning of stationary phase | 233 | 206 |
| | 500 | lag phase | 305 | 287 |
| | | beginning of stationary phase | 468 | 407 |

**Note:** Control – concentration of gibberellins in medium without erythritol.

Regardless of the growth substrate nature in the cultivation medium of *R. erythropolis* IMB Ac-5017, the increase in the content of the gibberellin biosynthesis precursor was accompanied by an increase in the concentration of the formed gibberellins $GA_3$ and $GA_4$. The maximum increase in the phytohormone amount during the cultivation of the strain IMB Ac-5017 on ethanol (361-380% of the control) was achieved in the presence of 400 mg/L of erythritol, and on refined oil (407-468% of the control) – in the presence of 500 mg/L of the precursor in the medium (see Table 3). It should be noted that the level of gibberellin synthesis depended not only on the erythritol concentration, but also on the moment of its introduction into the cultivation medium of *R. erythropolis* IMB Ac-5017.

Thus, the concentration of synthesised phytohormones was higher in the case of adding an exogenous precursor in the lag phase of the producer's growth on ethanol compared to that when adding erythritol at the beginning of the stationary phase (125–380 and 105–211%, respectively). In the process of growing the strain IMV Ac-5017 on refined oil, the gibberellins synthesis was higher when adding erythritol at the beginning of the stationary growth phase than at the start of the process (129-407 and 116-287%, respectively) (see Table 3). It should be noted that regardless of the erythritol concentration and the moment of its introduction into the cultivation medium of *R. erythropolis* IMB Ac-5017 with both ethanol and refined oil, an almost identical increase in the synthesis level of both biologically active gibberellins $GA_3$ and $GA_4$ was observed.

At the next stage, the key enzyme of gibberellin biosynthesis activity was determined in *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017 cells grown on various substrates in the presence of erythritol (Table 4).

**Table 4**

**Effect of erythritol on the key enzyme of gibberellin biosynthesis activity in *Acinetobacter calcoaceticus* IMB B-7241 and *Rhodococcus erythropolis* IMB Ac-5017**

| Strain | Substrate | Erythritol concentration, mg/L | 2-C-methyl-D-erythritol-4-phosphatecytidyl transferase activity, nmol/min·mg protein |
|---|---|---|---|
| *Acinetobacter calcoaceticus* IMB B-7241 | Refined sunflower oil | 0 | 5263±263 |
| | | 300 | 7519±375 |
| | Biodiesel production waste | 0 | 667±33 |
| | | 500 | 1071±53 |
| *Rhodococcus erythropolis* IMB Ac-5017 | Refined sunflower oil | 0 | 278±13 |
| | | 500 | 484±24 |
| | Ethanol | 0 | 103±5 |
| | | 400 | 159±7 |

**Note:** Erythritol was added to the culture medium at the beginning of the stationary phase of growth of strains IMV B-7241 and IMV Ac-5017.

The data presented in Table 4 indicate that the exogenous precursor is involved in the gibberellins synthesis in the methyl-erythritol-4-phosphate pathway: 2-C-methyl-D-erythritol-4-phosphatecytidylyl transferase activity in cells of both strains grown under conditions of maximum increase in the gibberellin phytohormones synthesis (see Tables 2 and 3) was 1.4–1.7 times higher than during the cultivation of *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017 in a medium without erythritol.

In our work (Pirog et al., 2025), it was noted that, unlike a fairly large number of publications on the auxin biosynthesis intensification in the presence of the precursor tryptophan, there is no information about the effect of erythritol on the formation of gibberellins by microorganisms. In our opinion, one of the reasons for this state of affairs is the fact that at present the main highly efficient producers of gibberellins are the fungi *Gibberella fujikuroi* (now reclassified as *Fusarium fujikuroi*) and *Fusarium moniliforme*, in which the synthesis of these phytohormones is carried out via mevalonic acid (Salazar-Cerezo et al., 2018), and not in the methyl-erythritol-4-phosphate pathway, as in bacteria. In addition, the fungi *F. fujikuroi* and *F. moniliforme* are industrial producers of gibberellic acid ($GA_3$); therefore, primary scientific research today focuses on increasing the efficiency of biotechnology based on these strains.

Thus, at present, the main approaches to intensification of gibberellin synthesis by the fungi *F. fujikuroi* and *F. moniliforme* are the conditions for cultivating producers optimisation and their improvement by metabolic and genetic engineering methods (Cen et al., 2020; 2023; Hernández Rodríguez et al., 2024; Peng et al., 2020; Shani et al., 2024; Wang et al., 2023).

Previously (Pirog et al., 2025), we found that the introduction of 300-400 mg/L erythritol into the cultivation medium of the surfactant producer *Nocardia vaccinii* IMV B-7405 was accompanied by a 2–14 times increase in the concentration of biologically active gibberellins $GA_3$ and $GA_4$ compared to the synthesis indicators in the medium without the precursor. This work is a logical continuation of our previous studies on enhancing integrated microbial technologies for plant production. In addition, these studies suggest that introducing exogenous precursors of the biosynthesis of the target product into the cultivation medium of the producer is a simple and highly effective method for intensifying the synthesis of practically important metabolites.

**Effect of erythritol on surfactant synthesis in *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017**

At the final stage, the level of surfactant synthesis was determined during the cultivation of producers in a medium with erythritol (Table 5).

The data in Table 5 show that the presence of erythritol in the cultivation medium of both strains with different growth substrates didn`t affect the indicators of surfactant synthesis.

Surfactants as well as phytohormones are classified as secondary metabolites. The producer's cultivation conditions determine the composition and properties of these compounds. Therefore, it is impossible to guarantee that preparations synthesised in the presence of erythritol will possess the biological properties necessary for their effective use in plant production, in particular, antimicrobial activity against phytopathogenic bacteria. The main component of the surfactant complex of *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017, responsible for antimicrobial activity, is aminolipids (Pirog et al., 2020). The results of enzymatic studies showed that during the cultivation of both strains under conditions that ensure maximum gibberellins synthesis, the key enzyme activity of surface-active aminolipids was 1.4–3.6 times higher than during cultivation in a medium without a precursor (Table 6). The activity of trehalose phosphate synthase, the key enzyme of glycolipid (trehalose mycolate) biosynthesis in *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017, was practically the same under the growth conditions of both strains in the presence and absence of erythritol.

**Table 5**

**Surfactant synthesis by *Acinetobacter calcoaceticus* IMB B-7241 and *Rhodococcus erythropolis* IMB Ac-5017 in the presence of erythritol**

| Strain | Substrate | Erythritol addition (growth phase) | Erythritol concentration, mg/L | Surfactant concentration, g/L |
|---|---|---|---|---|
| *Acinetobacter calcoaceticus* IMB B-7241 | Refined sunflower oil | – | 0 | 1.74±0.09 |
| | | lag phase | 300 | 1.63±0.08 |
| | | beginning of stationary phase | 300 | 1.81±0.09 |
| | Biodiesel production waste | – | 0 | 2.84±0.14 |
| | | lag phase | 500 | 2.71±0.13 |
| | | beginning of stationary phase | 500 | 3.08±0.15 |
| *Rhodococcus erythropolis* IMB Ac-5017 | Ethanol | – | 0 | 1.89±0.09 |
| | | lag phase | 400 | 1.62±0.08 |
| | | beginning of stationary phase | 400 | 1.71±0.09 |
| | Refined sunflower oil | – | 0 | 1.22±006 |
| | | lag phase | 500 | 1.05±005 |
| | | beginning of stationary phase | 500 | 1.12±0.05 |

**Table 6**

**Erythritol effect on key enzyme of surface-active glyco- and aminolipid biosynthesis activity in *Acinetobacter calcoaceticus* IMB B-7241 and *Rhodococcus erythropolis* IMB Ac-5017**

| Strain | Substrate | Erythritol concentration. mg/L | Activity, nmol/min·mg protein | |
|---|---|---|---|---|
| | | | $NADP^+$-glutamate dehydrogenase | trehalose phosphate synthase |
| *Acinetobacter calcoaceticus* IMB B-7241 | Refined sunflower oil | 0 | 2608±130 | 19±0.8 |
| | | 300 | 5630±280 | 23±1.2 |
| | Biodiesel production waste | 0 | 708±35 | 33±1.6 |
| | | 500 | 986±49 | 39±2.2 |
| *Rhodococcus erythropolis* IMB Ac-5017 | Refined sunflower oil | 0 | 556±27 | 23±3 |
| | | 500 | 1250±62 | 16±0.8 |
| | Ethanol | 0 | 82±4 | 10±0.5 |
| | | 400 | 298±14 | 10±0.5 |

**Note:** Erythritol was added to the culture medium at the beginning of the stationary phase of strains IMV B-7241 and IMV Ac-5017 growth.

Currently, there are few reports describing the ability of surfactant-producing microorganisms to synthesise gibberellin phytohormones (Abdelmoteleb et al., 2022; Chen et al., 2021; Hao et al., 2019). For example, Abdelmoteleb et al. (2022) isolated three strains of *Bacillus subtilis* that simultaneously synthesised the lipopeptide iturin and produced 0.53–1.65 mg/L of the auxin phytohormone indole-3-acetic acid and 1.64–1.97 mg/L of gibberellin $GA_3$. Similarly, Chen et al. (2021) demonstrated that *Bacillus atrophaeus* B44 is capable of concurrent production of an aminolipid complex with antifungal activity and the biologically active gibberellin $GA_3$ at concentrations of 7.7–23.1 mg/L.

In 2024, Ding et al. (2024) reported that *Bacillus amyloliquefaciens* MG-2 simultaneously synthesised the lipopeptides fengycin, iturin, and surfactin, along with a complex of auxin-type phytohormones, cytokinins, and the biologically active gibberellins $GA_1$ and $GA_3$, as determined by high-performance liquid chromatography.

Bacteria of the genus *Bacillus*, as shown in previous studies (Abdelmoteleb et al., 2022; Chen et al., 2021; Ding et al., 2024; Hao et al., 2019), are predominantly plant-associated microorganisms that naturally synthesise phytohormones and lipopeptides. In contrast, *A. calcoaceticus* IMB B-7241 and *R. erythropolis* IMB Ac-5017 are free-living soil bacteria, and information on the ability of such microorganisms to synthesise a surfactant complex together with phytohormones of three classes (auxins, cytokinins, and gibberellins) is currently lacking. A further advantage of *A. calcoaceticus* IMB B-7241 and *R. erythropolis* IMB Ac-5017 is their capacity to produce metabolites of practical relevance for plant production using inexpensive substrates. Moreover, the surfactants synthesised by these strains exhibit antimicrobial activity against phytopathogenic bacteria.

## Conclusions

Thus, the results demonstrated that phytohormone gibberellin biosynthesis in the surfactant-producing strains *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017 occurs via the methylerythritol 4-phosphate (MEP) pathway. Supplementation of the cultivation medium with erythritol—an intermediate in isoprenoid biosynthesis within this pathway—led to a marked increase in the synthesis of biologically active gibberellins. In the presence of 300–500 mg/L of this exogenous precursor across various growth substrates, the concentrations of $GA_3$ and $GA_4$ produced by strains IMV B-7241 and IMV Ac-5017 increased by 1.5–16-fold relative to cultures without erythritol.

These findings provide a foundation for the development of a highly efficient, integrated technology for the simultaneous biosynthesis of surfactants and phytohormones for application in plant production.

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