Histidine Production by a Regulatory Mutant of
Streptomyces coelicolor

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Streptomyces coelicolor mutant RF-59, isolated as a revertant of a histidine
auxotroph after mutagenic treatment with N-methyl-N'-nitro-N-nitrosoguanid-
ine, was found to accumulate L-histidine. The mutant was sensitive to 2-thiazo-
lealanine and 1,2,4-diaminobutyric acid and partially sensitive to α-methylhisti-
dine but resistant to 1,2,4-triazolealanine, indicating that repression of the histi-
dine operon was modified in the mutant. Culture conditions were investigated,
and optimal media for L-histidine production were developed, resulting in L-
histidine accumulation of 2.1 to 3.5 g/liter.

It has been shown that histidine analog-resistant mutants of Salmonella typhimurium (21),
Escherichia coli (16), Bacillus subtilis (13), and Corynebacterium glutamicum (2) are able to excrete L-histidine in the medium, and C. glu-
tamicum has been used for the microbial production of this amino acid (2). This phenomenon
is thought to be due to mutations in feedback sensitivity of an enzyme of L-histidine biosyn-
thesis or in regulatory genes controlling repression of L-histidine biosynthetic enzymes. Strept-
omyces are not known as L-histidine producers, although regulation of the L-histidine biosynthetic pathway has been described in Streptomyces coelicolor (11). Clustering of his
genes on the circular chromosome of S. coelicolor A3(2) (9, 12, 17) is an indication that an operon is involved in the regulation of L-histidine biosynthesis in this microorganism. Genetic and biochemical evidence obtained with a constitutive mutant by Carere et al. (7) and Russi et al. (19) supports this hypothesis.

This paper describes the isolation and characterization of a derepressed mutant of S. co-
elicolor A3(2) and conditions for L-histidine production.

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Germany, 1976.)

MATERIALS AND METHODS

Microorganisms. The auxotrophic mutant hisB12
of S. coelicolor A3(2) was kindly provided by D. A.
Hopwood, John Innes Institute, Norwich, England.
All mutants were derived by mutagenic treatment with N-methyl-N'-nitro-N-nitrosoguanidine according to the procedure of Delić et al. (8).

Culture method. Minimal medium was of the fol-
loowing composition (grams per liter): NaNO3, 3.3;
MgSO4, 0.5; KCl, 0.5; KH2PO4, 1.0; FeSO4, 0.01; and
glucose (autoclaved separately as a 50% solution), 41.0.
Sterilization was carried out for 20 min at 110°C. A
spore suspension was prepared from a 7-day agar slant
of complete medium (10) and was used for inoculation
of liquid medium (1 ml containing 10⁶ spores). Growth
inhibition by L-histidine analogs was studied in 100-
ml Erlenmeyer flasks containing 25 ml of minimal medium. The analogs were added to growing cultures at the beginning of the exponential growth phase, 22
h after inoculation.

Growth was measured every 3 to 4 h for the next
16 h. Growth was estimated by centrifugation of 25
ml of the fermentation broth (10 min at 2,500 rpm)
and expressed as packed-cell volume in milliliters per
25 ml. Fermentation was carried out in 500-ml Erlen-
meeyer flasks with 100 ml of medium for 144 h at 28°C
on a New Brunswick gyratory shaker, model-G 25, at
230 rpm.

Analysis. L-Histidine was determined by evapo-
ration of 2 ml of filtered broth and dissolution of the
residue in 0.5 ml of distilled water. Ten to 20 μl of
the solution was applied to Avicel plates followed by
development with methanol-water-10 N HCl-pyridine
(80:17.5:2.5:10, vol/vol). In this solvent system, good
separation of L-histidine from other amino acids in
our media was obtained. L-Histidine was detected with
ninhydrin. The ninhydrin-positive blue spot was
scraped from the plate, treated with a few drops of
Cu(NO3)2 solution to develop a stable pink color, and
eluted with 75% ethanol. The absorbance of the sam-
ple was measured in a Hitachi Perkin-Elmer double-
beam spectrophotometer (model 124) at 504 μm with
an appropriate blank. The concentration was calcu-
lated from a standard curve established with L-histi-
dine prepared in the same way.

Chemicals. 1,2,4-Triazolealanine (TRA) and 2-
thiazolealanine (TA) were obtained from R. G. Jones,
Eli Lilly and Co., Indianapolis, Ind. DL-α-Methylhisti-
idine (MH) was kindly provided by C. W. Mushet,
Merck Sharp and Dohme Research Laboratories,
RESULTS

Isolation and characterization of an L-histidine-excreting mutant, *S. coelicolor* RF-59. Spores of *S. coelicolor* hisB12 were treated with N-methyl-N'-nitro-N-nitrosoguanidine (1 mg/ml, 30 min at pH 9). After mutagenesis, 1 ml of spore suspension was added to empty petri dishes, followed by 15 to 20 ml of molten minimal medium and mixing. Revertant colonies (*his to his*) were scored after 5 days, the frequency being 20 to 30 prototrophic colonies per 10⁶ treated spores. In five experiments, 32,000 revertants were checked, and one showed excretion of L-histidine as revealed by hisB12 growth as a halo around the revertant colony. This colony, designated RF-59, was restreaked on minimal medium agar and checked for L-histidine excretion ability by using the hisB12 strain as a test microorganism. The majority of L-histidine-excreting mutants of bacteria isolated by other investigators have been obtained on media containing histidine analogs and were resistant to them. Since *S. coelicolor* RF-59 was isolated without analogs, an attempt was made to determine whether the RF-59 mutant was resistant to analogs that interfere with the histidine biosynthetic pathway. Four L-histidine analogs were used; two of them are known to inhibit the first enzyme of the pathway (TA and DAB) and the other two (TRA and MH) affect repression of the biosynthetic pathway. *S. coelicolor* A3(2) was used as a control.

TA has been shown to be a strong inhibitor of *E. coli* (15) and *S. typhimurium* (18), but growth of *S. coelicolor* A3(2) has been reported to be less affected (6). The addition of TA to exponentially growing cells of *S. coelicolor* A3(2) caused immediate inhibition (Fig. 1a). However, after a relatively short interval, exponential growth resumed at the same rate as the uninhibited control. With *S. coelicolor* RF-59, TA caused transitory inhibition of growth for 3 to 4 h (Fig. 1b).

Almost the same growth response was observed after addition of DAB (Fig. 2), a weak inhibitor of *E. coli* (16) growth.

The parent strain A3(2) and the RF-59 mutant were inhibited to about the same extent. Two additional analogs, MH and TRA, inhibited *S. coelicolor* A3(2) much more strongly than did TA or DAB at the same concentrations. Growth of *E. coli* was also initially inhibited by MH, but resumed at a slightly reduced rate (20). The growth of *S. coelicolor* A3(2) was strongly inhibited for 8 h after MH addition (Fig. 3a), and then growth resumed at a significantly reduced rate. *S. coelicolor* RF-59 was also inhibited by the same concentration of MH, but growth resumed after a shorter period of time (3 h) and with a higher growth rate (Fig. 3b). TRA was also a very strong inhibitor of *S. typhimurium* (14). Here again, strong inhibition of *S. coelicolor* A3(2) was observed (Fig. 4a), but there was no growth inhibition of the RF-59 mutant (Fig. 4b).

Conditions for L-histidine production. In a search for optimal fermentation conditions, different media were used, and the effect of various substances on L-histidine production was investigated. A combination of corn starch and glucose as carbon source, casein hydrolysate as nitrogen source, and biotin and thiamine as vitamin additives provided the best results (Table 1).

Accumulation of L-histidine in F medium began after 96 h of cultivation (Fig. 5) and reached its maximum level after 144 h. The pH value rose gradually from pH 6.8 at the beginning of biosynthesis to 8.5 at the end. Since an increase in L-histidine productivity has been achieved in
C. glutamicum (1, 3) by inducing new mutations (auxotrophic or resistant to some analogs), S. coelicolor RF-59 was treated with N-methyl-N'-nitro-N-nitrosoguanidine to induce auxotrophic mutants. However, their L-histidine biosynthetic ability was poorer than that of their parent (Table 2).

DISCUSSION

The mechanisms by which S. typhimurium controls its rate of L-histidine biosynthesis are well known (4). Studies on the genetics (9, 11, 12, 17) and biochemistry (7, 19) of the his genes suggest that regulation of L-histidine biosynthesis in S. coelicolor A3(2) is also under an operon control mechanism. The mechanism of regulation in this biosynthetic pathway of S. coelicolor A3(2) is still far from known, however, because of the lack of particular regulatory mutants. Histidine analogs such as TA, DAB, MH, and TRA have been shown to be useful in the study of L-histidine regulation. The inhibition by TA of the first enzyme of L-histidine biosynthesis of E. coli (15) has been described. In the present study, the addition of TA to growing cultures of S. coelicolor A3(2) and RF-59 (Fig. 1a and b) caused an immediate growth inhibition of both strains, presumably by partially inhibiting the first enzyme of the pathway and lowering the endogenous level of histidine. Since TA is not thought to repress the synthesis of histidine biosynthetic enzymes, the pathway becomes derepressed. With the higher amounts of all the enzymes, including the first enzyme, the cells can produce enough histidine for growth even though the first enzyme is still partially

![Image](http://aem.asm.org/)

**FIG. 3.** Effect of MH on the growth of S. coelicolor A3(2) (a) and S. coelicolor RF-59 (b). Symbols: (●) no MH added; (▲) 2 x 10^{-3} M MH added. **FIG. 4.** Effect of TRA on the growth of S. coelicolor A3(2) (a) and S. coelicolor RF-59 (b). Symbols: (●) no TRA added; (▲) 2 x 10^{-3} M TRA added.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Amt (g/1,000 ml of tap water) in medium:</th>
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<tr>
<td></td>
<td>Minimal</td>
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<tr>
<td>Corn starch</td>
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<tr>
<td>Sucrose</td>
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<tr>
<td>Glucose</td>
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<tr>
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<tr>
<td>Corn steep liquor</td>
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<tr>
<td>Casein hydrolysate</td>
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<td>KH&lt;sub&gt;2&lt;/sub&gt;PO&lt;sub&gt;4&lt;/sub&gt;</td>
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<tr>
<td>(NH&lt;sub&gt;4&lt;/sub&gt;)&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
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<tr>
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<td>KCl</td>
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<td>Biotin</td>
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<td>Thiamine HCl</td>
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* L-Histidine produced (mg/ml) 0.0 0.2 0.35 0.75 0.88 3.5

**TABLE 1.** Effect of nutrients on L-histidine overproduction in S. coelicolor RF-59<sup>a</sup>

*a* L-Histidine production was determined after 144 h of fermentation.
inhibited. After that, cells can grow at the same rate as cells without the analog.

DAB action is also at the allosteric site of the first enzyme in E. coli (15). Since the growth of both strains in our study is inhibited (Fig. 2a and b), it indicates that S. coelicolor RF-59 has an unchanged initial enzyme and hence is sensitive to feedback inhibition.

The other two analogs, MH and TRA, cause repression of the histidine biosynthetic enzymes and thus inhibit the growth of E. coli (20) and S. typhimurium (14). MH inhibits histidine transfer ribonucleic acid synthetase (20) and TRA inhibits the growth of S. typhimurium by complementing with histidine transfer ribonucleic acid (22).

The growth of S. coelicolor A3(2) (Fig. 3a) was strongly inhibited by MH. Although S. coelicolor RF-59 was also inhibited by this analog (Fig. 3b), growth resumed after a short period of time, presumably due to overproduction of L-histidine. S. coelicolor A3(2) was very sensitive to TRA inhibition (Fig. 4a), but S. coelicolor RF-59 was resistant (Fig. 4b).

Our data suggest that the TRA resistance of S. coelicolor RF-59 involves a regulatory mutation and, therefore, the culture is not subject to repression by the analog; it thus escapes from growth inhibition. Thus, L-histidine excretion by S. coelicolor RF-59 is apparently caused by derepression of the histidine operon. Since this mutant was isolated as a revertant from his to his it is possible that two mutations took place. The first would be reversion of his to his, and the second, in a gene(s) involved in histidine regulation. An alternate possibility is that the structural gene or gene product affected by the reversion is also involved in repression (autogenous control) (5). Production of L-histidine by a TRA- and TA-resistant mutant of C. glutamicum in simple medium containing glucose and molasses has been described by Araki and Nakayama (2). S. coelicolor RF-59 produces L-histidine in a medium containing three times less carbon than C. glutamicum.

Biotin and thiamine play an important role in the biosynthesis of L-histidine in C. glutamicum. A similar influence of these vitamins on L-histidine production by S. coelicolor RF-59 was observed; the addition of vitamins increased the yield threefold (Table 1).

### ACKNOWLEDGMENTS

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### LITERATURE CITED


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