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The Glucose Repression of Aerial Mycelium Formation in *Streptomyces*

I. Characterization of Factors Involved in Glucose Repression of Aerial Mycelium Formation

Kim, Jae Heon, Ung Jin Kim, Hyen Sam Kang, Yung Chil Hah, and Soon Woo Hong

(Department of Microbiology, College of Natural Sciences, Seoul National University)

*Streptomyces*의 Aerial Mycelium 형성에 대한 Glucose억제 기작에 관한 硏究

I. Glucose억제에 관여하는 요인분석

金載憲 · 金雄鎭 · 姜炫三 · 河永七 · 洪淳佑

(서울大學校 自然科學大學 微生物學科)

ABSTRACT

We have demonstrated that both L-histidine as an amino acid factor and dextrin as a carbon source were required for the glucose repression. 1% glucose was sufficient to the glucose repression of aerial mycelium formation in *Streptomyces lavendulae* and *Streptomyces aureofacience*. The synthesized medium, KK, which is lack of all organic nutrients except dextrin was able to induce glucose repression, but the addition of 0.003% or more L-histidine recovers the capacity of glucose repression. 0.02% or more histidine was required for glucose repression of aerial mycelium formation in the absence of dextrin.

Treatments of 5μM or more ethidium bromide (EtBr) gave rise to bald mutants at high frequency in *Streptomyces aureofaciens*, and it is probable that the gene(s) for the function of aerial mycelium formation is linked to plasmid DNA in this species.

INTRODUCTION

The transition from substrate to aerial mycelium in *Streptomyces* provides a simple model system of prokaryotic differentiation (Kalakoutskii and N. Agre, 1976). The involvement of specific factors in the regulation of *Streptomyces* differentiation were reported. Factor-C isolated from *S. griseus* induced spore formation of mutant strain which did not form spores in liquid culture (Szabor, Bekesi & Vitalis, 1966). Scribner *et al.* (1973) reported that the dark purple pigment from *S. venezuelae*

induced sporulation in liquid media in which sporulation did not occur. Khokhlov *et al* (1973) isolated mutants devoid of aerial mycelium and also isolated Factor-A that induced aerial mycelium formation. Recently it was shown that the aerial mycelium formation of *Streptomyces* species was repressed by glucose on Hickey-Tresner agar media (Peggy *et al.*, 1976) and that the functions of aerial mycelium formation and other socendary metabolisms in *S. alboniger* are linked to plasmids(B.M.Pogell, 1979). Although the mechanism of glucose repression of aerial mycelium formation is still unknown, it is quite helpful for gaining the insight of the regulation metabolism of aerial mycelium formation to study the glucose repression in detail.

In our experiments Benett's agar and the synthesized KK agar were not able to induce glucose repression which were devoid of dextrin or all unknown organic nutrients except dextrin, respectively. It is considered that dextrin affects the glucose repression in some unknown ways and that there are some unidentified factors required for glucose repression in HT media which are not contained in the synthetic media. Among the possible constituents of HT medium, 20 amino acids, vitamins and several metabolic intermediates were tested for inducibility of glucose repression. The effects of each compounds on glucose repression were studied and the minimal concentrations that enables the glucose repression were determined. We have also treated 5μM or more concentrations of ethidium bromide to the broth cultures of *S. aureofaciens* and *S. lavendulae* to determine whether the function of aerial mycelium formation are plasmid-linked or chromosome-linked. After identifying the amino acid factor, L-histidine, further experiments were carried out to trace a possible pathway of L-histidine by adding such compounds as 5′,3′-cyclic adenosine monophosphate, adenosine, guanosine, 5-aminoimidazole-4-carboxamide ribonucleotide, urocanic acid, histidinol, and several other intermediates of histidine metabolism to KKG(KK+1% glucose)with 0.01% histidine, and observing the effects of each compounds on glucose repressions. Figure 1 is a model for summarized metabolic pathways of L-histidine.

Materials and Methods

1. Microorganisms

Streptomyces lavendulae (NRRL B 2036) and *Streptomyces aureofaciens*(NRRL R 2209) were used throughout this experiment.

2. Growth media

Hickey-Tresner(HT) agar(Hickey & Tresner, 1952) with a little modification and Benett's agar were used as complex media and KK agar was used as a minimal medium for the analysis of factors that affects on the glucose repression of aerial mycelia formation. Their compositions are shown in Table 1. Each amino acid and metabolic intermediate was supplemented to the minimal media at the concentration of 0.01% (w/v). Media were adjusted to pH 6.8~7.0 with 1N NaOH and

Table 1. Composition of Hickey-Tresner, Benett's and KK media

Compounds	HT agar	Benett's agar	KK agar
Dextrin	1%	—	1%
Glucose	—	1%	—
Beef extract	0.1%	0.1%	—
Yeast extract	0.1%	0.1%	—
Casitone	0.2%	0.2%	—
NH_4Cl	—	—	0.1%
K_2HPO_4	—	—	0.05%
$MgSO_4 \cdot 7H_2O$	—	—	0.05%
NaCl	—	—	0.05%
$CaCO_3$	—	—	0.02%

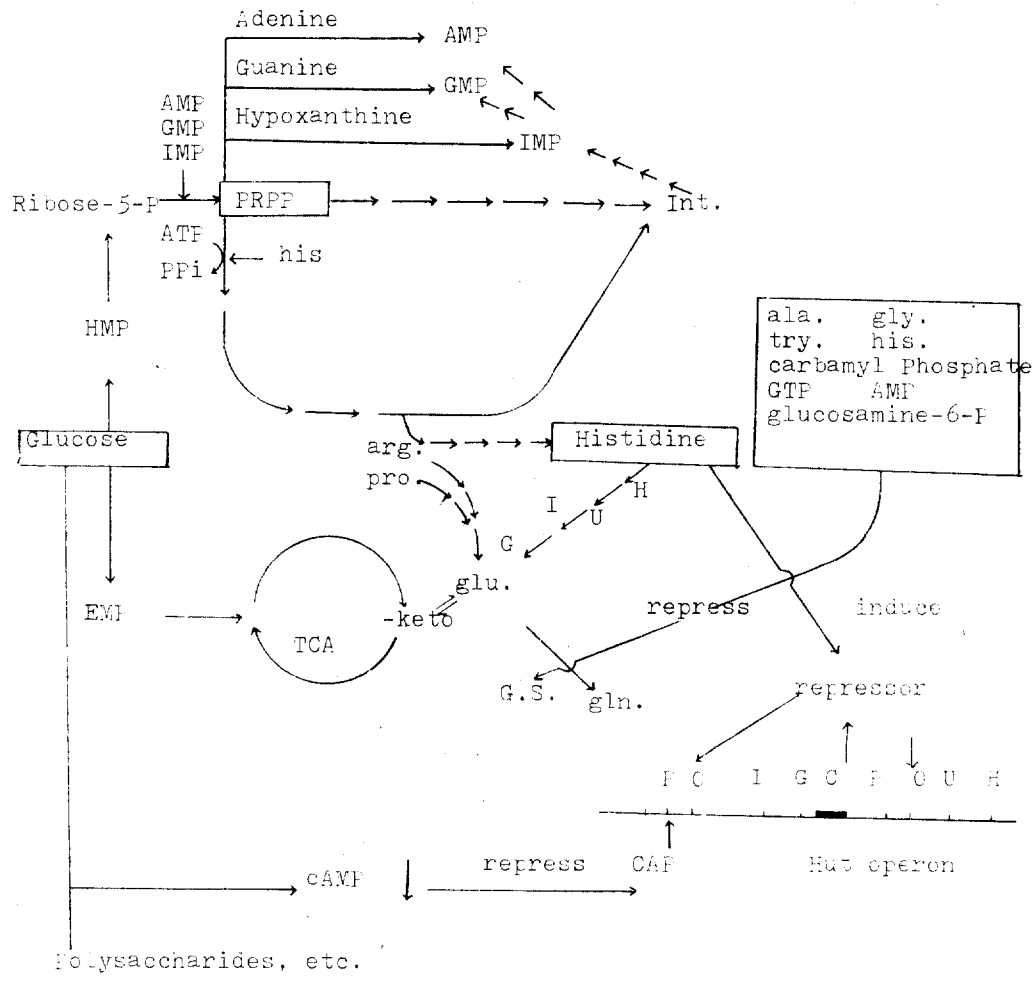

Fig. 1. The metabolic pathways of histidine biosynthesis and degradation

autoclaved at 15 lb, 120°C for 15 minutes. Glucose solution was autoclaved seperately and added to the media at the desired concentration.

3. Preperation of spore

Spore solution was made by the procedure described Peggy, *et al* (1979). Spore suspension in 0.1% Tween 80 was prepared by scraping the cultures growing in HT agar and vortexed for 10 minutes. The suspension was filtered through Toyo No. 1 filter paper. The filterate was centrifuged and the supernatant was decanted and the spore pellet was resuspended in 0.1% Tween 80. This suspension was diluted properly. 30~100 colony forming units were inoculated on the petri-dish and incubated at 30°C.

4. Treatment of spore with ethidium bromide

Spore-rich preperations of *Streptomyces lavendulae* and *Sterptomyces aureofaciens* were harvested by scraping cultures grown in HT agar. Spores were diluted into 100m*l* of HT medium (control) or 100m*l* of medium containing the appropriate concentration of ethidium bromide (5 μM or 50 μM). Cultures were grown at 30°C on a shaker. After grown for 3 days, cells were harvested, diluted and plated on HT agar and the aerial mycelium

Table 2. Glucose repressibilities of each media with or without 1%(w/v) glucose

Media	HT		Benett's		KK	
Glucose	−	+	−	+	−	+
Aerial mycelia	am^+	am^-	am^+	am^-	am^+	am^+

* am^+; aerial mycelia was formed
am^-; aerial mycelia formation was repressed

Table 3. Effects of amino acids on glucose repression. (Each amino acid was added to KK media at the concentration of 0.01%)

Amino acid	pro.	arg.	ala.	asp.	aspn.	gly.	leu.
Repression	$+^w$	$+^w$	$-^a$	−	−	$+^b$	−
Amino acid	lys.	ser.	glu.	gln.	met.	val.	tyr.
Repression	−	$-^b$	$+^w$	ND	−	−	−
Amino acid	trp.	phe.	ileu.	cys.	cys	his.	
Repression	−	−	−	$-^a$	ND	+	

* w; weakly repressed. All of these were reversed after long-term culture.
ND; not dated a; maturation was delayed b; unclear

negative(am^-) colonies were observed.

RESULTS

Glucose-repressibilities of aerial mycelia formation of each medium by the addition of 1% (w/v) glucose is shown in Table 2. Formations of aerial mycelium were repressed on HT agar plate by the addition of 1%(w/v) glucose but not on Benett's agar of KK agar. The compositional difference between HT and Benett's agar showed that dextrin as a carbon source was clearly a factor for the glucose repression. And it was considered that there were some other factors required for the glucose repression in HT media which were not contained in KK media. For the analysis of various organic compounds contained in HT media, as described in Materials and Methods, each 20 different amino acid was added to KK agar containing 1%(w/v) glucose(KKG). The results are described in Table 3. Proline, arginine and glutamate caused partial repressions of aerial mycelium formation with 1% (w/v) glucose in the minimal media but in these cases, every colony was reversed after prolonged culture.

The glucose repression of aerial mycelium formation was clearly observed in the presence of 0.01%(w/v) L-histidine and 1%(w/v) glucose(Fig. 2). To study the *in vivo* role of L-histidine in the glucose repression of aerial mycelium formation, several metabolites which are related to histidine biosynthesis and degradation metabolisms, such as 5-aminoimidazole-4-carboxamide ribonucleotide, L-ketoglutarate, histidinol, urocanic acid, phosphoenol pyruvate and ornithine, were tested in the same way as described above. The results are shown in Table 4. None of these intermediates caused a remarkable effect on the glucose repression of aerial mycelium formation. We also determined the combinations of the minimal concentrations of three factors (L-histidine, dextrin, glucose) in which aerial mycelium formation could be repressed and the results are sum-

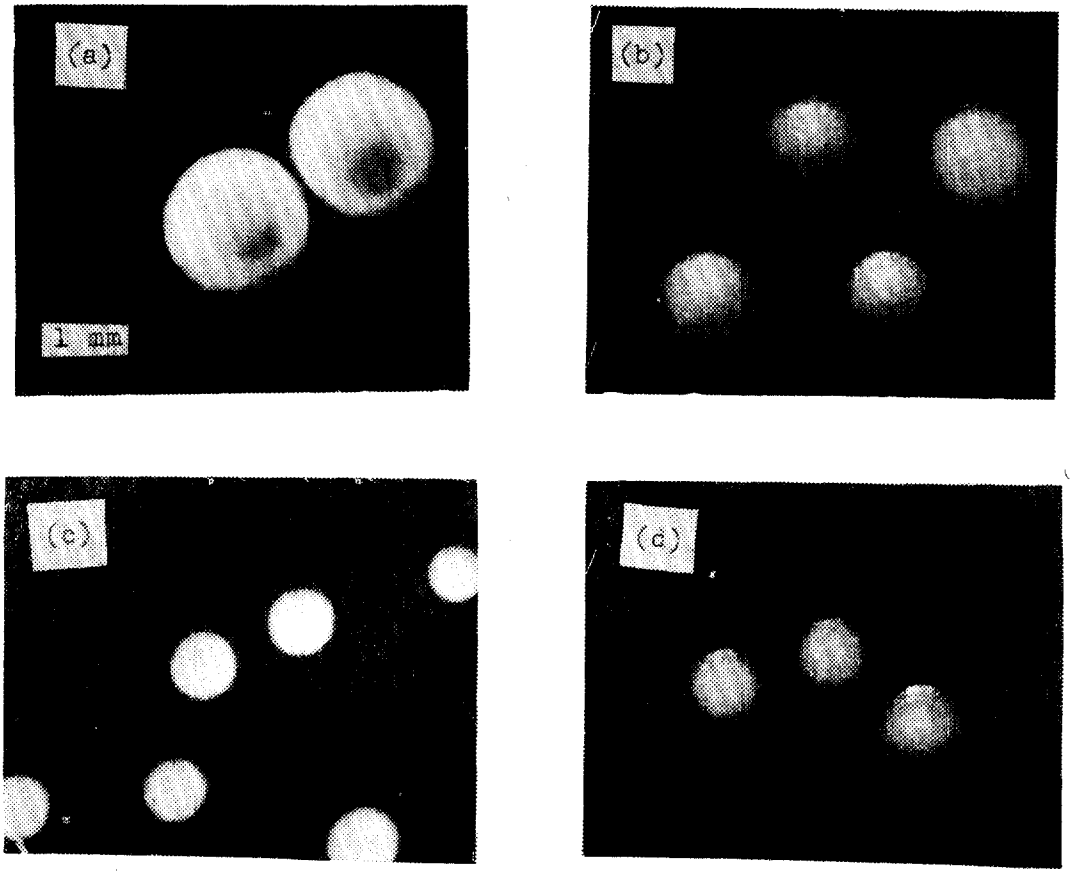

Fig. 2. Morphologies of colonies grown on 4 different agar media for 3 days. (a) Aerial mycelium are fully developed on HT. (b) Colonies are bald on HTG. (c) Aerial mycelium are fully developed on KKG. (d) Colonies are bald on KKG+0.01% (w/v) L-histidine.

Table 4. Effects of the intermediates of histidine metabolism on glucose repression

Compounds	Ornithine	α-ketoglutarate	5-aminoimidazole-4-carboximide ribonucleotide
Repression	+*	+*	–
Compounds	Histidinol	Urocanate	Phosphoenol pyruvate
Repression	–	–[a]	+*

* w; weakly repressed

a; weak repression was observed at the concentration of 0.02%

Each compound was added to KK media at the concentration of 0.01%.

Table 5. Effects of dextrin and glucose in HT agar on the formation of aerial mycelia. The concentration of L-histidine was fixed at 0.01% (w/v).

Dextrin \ Glucose	0%	0.5%	1.0%	2.0%
0%	NR	NR	NR	NR
0.2%	NR	+*	+*	+*
0.5%	NR	+	+	+
1.0%	NR	+	+	+

* +; repressed NR; not repressed

w; weakly repressed and reversed after long-term culture.

Table 6. Minimal concentration of dextrin, glucose, and histidine for the aerial mycelia repression in *S. lavendulae*

Dextrin	Glucose	Histidine
1%	1%	0.003%
0.2%	1%	0.01%
0%	1%	0.02%
1%	0.2%	0.01%

marized in Table 5 and 6.

At least 0.02%(w/v) L-histidine was required for the repression of aerial mycelium formation with 1% (w/v) glucose in the absence of dextrin.

Previous reports suggested that some functions for secondary metabolisms and formation of aerial mycelium in *Streptomyces* species are linked to plasmids(R. Kirby *et al.*, 1975, Peggy *et al.*, 1976, M. Okanishi *et al.*, 1979, Y. Parag *et al.*, 1979, D.A. Hopwood et al., 1978).

In order to check the same possibilities, 5μM or 50μM membrane-filtered ethidium bromide were added to each HT broth cultures of *S. lavendulae* and *S. aureofaciens*, and cultured on a shaker at 30°C for 3 days. In case of *S. lavendulae*, bald mutants (am^-) were observed in the 50μM EB-treated cultures at high frequency (about 20%) but they were reversed after prolonged incubation. On the other hand, *S. aureofaciens* grown both in 5μM and 50μM EB-treated broth cultures showed high frequency emergences (about 30%) of bald mutants and they were never reversed.

DISCUSSION

Our studies have confirmed that L-histidine is a nutritional factor which is essential for the glucose repression of aerial mycelium formation in *Streptomyces*. Partial effects were observed with arginine, proline, glutamate and some other metabolites which are related to histidine metabolism. Since the permeability of each compound into the cell was not tested, and the roles of L-histidine and glucose in the repression of aerial mycelium formation are entirely unknown, it is not obvious that these compounds are non-essential for glucose repression. Peggy *et al* (1976) reported that adenine and guanine at low concentration reversed the effect of glucose on aerial mycelium formation but we could not observe any remarkable effect of adenine and guanine with *S. lavendulae* and *S. aureofaciens*. It could be explained for the difference in species used.

Dextrin seems to be non-essential for glucose repression since the elevation of the concentration of L-histidine could recover the glucose-repressibility(Table 6). The interrelationship between L-histidine and dextrin and the molecular basis for glucose repression of aerial mycelium formation have never been tested nor been discussed in any other paper.

Genetic approaches, however, have suggested that the genes controlling the formation of aerial mycelium in some *Streptomyces* species are located in extrachromosomal DNA(Peggy *et al.*, 1976 M. Okanishi *et al.*, 1979, Y. Parag *et al.*, 1979) Ethidinum bromide may possibly bring about the curing of plasmids in *Streptomyces* species(Kahler, R., and D. Noack, 1974, Okanish, M., *et al.*, 1970).

As described in Results, *S. aureofaciens* showed high frequency bald mutation (am^-) after treatment of ethidium bromide and it suggests that the genes for aerial mycelium in *S. aureofaciens* are extrachromosomally linked.

It is not quite convincing that the functions of aerial mycelium formation and/or glucose repression are linked to plasmids in *S. aureofaciens*.

We can only guess the possibility of plasmid-linkage with our data. A rapid method for

the identification of plasmid DNA in bacteria (T. Eckhardt, 1978) will clearly show whether the bald mutations are extrachromosomal or not.

In conclusion, the discovery of the involvement of L-histidine in glucose repression is significant in two respects. Firstly, aerial mycelium formation of *Streptomyces* can be controlled easily on the defined media (KK agar) and it may facilitate further studies of differentation in *Streptomyces*. Secondly, L-histidine metabolism in itself seems to have a role in glucose repression.

Further studies on the relationship between glucose repression and histidine metabolism must be followed and it will be helpful for the elucidation of the molecular events involved in the mechanisms of aerial mycelium development in *Streptomyces*.

摘 要

*Streptomyces lavendulae*와 *S. aureofacience*의 aerial mycelium 형성이 Hickey-Tresner 한천배지에서 1% glucose에 의하여 억제되었으며, 이러한 glucose 억제에는 L-histidine와 dextrin이 반드시 필요한 것임을 확인하였다. Dextrin을 포함하는 배지에서 0.003% 혹은 보다 높은 농도의 L-histidine는 glucose 억제현상을 상실시켰으나 dextrin이 포함되지 않은 배지에서는 0.02% 혹은 보다높은 L-histidine은 glucose 억제효과를 일으켰다. 5μM 이상의 ethidium bromide로 상기균을 처리하여 aerial mycelium이 형성되지 않는 돌연변이주를 유발하였다. 돌연변이 유발빈도는 약 20%였다. 이는 aerial mycelium 형성에 관계하는 유전자들 중에서 적어도 일부가 *Streptomyces*의 유전물질외의 plasmid에 존재한다는 것을 시사한다.

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