балаусада кумарин шамасы 1,14 %, 20° температурада кептірілген шөпте – 0,65 %, 60° температурада белсенді желдету кезінде 0,48% құрады.

Кілт сөздер: Түйежоңышқа, мал азығының сапасы, кумарин.

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ВЛИЯНИЕ ПРИЕМОВ СНИЖЕНИЯ СОДЕРЖАНИЯ КУМАРИНА НА УРОЖАЙНОСТЬ И КАЧЕСТВО КОРМОВОЙ МАССЫ ДОННИКА В УСЛОВИЯХ СЕВЕРНОГО КАЗАХСТАНА

Аннотация

В статье приведены результаты лабораторных и полевых опытов по изучению влияния различных приемов снижения содержания кумарина в доннике. Цель исследований – разработать безопасный для животных способ заготовки сена из донника в связи с содержанием в листостебельной массе глюкозида кумарин. Во исполнение решения поставленных задач проведены полевые работы на опытном участке Северо-Казахстанского НИИ сельского хозяйства в 2015-2016 гг. Содержание кумарина в зеленой массе донника составляло 1,14%, в сене высушенном при температуре 20° - 0,65% и при температуре 60° - 0,48%.

Ключевые слова: Донник, качество корма, кумарин.

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MEDIUM OPTIMIZATION FOR PRODUCTION ANSAMITOCIN P-3 BY *ACTINOSYNNEMA PRETIOSUM*

Abstract

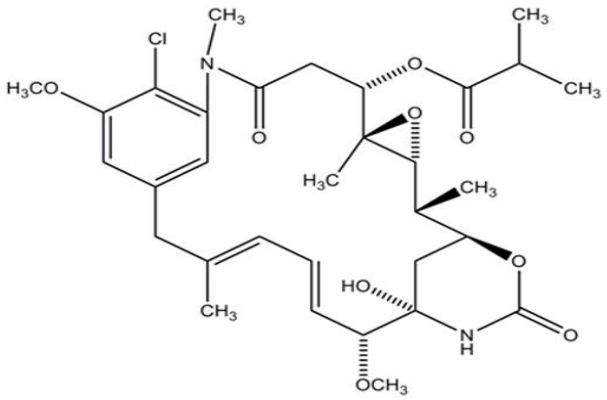
Ansamitocins belong to the group of ansamycin macrolactam antibiotics, which are produced by Actynosynnema spp. In the present studies, inexpensive carbon sources in an attempt to increase ansamitocin P-3 production were investigated. Dry powder of Jerusalem artichoke was the best carbon sources for production of ansamitocin P-3. In the medium, which contains Jerusalem artichoke powder combining with glycerol and additionally adding isobutanol, the AP-3 was reached about 60 mg/l.

Key words: Actinosynnema pretiosum, ansamitocin P-3, fermentation, carbon sources.

Introduction

Ansamitocins are potent antitumor agents produced by Actinosynnema pretiosum.[1]

Actinosynnema pretiosum is a commercially important organism due to its ability to produce ansamitocin P-3. The cytotoxicity of ansamitocin has prompted its use as a toxic "warhead" in immuno-toxin conjugates. Several of these conjugates are currently in late-phase clinical trials as therapeutic agents against solid tumors [2]



Ansamitocin P3

Fig.1 - Structure of ansamitocin is shown.

Biosynthesis of ansamycin antibiotics involves the formation of 3-amino-5-hydroxybenzoate (AHBA) as the starter unit for the assembly of the polyketide chain. The first three steps of the AHBA pathway are very similar to the shikimate pathway, but later steps are differentiated by the introduction of glutamine-derived nitrogen, to give 3,4-dideoxy-4-amino-D-arabino-heptulosonic acid 7-phosphate (aminoDAHP). Cyclization and dehydration of amino-DAHP generates 5-deoxy-5-amino-3-dehydroquinic acid (aminoDHS), which is finally aromatized, by the enzyme AHBA synthase to give AHBA [3].

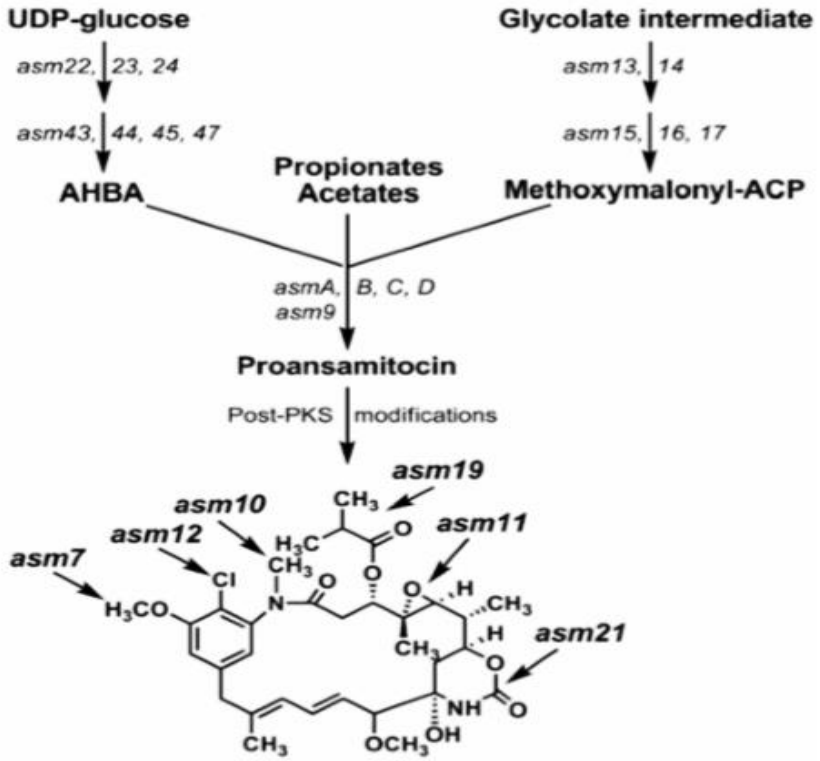


Fig.2 - Key steps for ansamitocin biosynthesis.

In the recent years, considerable effort has been focused on enhancing AP-3 production with medium development and genetic modification because of its high commercial value. Manipulation of fermentation process is essential to improve the productivity of secondary metabolites.

Production of secondary metabolites is a process influenced by several physico-chemical factors including nutrient supply, oxygenation, temperature and pH. These factors have been traditionally controlled and optimized in industrial fermentations in order to enhance metabolite production [4]. The investigation of medium component influence is the classical method for improvement second metabolite production. Several studies investigate the effect of medium components and some of them had good results. Glucose, glycerol and fructose used as major carbon source and medium with fructose obtain significantly higher secondary metabolite titer, indicating that there was great influence on the accumulation and conversion of intermediates to biosynthesis ansamitocin, particularly on the transcription of genes responsible for ansamitocin biosynthetic pathway. In that study significant increase in *asm8* gene expression in the presence of fructose which might result in the up-regulation of AHBA synthetic genes has mentioned, and enhanced biosynthesis of ansamitocin by A. pretiosum has also resulted in [5].

Glycerol is widely used in microbiology fermentation, and considered as cheap carbon sources.[6] It was discovered that combination of glucose and glycerol could be an appropriate strategy for enhancing AP-3 production by Actinosynnema pretiosum. When the weight ratio of glucose/glycerol was 5:5, the production and yield of AP-3 was 8.4 mg l^{-1}, and 2.5 mg l^{-1} , respectively, which was 3.3- and 1.8-fold higher than the amount, which was obtained in the medium containing only glucose as the main carbon source [7]. In the study by Qian, S has been reported that inulin had positive effect on bacillomycin D biosynthesis by Bacillus subtilis[8].

In industrial level, secondary metabolite production costing is very important. Nowadays, a lot of work in a way to find cheap carbon and nitrogen sources has done. Vegetable oils were investigated to evaluate their potential to act as the sole carbon source for production of cephamycin C [9, 10]. Soybean oil was the best carbon source for cephamycin C production. Bioautography and HPLC analyses showed that cephamycin C was exclusively produced even though when soybean oil as the sole carbon source was used. Soybean oil was the best carbon source for cephamycin C production. When the initial oil concentration was 7 g/l, the product yield showed the maximum at 296 mg cephamycin C[10].

This study investigates effect of soybean oil, buckwheat starch and dry powder of Jerusalem artichoke on dry cell weight and AP-3 production by Actinosynnema pretiosum.

Materials and methods

A.pretiosum ATCC 31565 was used in this study. The stock cell suspension was prepared by incubating. The strain was cultivated on the medium T1 containing (in g/L, pH 7.4) glucose (10), glycerol (10) yeast extract (5), and peptone (10) on a rotary shaker (200 rpm) at 28 °C for 48 h, and maintained in 20% (v/v) glycerol at −40°C. The fermentation experiment was carried out as follows: the preculture was prepared by inoculating 60 μL of the stock cell suspension into 250 mL Erlenmeyer flasks containing 50 mL of T1 seed medium, followed by the incubation in a rotary shaker (220 rpm) at 28 °C for 48 h in term of which 500 μL of the obtained culture was transferred into 50 mLT1 seed medium and cultivated for another 24 h. The cells were harvested and inoculated into 50 mL of fermentation medium with in an inoculum size of 500μL and cultivated at 28°C for 8 day. Fermentation medium consisted of (in g/L, pH 7.4) distilled water, yeast extract 7; $CaCO_3$ 5; K_2HPO_4 0.25; $FeSO_4 \cdot 7H_2O$ 0.01; and NH_4Cl 1; Appropriate amount of soybean oil, dry powder of Jerusalem artichoke and buckwheat starch were adjusted where necessary. Jerusalem artichoke tuber was washed and cut to small pieces. Then, they were dried at 65 °C until reaching a constant weight and milled to powder.

Growth Measurement and Ansamitocin P-3 Determination 2 mL of fermentation broth was centrifuged at 10,000 rpm for 5 min. For AP-3 assay, 1 mL supernatant with equal volume of ethyl acetate was extracted 3 times. The extracts were subsequently combined, evaporated and dissolved with 1 mL of methanol. AP-3 was analyzed by Shimadzu LC-20AD HPLC system (Shimadzu Corporation, Tokyo, Japan) with SinoChrom ODS-BP column (4.6 × 250 mm, 5 μL)

and UV detection at 254 nm. The mobile phase was 85% methanol and 15% water with a flow rate of 0.6 mL/min. Purified AP-3 (purity > 98%) was applied to perform quantification by external calibration. The precipitate after centrifugation was washed twice with 0.1 M HCl to remove the residual $CaCO_3$. The remaining cells were then dried to constant weight at 105 °C to obtain dry cell weight (DCW).

Results and discussion

Effect of different carbon sources on the growth and AP-3 production

The effect of three different carbon sources on cell growth and AP-3 production was tested in flask culture, the results are shown in Fig. 3. All carbon sources were used in concentration of 30 g/l and tested in the medium with and without yeast extract. The maximum dry cell weight was obtained in the medium with buckwheat and yeast extract. When dry powder of Jerusalem artichoke and soybean oil as the carbon sources were used the DCW remained the same. The highest AP-3 production 32.97 mg/l was gained in the medium containing buckwheat at the concentration of 30 g/l. When soybean oil and dry powder of Jerusalem artichoke were used as carbon sources, there was no difference in AP-3 production. Interestingly, in the medium with highest DCW was no AP-3 production found.

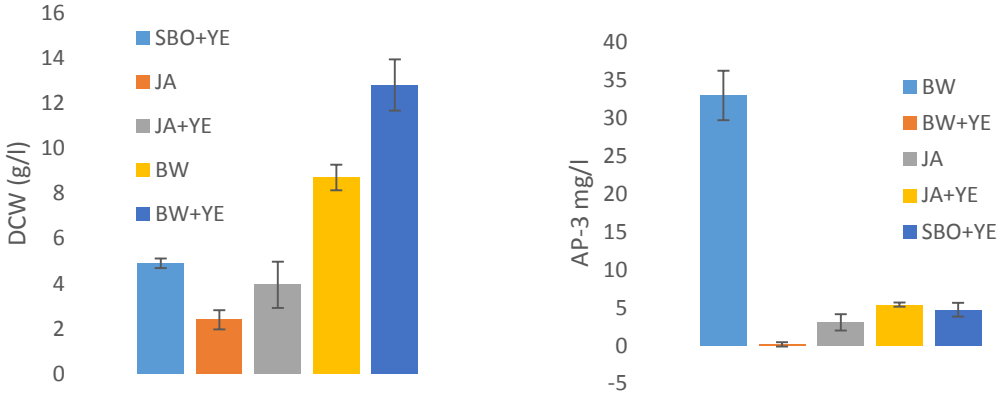


Fig.3 - Effect of different carbon sources on the growth and AP-3 production.

The contribution of glycerol and glycose to AP-3 in the medium with different carbon sources was tested. When the glucose was added at 10g/l in the medium with Jerusalem artichoke dry powder and soybean oil production of ansamitocin was higher two times compare with previous results without glucose. However, the production titer was still very low. Addition added glycerol in the medium with dry powder of Jerusalem artichoke and soybean oil had negative effect on production of AP-3.

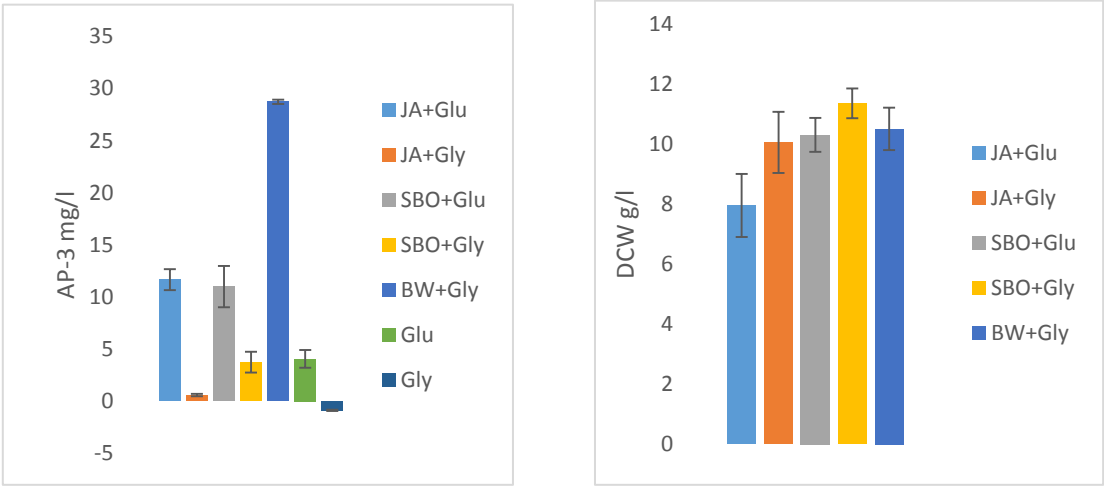


Fig.4 - Plot of DCW and AP-3 production in the medium with mixed carbon sources.

Effect of isobutanol on ansamitocin production.

It was found that addition isobutanol at optimal condition to medium can increase AP-3 production up to four fold. In addition, supply isobutanol reduce AP-2 production thereby simplifying separation process. [11] In our experiment, we have added isobutanol at the 3rd day in the concentration of 36mM to 4 different medium with various mixed carbon sources. The maximum amount of ansamitacin was obtained in medium M4, AP-3 production was 56.6 mg/l. Production of AP-3 in the isobutanol supplemented mediums are shown in Fig 5.

Table 1. Medium components

Medium	Carbon sources
M1	Soybean oil 30g/l, glycerol 26.8
M2	Soybean oil 30g/l, glucose 10 g/l
M3	Buckwheat 30g/l, glycerol 26.8g/l
M4	Dry powder of Jerusalem artichoke 30 g/l, glycerol 26.8 g/l

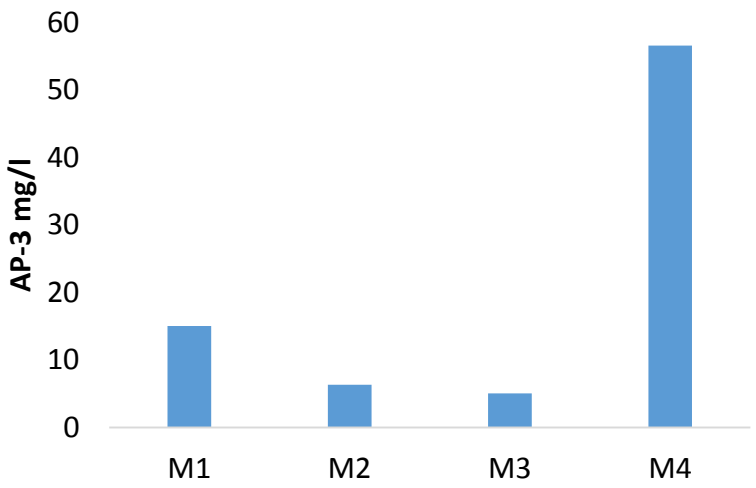


Fig.5 Production of AP-3 in the isobutanol supplemented mediums

Conclusion

Cost of fermentation medium is very important aspect in antibiotics production. Finding alternative carbon sources can be cost-effective solution in the production of ansamitocin. Dry powder of Jerusalem artichoke was found as the good carbon source supplemented with isobutanol for ansamitocin production. In the future, there has a priority to investigate nitrogen sources and optimize the medium for further improvement of AP-3 production

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ОПТИМИЗАЦИЯ ПИТАТЕЛЬНОЙ СРЕДЫ ДЛЯ ПОЛУЧЕНИЯ АНСАМИТОЦИНА Р-3 ИЗ ACTINOSYNNEMA PRETIOSUM

Аннотация

Ансамитоцины относятся к группе макролактамных антибиотиков ансамицинам, продуцентами которых являются бактерии рода Actynosynnema. В данном эксперименте были исследованы дешевые источники углевода для повышения выхода ансамитоцина. Сухой порошок топинамбура оказался лучшим источником для биосинтеза ансамитоцина. В среде, содержащей 30 г/л порошка томинамбура в сочетании с глицерином и, добавленным на третий день, изобутанолом было получено 60мг/л ансамитоцина Р-3.

Ключевые слова: Actinosynnema pretiosum, ансамитоцин Р-3, ферментация, источники углевода.

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ACTINOSYNNEMA PRETIOSUM ТҮЗЕТІН АНЦАМИТОЦИН ҮШІН ҚОРЕКТІК ОРТА ОПТИМИЗАЦИЯСЫ

Аңдатпа

Ансамитоциндер макролактам тобының ансамицин тобына жатады, анцамициндерді тузетін негізінен Actynosynnema тобының бактериялары. Осы зерттеулер барысында ансамитоцин көлемін көбейту үшін арзан көміртегі көздері қарастырылды. Топинамбур ұнтағы ансамитоцин биосинтезіндегі тиімді көміртегі көзі екендігі анықталды. 30 г/л

концентрациясындагы топинамбур ұнтағы және глицирин мен изобутанол қосылған қоректік ортадан ,60 мг/л анцамитоцин Р-3 бөлініп алынды.

Кілт сөздер: Actinosynnema pretiosum, ансамитоцин Р-3, ферментация, көміртегі көзі.

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АВСТРАЛИЯЛЫҚ ЭМУ ТҮЙЕҚҰС ҰШАСЫ МЕН АҒЗАЛАРЫН ВЕТЕРИНАРИЯЛЫҚ САНИТАРИЯЛЫҚ САРАПТАУ ЖӘНЕ ЕТІНІҢ ХИМИЯЛЫҚ ҚҰРАМЫ

Аңдатпа

Мақалада рационына минералды азықтық қоспа қосып азықтандырылған австралиялық түйеқұс эмуды сояр алдындағы қарап тексеру, ұшасы мен ағзаларын ветеринариялық санитариялық сараптау жүргізу және етінің химиялық құрамы нәтижелері көрсетілген.

Кілт сөздер: Австралиялық эму түйеқұс, минералды азықтық қоспа, ұша, нәруыз, ылғал, энергетикалық құндылық, дәрумен, минералдық заттар, май, химиялық құрамы.

Кіріспе

Ауыл шаруашылығының тиімді саласының бірі - құс шаруашылығы. Адамзатты нәруызды тағаммен қамтамасыз етуде құстан алынатын жұмыртқа мен еттің маңызы ерекше болып табылады.

Қазір Қазақстанда құс өсірумен асыл тұқымды құс зауытттары мен құс фермалары айналысады. Соның бірі, экзотикалық ет түрімен жоғары өнімді еті мен жұмыртқа, тері, қауырсыны ретінде ерекшеленетін түйеқұс шаруашылығы бой көтеруде.

Реңі ашық қоңыр, қара сұр түсті, биіктігі 1,8 м, салмағы 40–55 кг болатын австралиялық эму түйеқұс еті өзінің ерекшелігімен, диеталық азық ретінде танылa бастады. Түйеқұсты өсіріп-бағуда дәрумен, гормон, минералдық заттардың жеткіліксіздігі зат алмасу үдерісінің бұзылуларына әкеп соғады. Нәтижесінде қауырсындары түсіп, жұмыртқалау көрсеткіші төмендеп, олардың тағамдық құндылығы нашарлап, инкубаторлық бағытта балапандардың өміршеңдігі төмендейді.

Елімізде түйеқұстарды алып келгелі 15 жылдан асты, австралиялық түйеқұс жайлы жаппай Еуропа, АҚШ және ТМД елдерінде, сонымен бірге Ресейде көптеген зерттеулер жүргізілді. Оларды жерсіндіру мәселелерін, етінің құрамын, олардан жасалған өнімдерді, дайын биологиялық белсенді заттардың түйеқұс ағзасына әсерін зерттегені жайлы бірқатар мақалалар жарияланды. Дегенімен, бұл ғылыми - зерттеу жұмыстары австралиялық түйеқұс жайлы толық мәліметтерді бере алмайды.

Зерттеу жұмысының мақсаты: түйеқұстарға арналған минералды азықтық қоспаны австралиялық эму түйеқұс рационына қосып азықтандырғаннан кейін, ұшасы мен ағзаларын ветеринариялық санитариялық сараптау және етінің химиялық құрамын анықтау . Қойылған мақсатқа байланысты мынадай міндеттер айқындалды:

1. Сояр алдындағы клиникалық зерттеу жұмыстарын жүргізу;
2. Союдан кейінгі ұшасын ветеринарлық санитарлық сараптап, сойыс шығымы мен мүшелерінің салмағын анықтау;