Exogenous and Endogenous Induction of the Histidin-degrading Enzymes in Aerobacter aerogenes

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Three different imidazole compounds are known to induce the enzymes that degrade histidine in Aerobacter aerogenes (1-4). Two of them, histidine and urocanate, are, respectively, the substrate and product of the first enzyme, histidine ammonia-lyase; the third, imidazolepropionate, is a nonmetabolizable analogue of urocanate.

Previous studies with this system did not discriminate between the possibility that histidine and urocanate act as inducers independently, or that histidine is an inducer solely by virtue of its conversion to urocanate. In order to distinguish between these alternatives a number of mutants of A. aerogenes unable to degrade histidine were isolated. Some of the mutants are impaired in the ability to convert histidine to urocanate because of a low level of the histidine ammonia-lyase: in these mutants histidine is a poor inducer, whereas urocanate and imidazolepropionate behave as in the parent organism. Other mutants lack the enzyme urocanase; these mutants have high levels of the other histidine-degrading enzymes even in the absence of added inducer.

These observations suggested that exogenously supplied or endogenously produced urocanate, rather than histidine, is the inducer. This view is strongly supported by a detailed biochemical study of these mutants.

EXPERIMENTAL PROCEDURE

Chemicals—In addition to those listed in the previous papers (1-3) adenine-2-14C was procured from Schwarz.

Growth and Enzyme Induction—The cells were grown and the enzymes induced as described previously (1, 2). The media, unless stated otherwise, contained 0.2% sodium succinate as the source of carbon and 0.2% ammonium sulfate as the source of nitrogen; the temperature was 37°.

Isolation and Selection of Mutants—The wild-type organism, A. aerogenes, strain 35, has been described previously (1, 2). The following mutagenic agents were employed: ultraviolet light, and the chemicals N-methyl-N-nitrosoguanidine (5), ethylmethane sulfonate (6), and 2-aminopurine (7). Penicillin selection was used for the isolation of mutants unable to degrade histidine (8, 9). The mutagenized cultures were grown overnight in medium containing 0.2% glycerol, 0.2% ammonium sulfate, and 0.2% histidine. This was done to prevent the long lag period that ensues when the culture is transferred directly from glycerol to histidine. Before penicillin treatment the cells were centrifuged, washed, and resuspended in fresh medium containing either 0.2% histidine as the carbon source, or 0.2% histidine as the nitrogen source and glycerol as the carbon source. The latter medium was used in selecting mutants that lack histidine ammonia-lyase. The penicillin selection was repeated three to four times. Between 100 and 300 colonies were then plated on a mineral agar plate containing 0.2% histidine plus 1% L broth (10). Those cells which are unable to utilize histidine as a carbon source form small colonies because of the limiting amount of broth present. The small colonies were isolated and their ability to grow with histidine either as a carbon or nitrogen source was determined. Revertants of these mutants were isolated by spreading between 10^8 and 10^7 mutant cells on an agar plate containing 0.2% histidine as the only carbon source.

Enzyme Assays—The assays for histidine ammonia-lyase, histidine biosynthetic enzymes, imidazolepropionate permease, and formiminoglutamate hydrolase have been described in previous papers (2-4). In order to increase the sensitivity of the assay for formiminohydrolase the concentration of the substrate was changed from 2 mM to 4 mM. For the measurement of the initial kinetics of urocanase induction a more sensitive assay for this enzyme was devised: between 2 and 3 ml of the culture were pipetted into a chilled centrifuge tube containing either sodium azide (final concentration, 0.1 mM). The cells were collected by centrifugation, washed once with 0.85% sodium chloride, and resuspended to the same volume with 50 mM phosphate buffer, pH 7.5. A portion of these cells was added to a cuvette containing either sodium azide (final concentration, 5.0 mM) or CTB (final concentration 0.1 mM). The cells were collected by centrifugation, washed once with 0.85% sodium chloride, and resuspended to the same volume with 50 mM phosphate buffer, pH 7.5. A portion of these cells was added to a cuvette containing a mixture of 150 μmoles of phosphate buffer, pH 7.5, 150 μg of CTB, and 0.15 μmole of urocanate in a final volume of 3.0 ml at 37°. The quantity of cells added to the cuvette should be varied depending on the amount of the enzyme present in the culture. The initial rate of urocanase activity at 37° was measured by the loss in absorbance at 277 μm in a Beckman spectrophotometer equipped with a Gilford recorder. The linearity of the assay as a function of bacterial cell concentration is shown in Fig. 1. A unit is

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1 The abbreviation used is: CTB, cetyltrimethylammonium bromide.
defined as the disappearance of 1 μmole of urocane per min at 37°.

Incorporation of Radioactive Leucine into Protein—This procedure has been described by Hartwell and Magasanik (11).

RESULTS

Mutants Unable to Degrade Histidine—In A. aerogenes histidine is degraded by the series of reactions depicted in Fig. 2. The deamination of histidine to form urocane and ammonia allows histidine to be utilized as a source of nitrogen, whereas all of the reactions must occur for histidine to be utilized as a source of carbon. Different mutants selected for their inability to degrade histidine are listed in Table I. Mutants A12 and A21 cannot utilize histidine either as a carbon or nitrogen source, but are able to grow with urocane as the source of carbon; they lack the enzyme histidine ammonia-lyase. Mutants A10 and M24 can use histidine as a nitrogen source but not as a carbon source; they lack the enzyme urocane.

Another mutant, M2, has very low level of the two coordinately controlled enzymes, histidine lyase and urocane (1), under all conditions of induction; revertants selected for the ability to use histidine as a nitrogen source regain both enzyme activities. This mutant may be of the type described as a polar mutant in other systems (13): in these mutants the synthesis of several coordinately controlled enzymes is affected by a single mutation.

Both mutants A15 and N5 have the three enzymes in the pathway whose activities have been assayed, the presence or absence of imidazolopropionate amidohydrolase has not yet been determined. Mutants N5 and M1 have in common the property that their growth in minimal medium with succinate as the carbon source and ammonium sulfate as the nitrogen source is inhibited by histidine. M1 is further characterized by the lack of formiminoglutamate hydrolase. These mutants have not been studied in detail and the reason for the inhibition exerted by histidine is not known.

Induction of Urocane in Histidine Lyase-Deficient Mutant—In preliminary experiments, mutant A12, whose level of histidine ammonia-lyase is only 1 to 5% that of the parent strain, formed very little urocane in response to histidine, unless it was exposed to the amino acid for several generations. The induction of urocane by histidine or by imidazolepropionate was similar to that observed in the parent organism. These results suggested that histidine might induce the histidine-degrading enzymes only by virtue of its conversion to urocane. In order to obtain further evidence for this hypothesis the initial kinetics of urocane formation was studied.

In the parent organism the kinetics of induction of urocane with 0.1 mM histidine or with 0.1 mM urocane was very similar (Fig. 3). Identical induction curves were observed with either 0.1 mM or 1.0 mM histidine. The length of the lag period before enzyme formation and the rate of enzyme synthesis do not vary over this range of histidine concentration. A similar finding has been reported previously for the induction of histidine ammonia-lyase (2).

An entirely different result was obtained when the kinetics of induction of urocane was examined in the mutant lacking histidine lyase (Fig. 3). In this mutant histidine at a concentra-

![Fig. 1.](http://www.jbc.org/figure1.png)

**Fig. 1.** The activity of urocane as a function of cell concentration. The culture was induced for 2 hours with 1.0 mM urocane. The assay for urocane is described in the text. The dry weight was determined from a standard curve relating dry weight to optical density at 606 nm on a Zeiss spectrophotometer (an absorbance of 0.6 is equivalent to 0.4 mg per ml, dry weight).

![Fig. 2.](http://www.jbc.org/figure2.png)

**Fig. 2.** The pathway of histidine degradation in A. aerogenes. A number of mutants have been isolated which lack one or more of the enzymes in this pathway. The mutants for which the enzyme defect is known are noted at the missing enzyme.
tion of 0.1 mM did not induce urocanase. At a 10-fold higher concentration a delayed induction was observed. On the other hand, the induction obtained with urocanate or with imidazolepropionate (not shown) was exactly like that found in the parent strain. The most likely explanation for this result is that histidine must be converted to urocanate in order to induce urocanase. However, other alternatives were also considered.

1. Histidine might inhibit protein synthesis in this mutant. Although this possibility would seem unlikely it had to be considered since histidine actually prevents the growth of several other mutants of A. aerogenes when they are grown with succinate as carbon source (see Table I). The effect of histidine on growth was determined in both the parent and the histidine lyase negative mutant by following the incorporation of radioactive leucine into protein. As shown in Fig. 3 there was no difference in the rate of protein synthesis when histidine and urocanate were used as inducers.

2. The mutant may be impaired in its ability to take up histidine. If this explanation were true, the amount of histidine entering the cell might be too low to allow induction to occur. Fig. 4 shows the results of an experiment in which the uptake of histidine was measured in uninduced cultures of both the parent and mutant organism. The experiment was performed under the same conditions as was the induction, except that chloramphenicol was added to prevent the incorporation of histidine into protein. The uptake of histidine by the two organisms was identical.

3. The enzyme urocanase might normally be stabilized by its interaction with other proteins. If this explanation were true, the amount of histidine entering the cell might be too low to allow induction to occur. Fig. 4 shows the results of an experiment in which the uptake of histidine was measured in uninduced cultures of both the parent and mutant organism. The experiment was performed under the same conditions as was the induction, except that chloramphenicol was added to prevent the incorporation of histidine into protein. The uptake of histidine by the two organisms was identical.

Table I

Properties of mutants of A. aerogenes unable to utilize histidine as carbon or nitrogen source

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Mutagenic Agent</th>
<th>Growth</th>
<th>Enzymes</th>
<th>Imidazolepropionate permease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HN</td>
<td>HS</td>
<td>HSN</td>
</tr>
<tr>
<td>A12a</td>
<td>N-Methyl-N-nitrosoguanidine</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A21</td>
<td>N-Methyl-N-nitrosoguanidine</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A10</td>
<td>N-Methyl-N-nitrosoguanidine</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M24</td>
<td>Nitrous acid</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M2</td>
<td>Ethylmethane sulfonate</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A15</td>
<td>2-Aminopurine</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M1c</td>
<td>Ethylmethane sulfonate</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>N5c</td>
<td>N-Methyl-N-nitrosoguanidine</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* This mutant was isolated by Mr. Richard Cohen.
* M24 was selected by Dr. T. Berman for its inability to utilize inositol. It was also unable to use histidine as carbon source. Revertants selected for the ability to utilize histidine were still unable to metabolize inositol and vice versa (12).
* Grows in a medium containing succinate as source of carbon and ammonium sulfate as source of nitrogen.
isolated in which an increase in the urocanase activity is paralleled by a decrease in the constitutive level of the histidine lyase.

The constitutivity could be attributed to a mutation which overlaps both the gene for urocanase and a specific regulatory gene, or it might be explained by endogenous induction. According to the latter hypothesis some of the internally synthesized histidine is normally degraded even in uninduced cells; if the complete degradation is prevented by a loss of urocanase, urocanate accumulates and induces the other histidine-degrading enzymes.

Evidence supporting this mechanism is the observation that strain A10 excretes, during growth in a medium containing succinate as the only carbon source, material capable of inducing histidine lyase in the wild strain (Fig. 3). The inducing material could be identified as urocanate by the following experiment, which takes advantage of the fact that carbon 2 of adenine is the source of carbon 2 of histidine (14); consequently, urocanate derived from endogenous histidine by cells growing in the presence of adenine-2-^14C should be radioactive.

A portion of adenine-2-^14C (10 pmoles, 1.04 μCi per μmol) as a source of carbon a surprising observation was made with

\[ \text{Histidine lyase mutant, A12} \]

\[ \text{Histidine} \]

\[ \text{Urocanate} \]

\[ \text{units/mg protein} \]

TABLE II

<table>
<thead>
<tr>
<th>Inducer</th>
<th>Formiminoglutamate hydrolase</th>
<th>units/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type, 35</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Histidine</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Urocanate</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Histidine lyase mutant, A12</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Histidine</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Urocanate</td>
<td>0.05</td>
<td></td>
</tr>
</tbody>
</table>

4. The loss of the ability of histidine to act as an inducer might be the result of a second mutation differing from the one causing the loss of histidine lyase. This appears unlikely, because in another, independently isolated histidine lyase-negative mutant, A21, 0.1 mM histidine also fails to induce urocanase. As a further test, several revertants capable of using histidine as a carbon source were isolated from strain A12. These revertants were found to have normal amounts of histidine lyase and their urocanase was as readily induced by histidine as that of the wild strain (Fig. 3).

The experiments, thus far, have been concerned only with the induction of urocanase. It was also important to determine how the loss of histidine lyase affects the induction of the fourth enzyme in the pathway, formiminoglutamate hydrolase. Since the assay for this enzyme does not have the sensitivity needed for kinetic studies, only the amount of enzyme formed 30 min after the addition of the inducer was determined. As seen in Table II, both histidine and urocanate give good induction in the wild-type organism, but in the mutant that lacks histidine lyase, histidine again is a very poor inducer.

\[ \text{Constitutivity and Loss of Urocanase Activity} \]

In the course of investigating other mutants of \( A. \) aerogenes unable to use histidine as a source of carbon a surprising observation was made with mutants lacking the enzyme urocanase. These mutants, A10 (Table III) and M24, contain high levels of the other histidine-degrading enzymes when grown in the absence of added inducer.

The constitutivity and the loss of urocanase are the result of a single mutation since in revertants selected from these strains for their ability to use histidine as a carbon source, the enzymes are no longer constitutive but are inducible as in the wild type (see Table III, strain A10R). Several partial revertants were also

2 Unpublished observations.
was added to a 100-ml culture of the urocanase-negative mutant containing succinate as carbon source. After 2 hours the cells were removed by centrifugation and 10 μmoles of unlabeled urocanate were added to the medium as a carrier. The medium was passed through a charcoal column (2 × 10 cm). The charcoal was washed with water until the eluate was free of radioactivity; it was then washed with 50% aqueous ethanol containing 1% ammonia until no more radioactive material was eluted. The ethanol and ammonia were removed by evaporation in a vacuum and an aliquot of the material was subjected to paper chromatography in sec-butyl alcohol-formic acid-H2O(8:1:1).

The urocanate which could be identified by the ultraviolet absorption of the added carrier material was eluted from the paper and the amount of its radioactivity was determined. Chromatography of this material in several solvents showed that the radioactive substance was identical with urocanate. It was calculated from this experiment that 0.02 μmole of urocanate was present per ml of medium. A slightly lower value of urocanate would be estimated from the induction experiment presented in Fig. 5.

The presence of urocanate in the medium of A10 is not necessarily the cause of the constitutivity; it might be only the result of the high level of histidine ammonia-lyase. If, however, the constitutivity is actually due to endogenous induction then the high rate of synthesis of the enzymes should be prevented by inhibiting the formation of urocanate. It was possible to eliminate the formation of urocanate by isolating a mutant from A10 which is deficient in histidine lyase as well as urocanase. This mutant (A01) no longer excretes into the medium a compound able to induce the lyase (Fig. 5); more important is the observation that fromiminoglutamate hydrolase and the permease are no longer constitutive, but are inducible (Table III).

The loss of the histidine lyase and of the urocanase are independent mutations since it was possible to isolate from A01 a mutant A012, which is able to use urocanate, but not histidine, as a carbon source (Table III). In this mutant urocanase is readily inducible only by urocanate or imidazolepropionate, just as in strain A12 that had been derived directly from the wild strain (see Table I).

Several attempts were made to inhibit the formation of urocanate by limiting the synthesis of histidine. A histidine-requiring mutant of M24 was isolated and either histidinol or carnosine was used as a source of histidine (15, 16). The growth of histidine-requiring mutants with either of these compounds is slower than growth in the presence of histidine. It might therefore be expected that the intracellular concentration of histidine would be low under such conditions. The presence of either of these compounds, however, still led to the excretion of urocanate and it was not possible to prevent induction in this manner.

Effect of Endogenous Induction on Growth The growth rate of mutants A10 and M24 is not appreciably different from that of the parent strain despite the fact that the mutants continually lose histidine in the form of urocanate to the medium.

It was found that the levels of the histidine biosynthetic enzymes (those responsible for the synthesis of compound III and histidinol dehydrogenase were examined) are not different from those in the parent strain. Therefore, the intracellular level of histidine in the mutants is sufficiently high to repress these enzymes.

The growth of the mutant is more sensitive to inhibition by 2-thiazolealanine, an inhibitor of histidine biosynthesis, than that of the wild type (Fig. 6, a and b). Addition of imidazolepropionate, a nonmetabolizable inducer of the histidine-degrading enzyme, enhances the sensitivity of the wild strain to 2-thiazolealanine (Fig. 6c). These results show clearly that when the rate of histidine biosynthesis is limited, histidine lyase can reduce the intracellular histidine to a level insufficient for rapid growth.

**DISCUSSION**

The results presented in this paper show clearly that in *A. aerogenes* the product, urocanate, and not the substrate, l-histidine, of histidine ammonia-lyase is the inducer of the enzymes responsible for the degradation of histidine to l-glutamate and formamide. This conclusion is based on the finding that mutants...
deficient in histidine lyase respond much more weakly than the parent strain to induction by histidine; both types of organisms are equally well induced by urocanate. The finding that in the parent strain histidine is superior to urocanate as inducer does not contradict this conclusion. It has previously been shown (1) that uracanate enters the cell much less quickly than histidine; consequently in cells endowed with a normal level of histidine lyase exogenous histidine is a better source of intracellular urocanate than exogenous urocanate. The prompt conversion of exogenous histidine to urocanate can be easily demonstrated: 1 min after addition of HCl-histidine to a culture of the wild type growing in a succinate medium radioactive urocanate can be extracted from the cells. This conversion is apparently mediated by the small amount of histidine lyase present in uninduced cells, since a rise in the level of this enzyme is only noted 3 to 4 min after the addition of the inducer. The possibility that histidine, itself, can induce the histidine lyase has not yet been excluded.

The induction of an enzyme by its product rather than its substrate is not an isolated phenomenon. In _Pseudomonas fluorescens_, kynurenine, and not tryptophan, induces the enzymes responsible for the oxidation of L-tryptophan (17). In _Escherichia coli_, L-α-glycerophosphate, and not glycerol, is the inducer of glycerokinase (18). The situation is similar in the case of the β-galactosidase of this organism: the most common substrate, lactose, can serve as inducer only because the enzyme, acting as a transgalactosidase, converts it to another galactoside (19).

Palleroni and Stanier (17) have proposed an hypothesis for the evolution of a control system where a product of tryptophan degradation, rather than tryptophan itself, is the inducer of the tryptophan-degrading enzymes; this hypothesis might also apply to the case of histidine. The amino acids are synthesized endogenously to serve as building blocks for proteins; in the absence of an exogenous supply of these amino acids it would be detrimental for the cell to lose a large portion of the endogenously synthesized compounds by degradation. The danger of induction of the degradative enzymes is reduced if the amino acid itself is not the inducer, and if the aminoacyl ribonucleic acid synthetase has greater affinity for the amino acid than the degradative enzyme. In that case a transient rise in the endogenous level of the amino acid will not allow sufficient product to be produced by the basal degradative enzyme to cause induction and, consequently, a large, potentially dangerous increase in the level of this enzyme.

It is of particular interest that in the case of the urocanase-deficient strain of _Aerobacter aerogenes_ endogenous induction of histidine lyase actually occurs. In this organism the urocanate formed from endogenous histidine is not further degraded, but builds up in the cell and induces the formation of additional histidine lyase. A vicious cycle results in which the increased level of histidine lyase causes an increased conversion of histidine to urocanate, and thus still better enzyme induction. This internal induction is not a necessary consequence of the lack of the enzyme required to remove the inducer; in the case of the tryptophan-oxidizing enzymes of _P. fluorescens_ the loss of kynureninase does not lead to the formation of the other enzymes in the absence of added tryptophan.

Internal induction has been postulated as an explanation for the constitutive production of the enzymes of galactose metabolism by mutants lacking the first of these enzymes, galactokinase (21). However, this explanation which proposes that galactose formed endogenously from glucose accumulates in the cell does not agree with a recent finding (22): mutants that lack in addition to the kinase, uridine diphosphate glucose pyrophosphorylase, an enzyme apparently essential for the synthesis of galactose from glucose, nevertheless produce the galactose-degrading enzymes in the absence of exogenous galactose. This is in contrast to the observations reported here concerning the production of formiminoglutamyl hydrolase by urocanase-less mutants of _A. aerogenes_; the enzyme is constitutive when only urocanase is lacking, but becomes inducible when histidine lyase, required for the production of uracanate, is lacking as well. It is surprising that the sustained attack on the endogenously produced histidine by the constitutive histidine lyase does not interfere seriously with the growth of the urocanase-deficient mutant. The wild strain and the mutant lacking urocanase grow at the same rate in histidine-free media. It can be calculated that during this growth the mutant excretes approximately as much uracanate as it incorporates histidine into protein. Consequently, the mutant produces histidine twice as fast as the wild type. This increased histidine production is accomplished without any increase in the level of the histidine biosynthetic enzymes. Apparently, the levels of these enzymes in cells growing in a histidine-free medium are sufficient to supply histidine at a rate which is at least 2-fold in excess of that required for protein synthesis. The fact that the wild strain does not excrete histidine is explained by the inhibitory effect of histidine on the first enzyme of its biosynthetic pathway (15, 24). It is only when this enzyme is inhibited by the histidine analogue 2-thiazolealane that the growth of the urocanase-deficient mutant can be shown to be more severely affected than that of the wild type. Apparently the inhibited enzyme cannot produce histidine rapidly enough to replace that destroyed by the endogenously induced histidine lyase.

**SUMMARY**

The study of mutants of _Aerobacter aerogenes_ deficient in enzymes required for the degradation of L-histidine has clarified the roles of L-histidine and urocanate as inducers of these enzymes. Mutants deficient in L-histidine ammonia-lyase, the enzyme whose substrate is L-histidine and whose product is urocanate, are readily induced by urocanate, but only poorly by L histidine. Mutants deficient in uroacnase, the enzyme responsible for the further metabolism of urocanate, are constitutive for the remaining L-histidine-degrading enzymes. There-

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4 This one minute after the culture had been induced with 0.1 mM radioactive histidine (38 μCi per μmole) the cells were filtered. Carrier urocanate was added to the cells and they were extracted with 50% aqueous acetone. The entire acetone extract was concentrated by the small amount of histidine lyase present in uninduced cells, since a rise in the level of this enzyme is only noted 3 to 4 min after the addition of the inducer. The possibility that histidine, itself, can induce the histidine lyase has not yet been excluded.

5 The K_m of histidinyl ribonucleic acid synthetase from _A. aerogenes_ has not been determined but the enzyme from _E. coli_ has a K_m of 0.005 mm (unpublished experiments). Values for the K_m of histidine ammonia-lyase from _Bacillus subtilis_ (11), _P. fluorescens_ (20), and _A. aerogenes_ vary between 3.0 mm and 20 mm.
fore, urocanate and not L-histidine is the actual inducer of these enzymes. L-Histidine serves as inducer only by virtue of its conversion to urocanate by L-histidine ammonia-lyase. When the further degradation of urocanate is not possible, sufficient urocanate derived from endogenously produced L-histidine accumulates in the cell to induce the L-histidine-degrading enzymes.

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