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REGULATION OF BIOSYNTHESIS OF 1-ACETYL-β-CARBOLINE IN *Streptomyces kasugaensis*

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SUMMARY

Biosynthesis of 1-acetyl-β-carboline, an enzyme inhibitor, in *S. kasugaensis* SK-619 was regulated by quality of the carbon source and by addition of organic acids into medium. Variation in phosphate concentration also affected production of this carboline.

INTRODUCTION

β-Carboline alkaloids originaly isolated from plants, are biosynthesized by microorganisms and they also occur in mammals. It has been found that these substances are biologically active and may play an important role as neuromodulators (Rommelspacher et al., 1991). Compounds of this structural group have hypnotic and antiepileptic (Desforges et al., 1989) activities. In previous works we identified 1-acetyl-β-carboline in cultivation broth of *S. kasugaensis* (Proksa et al., 1990) and observed inhibition of activity of β-galactosidase (40 %), β-glucosidase (30 %) and pancreatic elastase (20 %) at concentration of 1.10^{-5} mmol/l. (Šturdíková

et al., 1993). The main purpose of this study was to investigate the effect of sugars, phosphates and organic acids on production of the β-carboline.

MATERIALS AND METHODS

Producing organism *S. kasugaensis* SK-619 was obtained from the original strain IFO 13851 (Institute for Fermentation, Osaka, Japan) by active selection after UV irradiation combined with enzyme induction by addition of tryptophan into agar medium.

Seed medium (100 ml) containing (g/l) glucose 15, soybean 15, K_2HPO_4 1, $MgSO_4.7H_2O$ 0.5, NaCl 3, $CaCO_3$ 5 (pH 6.9) in tap water transferred into 500 ml cultivation flasks was sterilized at 120 ^oC for 15 min. This medium inoculated with a loopful of *S. kasugaensis* was incubated at 28 ^oC for 2 days.

Production medium consisting of (g/l) glucose 15, soybean 12, K_2HPO_4 1, $MgSO_4.7H_2O$ 0.5, NaCl 3 after sterilization and inoculation with vegetative inoculum (10 % v/v) was incubated on a rotary shaker (3.7 Hz) at 28 ^oC.

Product analysis. HPLC on a RP-18 column (Proksa et al., 1990) was used for analysis of 1-acetyl-β-carboline in cultivation media.

RESULTS AND DISCUSSION

Biosynthesis of secondary metabolites is controlled by internal (genetic) as well as by external (medium composition, stimulators, precursors) factors. Active selection after UV irradiation of the original strain *S. kasugaensis* and induction of the enzyme system resulted in formation of the mutant SK 619 which had enhanced ability to produce 1-acetyl-β-carboline (ABC). No significant differences in production of ABC were observed when glucose, fructose, maltose or glycerol in

concentration range 0.5 - 1.5 g/l were used as a carbon source. Glucose above 1.5 g/l in medium inhibited biosynthesis, on the other hand production of ABC was raised in medium with maltose. Similar effect of glucose was observed also in biosynthesis of ergot and carbazole alkaloids (Vining and Chatterjee, 1982). Amino acids serve as a nitrogen source in alkaloid formation by microorganisms (Nozawa et al., 1989; Kaneda et al., 1990; Reshetilova and Kozlovskii, 1990). In previous study we observed, that tryptophan and alanine are precursors of ABC (Proksa et al., 1990) and good correlation was observed between tryptophan in concentration range 5 - 20 mmol/l and ABC production; other aminoacids, which have no apparent conection with ABC biosynthesis enhanced production of this alkaloid as well (Tab. 1).

Table 1. Production of 1-acetyl-β-carboline (ABC) in presence of various aminoacids and glutathione.

Aminoacid [5 mmol/l]	Production of ABC [mmol/l]	Production vs. control [%]
control	0.77	100.0
proline	0.81	106.0
tyrosine	0.82	106.5
cystine	1.01	131.2
glutathione	1.37	177.9
tryptophan	1.73	224.1

Cystine and glutathione obviously take part in biosynthesis of aureothricin and thiolutin, sulfur-containing metabolites of *S. kasugaensis* (Šturdíková et al., 1990) and as a secondary effect only they stimulate also production of the ABC.

Another factor affecting biosynthesis of alkaloids is phosphate concentration (Pažoutová and Řeháček, 1984). Increasing

phosphate level in medium with glucose lowered production of ABC, contrary to medium with maltose where the opposite effect was observed up to phosphate concentration of 1.5 g/100 ml. Above this value decrease in ABC production occured.

Biosynthesis of ergot alkaloids by *Claviceps paspali* was regulated by quality and concentration of carbohydrates, phosphates and by addition of intermediates of the tricarboxylic acid cycle (Spalla et al., 1978). Aliphatic di- and tricarboxylic acids had no pronounced effect on production of ABC in *S. kasugaensis*; considerable enhancement was observed in presence of 2-oxoglutaric and aromatic acids (Tab. 2).

Table 2. Effect of organic acids on production of 1-acetyl-β-carboline (ABC)

Substrate [5 mmol/l]	Production of ABC [mmol/l]	% vs control
control	0.77	100.0
itaconic acid	0.41	53.2
citraconic acid	0.44	57.1
maleic acid	0.60	77.9
fumaric acid	0.69	89.6
citric acid	0.72	93.5
malic acid	0.76	98.7
phthalic acid	0.92	119.5
2-oxoglutaric acid	0.97	125.9
gallic acid	1.16	150.6

Addition of methionine into medium remarkably raised production of ABC. This aminoacid is the precursor of biosynthesis of 3-methylthioacrylic acid (Šturdíková et al., 1990), a metabolite accompanying ABC. The highest production of ABC was ob-

served in medium containing maltose (5 %), phosphate (0.1 %), tryptophan (5 mmol/l) and methionine (40 mmol/l) (Tab. 3).

Table 3. Production of 1-acetyl-β-carboline (ABC) by *S. kasugaensis* cultivated in various media

Glc [%]	Mal [%]	P [%]	Trp [mmol/l]	Met [mmol/l]	ABC [mmol/l]
1.5	-	0.1	-	-	0.20
1.5	-	1.0	-	-	0.12
1.5	-	0.1	5.0	-	0.42
1.5	-	1.0	5.0	-	0.16
1.5	-	0.1	-	40.0	0.09
1.5	-	1.0	-	40.0	0.11
1.5	-	0.1	5.0	40.0	0.39
1.5	-	1.0	5.0	40.0	0.11
-	5.0	0.1	-	-	0.17
-	5.0	1.0	-	-	0.26
-	5.0	0.1	5.0	-	0.74
-	5.0	1.0	5.0	-	0.82
-	5.0	0.1	-	40.0	0.50
-	5.0	1.0	-	40.0	0.39
-	5.0	0.1	5.0	40.0	2.40
-	5.0	1.0	5.0	40.0	2.06

Glc - glucose; Mal - maltose; P - phosphate; Trp - tryptophan; Met - methionine.

Formation of 1-acetyl-β-carboline was observed only with *S. kasugaensis*; the other actinomycetes (*S. griseus* CCM 3178, CCM 3179, *S.griseus* ATCC 1037, *S. rutgersensis* CCM 3196, *S. fradiae* CCM 3174, *S. vinaceus* CCM 3005, *S. anulatus* CCM 3158) did not synthesize ABC in presence of trpytophan.

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