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Activation of the *Bacillus subtilis hut* Operon at the Onset of Stationary Growth Phase in Nutrient Sporulation Medium Results Primarily from the Relief of Amino Acid Repression of Histidine Transport

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During growth of *Bacillus subtilis* in nutrient sporulation medium containing histidine (DSM-His medium), the expression of histidase, the first enzyme in the histidine-degradative pathway (*hut*), is derepressed 40- to 200-fold at the onset of stationary phase. To identify the gene products responsible for this regulation, histidase expression was examined in various *hut* regulatory mutants as well as in mutants defective in stationary-phase gene regulation. Histidase expression during growth in DSM-His medium was significantly altered only in a strain containing the *hutC1* mutation. The *hutC1* mutation allows the *hut* operon to be expressed in the absence of its inducer, histidine. During logarithmic growth in DSM-His medium, histidase levels were 25-fold higher in the HutC mutant than in wild-type cells. Moreover, histidase expression in the HutC mutant increased only four- to eightfold after the end of exponential growth in DSM-His medium. This suggests that histidine transport is reduced in wild-type cells during exponential growth in DSM-His medium and that this reduction is largely responsible for the repression of *hut* expression in cells growing logarithmically in this medium. Indeed, the rate of histidine uptake in DSM-His medium was fourfold lower in exponentially growing cells than in stationary-phase cells. The observation that the degradation of histidine is inhibited when *B. subtilis* is growing rapidly in medium containing a mixture of amino acids suggests that a hierarchy of amino acid utilization may be present in this bacterium.

When *Bacillus subtilis* cultures enter stationary growth phase in nutrient sporulation medium, the expression of gene products which allow the bacteria to adapt to their altered growth conditions is derepressed. Cells become motile, secrete a number of degradative enzymes, synthesize antibiotics, and, ultimately, can initiate the process of sporulation (28, 30, 32). All of these processes help the cells to survive under environmental conditions which are not optimal for growth.

We have been studying the regulation of the enzymes responsible for histidine utilization (*hut*) in *B. subtilis*. The genes encoding the four enzymes that catalyze histidine degradation are organized as a multicistronic operon in *B. subtilis* (5, 17, 26). The first open reading frame in the *hut* operon, *hutP*, encodes a regulatory protein, which activates *hut* expression in *trans* (10, 26). Downstream of the *hutP* gene is the *hutH* gene, which encodes histidase, the first enzyme in histidine degradation (26). A nucleotide sequence which could form a stem-loop structure lies between the *hutP* and *hutH* genes. Antitermination of *hut* transcription at this putative stem-loop structure has been proposed to mediate histidine-dependent induction of *hut* expression (26).

Expression of the *hut* operon is highly regulated in response to nutrient availability in *B. subtilis*. *hut* expression is induced by histidine and repressed by rapidly metabolizable carbon compounds such as glucose, glycerol, or malate (5). Growth in the presence of a mixture of amino acids strongly inhibits synthesis of the *hut* enzymes (2). Mutations which alter regulation of the *hut* operon have been isolated. The *hutC1* mutation allows expression of the *hut* enzymes in the absence of the inducer, histidine (5). *hut* expression is insensitive to regulation by catabolite repression in strains containing the *hutR4* mutation (2, 11). Both the *hutC1* and *hutR4* mutations are tightly linked to the *hutH* locus by transformation (5, 11).

In this article, we report that *hut* expression is repressed during exponential growth in nutrient sporulation medium, but its expression completely derepresses at the onset of stationary growth phase. The postexponential activation of *hut* expression in this medium was investigated to identify factors regulating gene expression during this period of environmental stress. Derepression of *hut* expression during stationary growth in nutrient sporulation medium appears to be mediated primarily by the relief of amino acid repression of histidine transport.

MATERIALS AND METHODS

Bacterial strains. *B. subtilis* 168 (*trpC2*) and SMY were from this laboratory. Strain QB5505 (*trpC2 sigL::aphA3*) and an isogenic 168 (*trpC2*) strain were obtained from M. Débarbouillé (7). Strains IS56 (*trpC2 lys-1 relA*) (29), IS58 (*trpC2 lys-1*), IS75 (*leuA8 metB5 hisA1*), IS75Δ*pai* (*leuA8 metB5 hisA1 Δpai::cat*), and IS432 (*leuA8 metB5 hisA1 Δsin::cat*) were provided by I. Smith. Strains IS708 (*leuA8 metB5 hisA1 Δhpr::cat*), JH648 (*trpC2 pheA1 spo0B136*), JH647 (*trpC2 pheA1 spo0E11*), and JH649 (*trpC2 pheA1 spo0F221*) were obtained from A. Grossman. Strains JH642 (*trpC2 pheA1*) and JH646 (*trpC2 pheA1 spo0A12*) were provided by M. Perego. Strain BH1 (*trpC2 Δspo0H::cat*), a strain 168 derivative, was obtained from J. Healy. *comA* and *comP* mutant strains MB225 [*ΔamyE::Φ(gsiA-lacZ)42 ermC*

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comPΔk1] and MB223 [*ΔamyE*::Φ(*gsiA-lacZ*)*42 ermC comA124*::pTV55Δ2*cat*] were provided by J. Mueller.

The *hutC1* mutation was moved into the strain 168 *trpC2* genetic background by transforming strain SH32 (*hutC1*) (5) to erythromycin resistance with SF1685 (*trpC2 hutU*::Tn*917-lacZ*) DNA (2) and screening for a transformant containing both the *hutC1* mutation and the *hutU*::Tn*917-lacZ* insertion. The *hutC1-hutU*::Tn*917-lacZ* fusion was then transferred into the strain 168 *trpC2* genetic background by transformation. SF168C, a strain 168 derivative containing the *hutC1* mutation, was obtained by transforming the 168 *hutC1-hutU*::Tn*917-lacZ* strain to Hut$^+$ with SH32 DNA. A similar procedure was used to transfer the *hutR4* mutation from SF34 (11) into strain 168, creating SF168R. A strain 168 *hutH1* derivative, SF168H, was constructed by transforming strain 168 cells to Trp$^+$ with SH3 (hutH1) (5) DNA and screening for histidine utilization on citrate-plus-histidine BSS plates (5).

Cell growth and media. Methods used for bacterial cultivation have been described previously (2). Difco sporulation medium (DSM medium), a nutrient sporulation medium (31), and the MOPS (morpholinepropanesulfonic acid) minimal medium of Neidhardt et al. (23) have been described previously. The carbon and nitrogen sources used in the MOPS minimal medium were the same as those described for BSS minimal medium (5). The 16-amino-acid mixture was described previously (2).

L-Histidine was freshly prepared for each experiment, filter sterilized, and added at 0.01% to DSM cultures (DSM-His medium) and at 0.1% to MOPS minimal medium to induce the histidine-degradative enzymes. The concentration of L-histidine in DSM medium is insufficient for full induction of the *hut* operon. When strain 168 cells were grown in DSM medium, histidase levels at 1 h after the end of exponential growth (T_1) were 2.5-fold lower than those seen in DSM-His cultures.

Cells used for histidine transport studies were grown in DSM medium supplemented with 0.01% L-histidine, 50 mM *N*-tris (hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES; pH 6.8), 10 mM potassium phosphate (pH 6.8), 50 mM NaCl, and 1 mM $MgCl_2$.

Enzyme assays. Histidase was assayed in cell extracts as described previously (2). One unit of histidase activity produced 1 nmol of urocanic acid per min. Proline oxidase was measured as described previously (12).

RNA isolation and primer extensions. RNA was isolated from *B. subtilis* cells by extraction with guanidine thiocyanate and CsCl centrifugation (2). Primer extensions were performed as described previously (13). Autoradiographs were scanned with a Molecular Dynamics personal densitometer by using ImageQuant software.

Histidine transport. To prevent degradation of radiolabeled L-histidine after uptake, the rate of L-histidine transport was measured in strain SF168H (*hutH1*), which synthesizes an enzymatically inactive histidase protein. At different times during growth, samples (1 to 2 ml) of a culture of SF168H cells were removed, harvested by filtration on a 0.45-μm-pore-size nitrocellulose filter (Millipore), and washed twice with 5 ml of wash buffer (50 mM TES and 30 mM potassium phosphate [pH 6.8] containing 50 mM NaCl, 1 mM $MgCl_2$, and 100 μg of chloramphenicol per ml). Cells were resuspended in 6 ml of wash buffer by shaking at 25°C for 5 min. Uptake was initiated by the addition of 200 μM L-histidine and 2 μCi of L-[^{14}C]histidine (New England Nuclear). Samples (0.5 ml) were removed every 30 s and filtered through 0.45-μm-pore-size filters which had been presoaked in the wash buffer. The filtered cells were washed twice with 10 ml of wash buffer and counted in Ecolite (+) (ICN). The initial rate of uptake was determined from the linear portion of the uptake curve.

Twenty millimolar sodium DL-α-glycerophosphate (Sigma) is typically added as an energy source for transport studies in stationary-phase cells pregrown in nutrient sporulation medium (6, 18). However, in our hands, the rate of L-[^{14}C]histidine uptake reached a plateau within 20 s when these cells were resuspended in wash buffer containing 20 mM sodium DL-α-glycerophosphate. In the absence of 20 mM sodium DL-α-glycerophosphate, kinetics of L-[^{14}C]histidine uptake were linear for 1 to 1.5 min. To determine whether sodium DL-α-glycerophosphate is generally required for amino acid transport in stationary-phase cells, the effect of this compound on L-[^{14}C]glycine uptake was examined in strain SF168H cells that had been harvested at the end of exponential growth and resuspended in uptake mixtures containing 100 μM glycine and 1 μCi of [^{14}C]glycine, neutralized with KOH (New England Nuclear). Since no stimulation of L-[^{14}C]glycine uptake by sodium DL-α-glycerophosphate was observed in these experiments, sodium DL-α-glycerophosphate was omitted from the L-[^{14}C]histidine uptake mixtures.

RESULTS

***hut* expression during stationary growth phase.** When cells of strain 168 were grown in DSM-His medium, the levels of histidase, the first enzyme in the histidine-degradative pathway, increased 40- to 200-fold at the onset of stationary growth compared with the levels seen in the same culture during early exponential growth (80 Klett units; Fig. 1A and data not shown). High levels of histidase activity were present in extracts of cells harvested at T_4 (Fig. 1A). In contrast, when strain 168 was grown in MOPS minimal medium containing NH_4Cl and a mixture of 16 amino acids as the nitrogen source, and either glucose or citrate as the carbon source, no increase in histidase expression was observed even at T_3 (data not shown). The level of proline oxidase, the enzyme catalyzing the first step in proline degradation, increased fivefold at the end of exponential growth in DSM medium containing proline and then declined to the level seen in exponentially growing cells by T_3 (data not shown).

The relative derepression of histidase activity during stationary growth in cells grown in DSM-His medium depended on the growth state of the overnight culture used for inoculation. When cultures were inoculated with stationary-phase cells (in which histidase expression had already been derepressed), the level of histidase present during exponential growth was 5- to 15-fold higher than that seen in cultures which had been inoculated with exponential-phase cells. Histidase specific activity reached the same absolute level during stationary growth phase in cultures inoculated with either logarithmic- or stationary-phase cells (data not shown). Thus, to ensure that cultures were inoculated with exponentially growing cells, overnight cultures were routinely diluted to a culture turbidity of 5 Klett units and grown for two to four generations before they were used for inoculation.

***hut* activation in regulatory mutants.** To determine whether any of the gene products known to alter gene expression during stationary growth phase also regulate *hut* expression, histidase expression was examined in strains containing mutations in the *comA* (9), *comP* (9), *degUS* (30), *sin* (28, 32), *pai* (16), *hpr* (32), *sigH* (28), *spo0A* (28), *spo0B* (28),

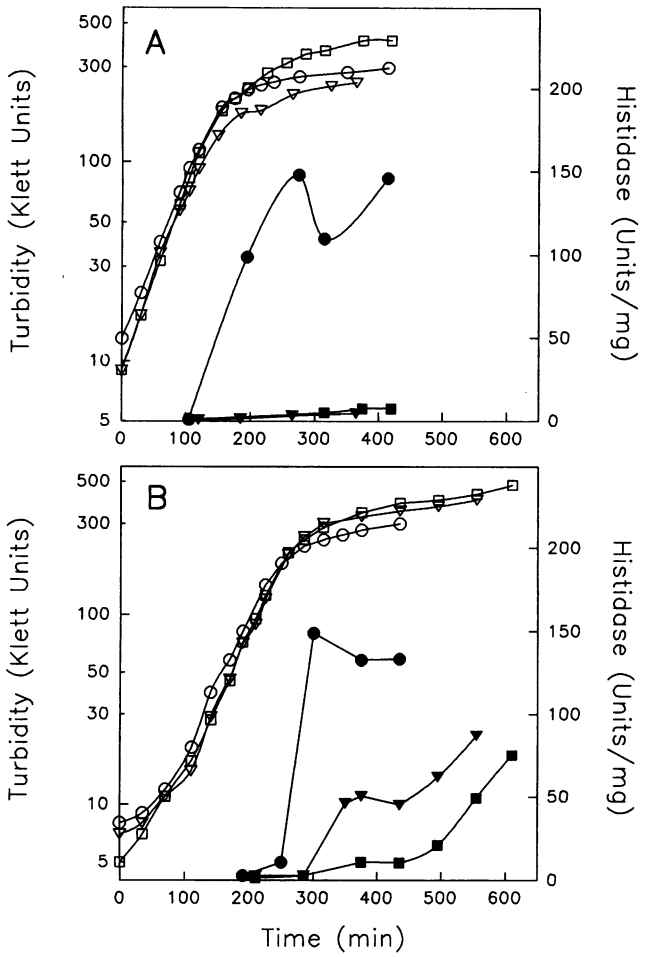

FIG. 1. Growth and histidase levels in strain 168 cells during growth in several versions of DSM-His medium. Samples were removed periodically, and histidase activity was assayed in cell extracts. Data from a typical experiment are shown. (A) Symbols: circles, DSM-His medium; squares, DSM-His-glucose medium; triangles, a version of DSM-His medium which lacked $MnCl_2$. Glucose was added to a final concentration of 0.5% in the DSM-His-glucose medium. (B) Symbols: circles, DSM-His medium; triangles, DSM-His medium containing 0.4% vitamin assay Casamino Acids; squares, DSM-His medium containing 1% vitamin assay Casamino Acids. Open symbols, Klett units; closed symbols, histidase specific activity.

spo0E (28), and *spo0F* (28) genes. Similar patterns of histidase expression were seen in these mutants and their isogenic wild-type strains during growth in DSM-His medium, although histidase was expressed at higher levels in the early-blocked *spo0* mutants than in strain JH642 (Spo^+) during stationary growth (data not shown). Elevated *hut* expression in *spo0* mutants was reported previously by Boylan et al. (4).

***hut* transcription during stationary growth phase.** The start point of *hut* transcription during vegetative and stationary growth in DSM-His medium was determined by primer extension. Since no new major *hut* start sites were seen with RNA isolated from stationary-phase cultures, *hut* transcription occurred from the same promoter during both growth phases (Fig. 2). This promoter is identical to the promoter identified by Oda et al. (25, 26). Densitometric scanning of

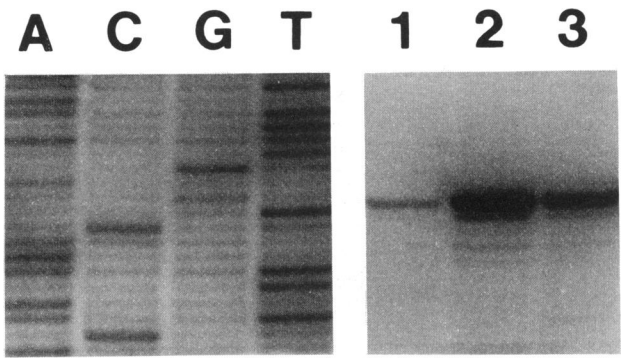

FIG. 2. Identification of the start point of *hut* transcription during growth. Primer extensions were performed as described previously (13) by using a 20-base oligonucleotide complementary to nucleotides +101 to +120 of the *hut* mRNA. The sequencing ladders (34), derived from the same primer, are shown in lanes A, C, G, and T. RNA was extracted from aliquots of the same culture harvested at various times during growth in DSM-His medium. Lanes (RNA sources): 1, $T_{-0.85}$ cells; 2, $T_{0.66}$ cells; 3, $T_{1.66}$ cells. T_0 corresponds to the end of exponential growth. Histidase specific activities (units per milligram of protein) measured in extracts of these culture aliquots were 4.3 (lane 1), 57.9 (lane 2), and 153 (lane 3).

the autoradiogram showed that the relative levels of *hutP* RNA were 1 in $T_{-0.85}$ cells (Fig. 2, lane 1), 38.6 in $T_{0.66}$ cells (lane 2), and 25 in $T_{1.66}$ cells (lane 3). This indicates that the derepression of histidase expression during stationary growth phase can be accounted for by changes in *hut* mRNA levels.

Regulation of *hut* expression by catabolite repression during stationary growth phase. *hut* expression in vegetative cells grown in minimal medium is regulated by catabolite repression (5). Since histidase levels in extracts of strain 168 grown in DSM-His medium containing 0.5% glucose increased only fivefold after the end of exponential growth (Fig. 1A and data not shown), *hut* expression during stationary growth is subject to regulation by catabolite repression.

The *hutR4* mutation causes *hut* expression to be insensitive to catabolite repression in cells growing exponentially in minimal medium (2, 11). When strain SF168R (*hutR4*) was grown in DSM-His medium (lacking added glucose), histidase expression became derepressed 25- to 85-fold during stationary growth. This extent of derepression is within the normal range but occurred about 15 to 20 min earlier in the strain SF168R cultures than in the strain 168 cultures (Fig. 3 and data not shown). The elevated levels of histidase seen in the SF168R (*hutR4*) culture during stationary growth were not unexpected because histidase levels are about twofold higher in HutR mutant cells than in wild-type cells during vegetative growth in citrate minimal medium (5, 10). When SF168R (*hutR4*) cells were grown in DSM-His medium containing glucose, histidase levels increased 45-fold during stationary growth phase (data not shown). This indicates that the *hutR4* mutation relieves catabolite repression during both vegetative and stationary growth phases.

Mn^{2+} dependence of *hut* activation. *B. subtilis* sporulates at high frequency in nutrient sporulation medium only in the presence of Mn^{2+}. This is due to the strict Mn^{2+} requirement of the *B. subtilis* glycolytic enzyme, phosphoglycerate phosphomutase (27, 33). DSM medium contains some glycerol and possibly other carbon compounds such as ribose and inositol (14). These carbohydrates are incompletely metabolized in the absence of Mn^{2+}, and the initiation of sporula-

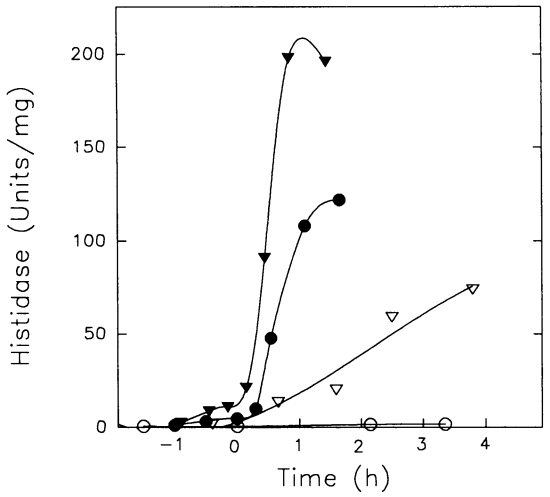

FIG. 3. Histidase levels in wild-type and *hutR* mutant strains during growth. Samples were removed periodically, and histidase activity was determined in extracts of wild-type cultures (circles) or HutR mutant cultures (triangles) grown in either DSM-His medium (closed symbols) or in a version of DSM-His medium which lacked $MnCl_2$ (open symbols). Data from a typical experiment are shown. T_0 corresponds to the end of exponential growth.

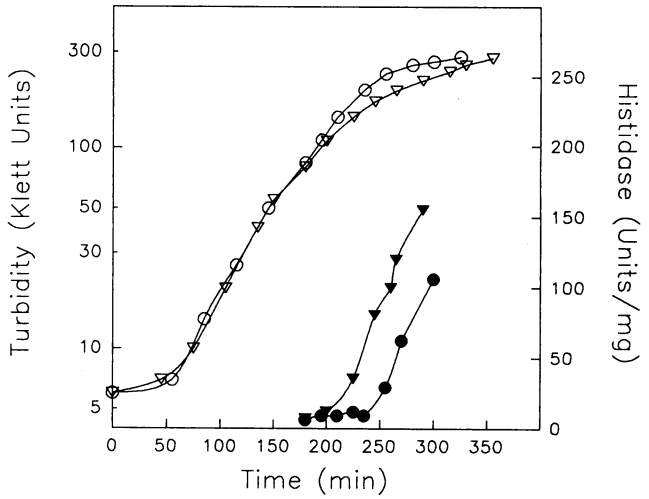

FIG. 4. Growth and histidase levels in strain 168 and QB5505 (*sigL::aphA3*) cells during growth in DSM-His medium. The strain 168 used in this experiment was obtained from M. Débarbouillé. Culture aliquots were removed periodically for histidase assays in extracts of strain 168 cells (circles) and strain QB5505 (*sigL::aphA3*) cells (triangles). Data from a typical experiment are shown. Open symbols, Klett units; closed symbols, histidase specific activity.

tion is thought to be inhibited by carbon catabolite repression (27, 33). When cultures of strain 168 were grown in a version of DSM-His medium which lacked Mn^{2+}, the culture entered stationary growth phase at a lower cell density than did DSM-His cultures and histidase levels increased only two- to fivefold during stationary growth (Fig. 1A and data not shown).

To determine whether the reduced level of histidase expression in DSM-His medium lacking Mn^{2+} is due to regulation by catabolite repression, histidase expression was examined in the HutR mutant. Histidase levels in SF168R (*hutR4*) cells grown in DSM-His medium lacking Mn^{2+} increased about 40-fold during stationary growth phase compared with the levels seen in vegetatively growing cells but did not reach the postexponential levels seen during growth in DSM-His medium (Fig. 3 and data not shown). Since histidase levels were higher in *hutR4* mutant cells than in wild-type cells during stationary growth in DSM-His medium lacking Mn^{2+}, catabolite repression must, in part, be responsible for the reduced levels of histidase seen when wild-type cells are grown in this medium. However, the incomplete derepression of histidase expression in the HutR mutant during stationary growth indicates that Mn^{2+} must play some additional role as well.

Amino acid utilization in DSM medium. One or more amino acids is thought to be depleted from nutrient sporulation medium before the onset of stationary growth phase because the intracellular pools of ppGpp and pppGpp increase transiently towards the end of exponential growth in stringent *B. subtilis* strains and, to a lesser extent, in relaxed mutants (21). If exponential growth in DSM medium is limited by amino acid availability, then the relief of amino acid repression may be responsible for the postexponential activation of *hut* expression. The *relA* gene product is not required for this activation because no defect in histidase expression during growth in DSM-His medium was seen in the RelA mutant strain IS56 (data not shown).

To determine whether exponential growth is restricted by amino acid availability in DSM medium, growth and histidase expression were examined in cultures of strain 168 grown in DSM-His medium supplemented with vitamin assay Casamino Acids (Difco Laboratories, Detroit, Mich.). When the DSM-His medium contained 0.1% Casamino Acids, the turbidity of the culture at the end of exponential growth was 25% higher than that of a culture grown in DSM-His medium, and histidase was completely derepressed by T_1 (data not shown). When DSM-His medium contained 0.4 or 1% Casamino Acids, the turbidity of the culture at the onset of stationary growth phase was about 50% higher than that seen in DSM-His medium, and histidase derepression during stationary phase growth was greatly reduced (Fig. 1B).

If the onset of stationary growth phase in DSM-His medium is due to utilization of amino acids from the growth medium, then the end of exponential growth should occur earlier in a mutant defective in amino acid utilization. *B. subtilis sigL* mutants are unable to grow on media containing arginine, ornithine, isoleucine, or valine as the sole nitrogen source (7). Both the end of exponential growth and the derepression of histidase synthesis occurred at a lower cell density in SigL mutant cultures than in strain 168 cultures (Fig. 4).

Amino acid repression of *hut* expression in *hutC* mutants. We previously proposed that amino acid repression of *hut* expression is unlikely to result from the inability to transport histidine because histidase expression was repressed to similar levels when strains SH and SH32 (*hutC1*) were grown in glucose minimal medium containing amino acids (2). The *hutC1* mutation causes the *hut* operon to be expressed in the absence of its inducer, histidine (5). However, when the *hutC1* mutation was transferred into the strain 168 genetic background, low but significant levels of histidase were present in extracts of SF168C (*hutC1*) cells grown in glucose minimal medium containing amino acids (Table 1). The addition of amino acids to citrate minimal medium repressed the synthesis of histidase 400-fold in strain 168 cells but only

TABLE 1. Histidase activity in wild-type strain 168 and *hutC1* mutant strain SF168C

Medium[a]	Histidase sp act[b]	
	Strain 168 (wild type)	Strain SF168C (*hutC1*)
Glucose + Glt + N	<0.1	8.5
Glucose + Glt + N + His	6.7	33.5
Glucose + Glt + N + AA + His	<0.5	2.7
Citrate + Gln	1.7	99.1
Citrate + Gln + His	161.4	312.4
Citrate + Gln + AA + His	0.4	22.9

[a] Cells were grown in the MOPS minimal medium containing the indicated nitrogen and carbon sources. Abbreviations: His, L-histidine; N, NH_4^+; Glt, L-glutamate; Gln, L-glutamine; AA, a 16-amino-acid mixture.

[b] Enzyme activity is expressed as units per milligram of protein. Each value is the average of two to nine determinations; values did not vary by more than 20%.

13-fold in SF168C (*hutC1*) cells (Table 1). Since the *hutC1* mutation relieves amino acid repression, at least in part, the mixture of amino acids probably interferes with the transport of L-histidine and thus reduces *hut* induction.

To determine whether a reduction in histidine transport is responsible for the low level of *hut* expression during exponential growth in DSM-His medium, histidase expression was examined in the SF168C (*hutC1*) strain. In DSM-His medium, histidase levels in logarithmically growing SF168C (*hutC1*) cells were 25-fold higher than those in strain 168 cells, and histidase expression became derepressed only 4- to 8-fold during stationary phase in strain SF168C (*hutC1*) cultures (Fig. 5 and data not shown). The elevated level of histidase expression seen in exponentially growing HutC mutant cultures indicated that histidine transport is likely to be the limiting factor for histidase expression in exponentially growing wild-type cells in DSM-His medium. As predicted, the rate of L-histidine uptake in SF168H (*hutH1*) cultures grown in DSM-His medium was fourfold lower in logarithmically growing T_{-1} cells (0.9 nmol/min/mg of protein) than in $T_{0.6}$ cells (3.7 nmol/min/mg of protein). This indicates that the synthesis of the histidine permease is inhibited in *B. subtilis* cells grown in the presence of amino acids.

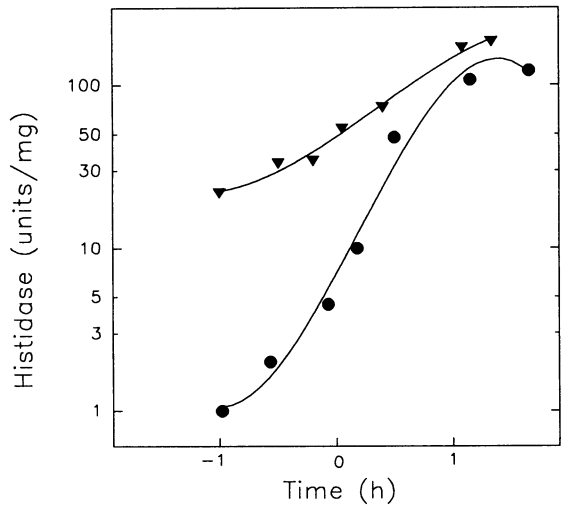

FIG. 5. Histidase levels in strain 168 and SF168C cells during growth in DSM-His medium. Samples were removed periodically, and histidase activity was determined in extracts of strain 168 (circles) and strain SF168C (*hutC1*) (triangles). Data from a typical experiment are shown. Histidase specific activity is shown on a logarithmic scale to emphasize the differences in histidase levels between the 168 and SF168C strains. T_0 corresponds to the end of exponential growth.

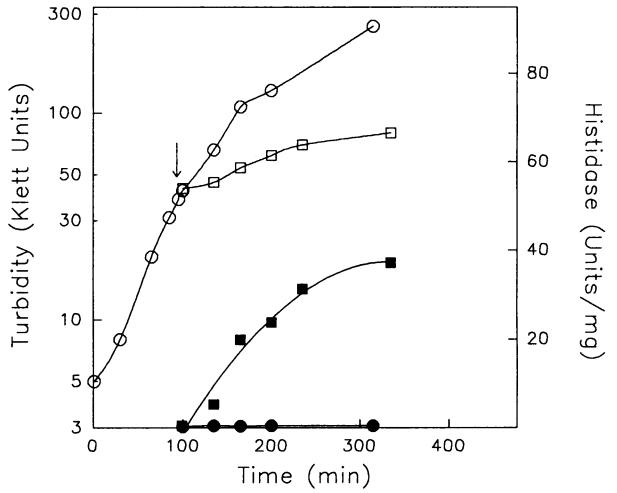

FIG. 6. Effect of decoyinine on growth and histidase expression in SF168R cells. Data from a typical experiment are shown. A SF168R (*hutR4*) culture growing exponentially in MOPS minimal medium containing glucose, L-glutamate, NH_4^+, L-histidine, and a 16-amino-acid mixture was diluted into two flasks containing the same growth medium at a cell density of 5 Klett units. When the turbidity of these two cultures reached 40 Klett units, decoyinine (50 mg/ml in 1 N KOH) was added to a final concentration of 1 mg/ml to one culture. As a control, the other culture received KOH at a final concentration of 0.02 N. Samples were removed periodically for histidase assays. Symbols: ●, histidase levels in cells grown in the absence of decoyinine; ■, histidase levels in cells grown in the presence of decoyinine; ○, turbidity of the culture not receiving decoyinine; □, turbidity of the culture to which decoyinine was added. The arrow indicates the time of decoyinine addition. Only the turbidity of the culture to which decoyinine was added is shown prior to decoyinine addition because the growth curves for the two cultures were identical and could be superimposed.

Decoyinine induction of *hut* expression. Sporulation is initiated in *B. subtilis* after the exhaustion of rapidly metabolizable nutrients from the growth medium (15, 28). During growth in medium containing excess carbon, nitrogen, and phosphate, sporulation can be induced by a partial depletion of intracellular guanosine nucleotide pools (15, 30). Under these conditions, the expression of many genes whose expression is activated at the onset of stationary growth phase in nutrient sporulation medium is also derepressed (15, 30). To determine whether histidase expression can be activated by depletion of guanosine nucleotide pools, decoyinine, an inhibitor of GMP synthetase (15), was added to exponentially growing cultures of strains 168 and SF168R (*hutR4*). Decoyinine treatment caused no alteration in histidase expression in wild-type cultures grown in glucose minimal medium containing either glutamate-NH_4Cl-histidine or histidine and a mixture of 16 amino acids as nitrogen sources (data not shown). However, histidase levels increased 40-fold after the addition of decoyinine to a HutR mutant culture growing exponentially in glucose minimal medium containing histidine and a mixture of 16 amino acids (Fig. 6 and data not shown). Histidase expression was not

examined in decoyinine-treated HutR mutant cultures grown in glucose minimal medium containing glutamate-NH_4Cl-histidine as a nitrogen source because the *hutR4* mutation causes histidase expression to be completely derepressed under these growth conditions.

The elevation of histidase synthesis in the HutR mutant culture, but not in the strain 168 culture, suggested that partial inhibition of guanosine nucleotide synthesis is able to overcome amino acid repression, but not carbon catabolite repression, of *hut* expression. If so, decoyinine treatment should induce histidase expression in strain 168 growing exponentially in citrate minimal medium containing amino acids because histidase expression is not subject to catabolite repression during growth in minimal medium containing citrate as a sole carbon source (Table 1). However, when strains 168R and 168 were grown in citrate minimal medium containing histidine and amino acids, the decoyinine treatment induced histidase synthesis in the HutR mutant culture but not in the wild-type culture (data not shown).

DISCUSSION

The end of exponential growth in nutrient sporulation medium was previously proposed to result from amino acid limitation because the stringent response is transiently induced before the onset of stationary growth (21). However, depletion of one or more amino acids could induce the stringent response without limiting growth. In this article, we show that the growth yield in nutrient sporulation medium is indeed influenced by the availability and utilization of amino acids. Supplementation with Casamino Acids increased the turbidity of the culture at the end of exponential growth. Moreover, a SigL mutant, which is unable to grow in media containing the amino acids arginine, ornithine, isoleucine, or valine as the sole nitrogen source (7), grew to a lower cell density in DSM-His medium than did an isogenic $SigL^+$ culture.

If amino acid limitation coincides with the end of exponential growth in DSM-His medium, then the activation of histidase expression at the onset of stationary growth in this medium is likely to result from the relief of amino acid repression. Since amino acid repression of *hut* expression is partially relieved in a HutC mutant growing logarithmically in amino acid minimal medium, and since histidase levels in exponentially growing HutC mutant cultures are 25-fold higher than in strain 168 cultures in DSM-His medium, amino acid repression is likely to mediate the repression of *hut* expression during exponential growth in DSM-His medium. Similarly, the reduced levels of proline oxidase in cells growing exponentially in nutrient sporulation medium most likely result from amino acid repression because expression of this enzyme is also regulated by amino acid repression during vegetative growth in minimal medium (2).

The partial relief of the repression of histidase expression in the HutC mutant strongly suggests that inhibition of histidine transport is, in part, responsible for amino acid repression of *hut* expression in minimal medium. Our observation that the rate of histidine uptake is fourfold lower in cells growing exponentially in DSM-His medium than in stationary-phase cells indicates that synthesis of the histidine permease is reduced during exponential growth in this medium. In addition, the presence of the 16 amino acids in the L-[^{14}C]histidine uptake mixture strongly inhibits L-histidine transport in *B. subtilis* 168H cells harvested during exponential growth in minimal medium containing amino acids (10). Thus, the activity of the L-histidine transport system may be inhibited in amino-acid-grown cells.

Alterations in the rate of transport of other nitrogen-containing compounds during growth in nutrient sporulation medium have been described previously. Cooney et al. (6) reported that the rate of uptake of nonmetabolized amino acid analogs of alanine and aspartate and of ^{14}C from a mixture of radiolabeled amino acids increased towards the end of exponential growth in nutrient sporulation medium. Expression of the *dciA* dipeptide transport operon, which is also regulated by amino acid repression, is induced at the onset of stationary growth in DSM medium (22). Increased transport of L-histidine following the end of exponential growth in sporulation medium has been reported in *Bacillus brevis* (19). During growth in nutrient sporulation medium, the rate of proline, alanine, and glutamate uptake was three- to fourfold higher in membrane vesicles isolated from stationary-phase *B. subtilis* W23 cells than in membrane vesicles prepared from exponentially growing cells (3).

The elevated histidase levels seen in strains containing the *hutC1* mutation during exponential growth in DSM-His medium suggest that inhibition of histidine transport is largely responsible for the repression of *hut* expression during exponential growth in DSM-His medium. It is unclear what mediates the four- to eightfold derepression of *hut* expression at the onset of stationary growth phase in the HutC strain. One possible explanation is that this regulation is due to additional *hut* induction by histidine. The *hutC1* mutation causes *hut* expression to be only partially constitutive. Thus, the derepression of histidase expression in the HutC mutant cultures during stationary growth phase might result from increased histidine transport. By this line of reasoning, inhibition of histidine transport would account entirely for the repression of *hut* expression seen during exponential growth in DSM-His medium.

Alternatively, the four- to eightfold derepression of *hut* expression in HutC mutant cultures during stationary growth in DSM-His medium may result from regulation by other systems. For example, the observation that histidase derepresses earlier in HutR mutant cultures than in wild-type cultures after the end of exponential growth in DSM-His medium suggests that *hut* expression may be regulated by catabolite repression during logarithmic growth in this medium. In addition, since histidase expression is still repressed 13-fold by amino acids in the HutC mutant during vegetative growth in minimal medium, amino acid repression of *hut* transcription must be mediated by two distinct mechanisms during growth in amino-acid-containing medium, regulation of L-histidine transport and a second unidentified regulatory system. The latter system could also partially repress *hut* expression during exponential growth in DSM-His medium.

The observation that synthesis of the first enzyme in the degradative pathways for histidine, proline, and arginine (8) is derepressed at the onset of stationary growth in nutrient sporulation medium indicates that these amino acids are not utilized as major sources of NH_4^+ or glutamate during exponential growth in this medium. This suggests that *B. subtilis* has evolved regulatory mechanisms which allow preferential utilization of certain amino acids during rapid growth on a mixture of amino acids. An examination of the extracellular concentrations of amino acids during the growth of a *B. subtilis* culture in glucose minimal medium containing NH_4Cl and Casamino Acids as nitrogen sources showed that glutamate, aspartate, serine, and alanine were depleted from the growth medium at similar rates throughout

exponential growth (20). In contrast, 65 to 85% of the extracellular arginine, glycine, and proline was taken up only during late exponential growth, while histidine, isoleucine, threonine, and valine were not significantly utilized until the onset of stationary growth (20). These observations suggest that exponential growth may be limited by the availability of a small subset of amino acids. Preferential utilization of these amino acids during exponential growth would ensure that other amino acids are available for adaptation and survival under conditions which are not optimal for growth in *B. subtilis*.

Indeed, amino acids and other nitrogen-containing compounds do not appear to be completely exhausted from the nutrient sporulation medium at the onset of stationary growth phase. During logarithmic growth in minimal medium where growth is nitrogen restricted, β-galactosidase levels in the *nrg-29*::Tn*917-lacZ* insertion strain is 4,000-fold higher than the levels seen in cells grown in the presence of excess nitrogen (1). However, during exponential growth in DSM-His medium, repressed levels of β-galactosidase were seen in the *nrg-29*::Tn*917-lacZ* insertion strain, and these levels did not increase by T_3 (10). This argues that nitrogen limitation does not occur at the end of exponential growth in nutrient sporulation medium. The reduced levels of β-galactosidase expression from the *nrg-29*::Tn*917-lacZ* fusion during stationary phase in DSM medium do not result from inability to activate *nrg-29* expression during stationary phase. During growth in DSM medium containing 2% glucose, β-galactosidase expression in the *nrg-29*::Tn*917-lacZ* insertion strain was repressed during early logarithmic growth but completely derepressed by T_0 (10).

The repression of sporulation in *B. subtilis* cultures grown in DSM-His medium lacking Mn^{2+} has been proposed to result from catabolite repression due to the accumulation of 3-phosphoglycerate and other glycolytic intermediates (27, 33). The observation that *hut* expression is partially derepressed in HutR mutant cultures but not in wild-type cultures is the first demonstration that catabolite repression occurs in DSM medium lacking Mn^{2+}. However, the inability to fully derepress *hut* expression in HutR mutant cultures grown in DSM-His medium lacking Mn^{2+} suggests that other aspects of cellular metabolism are altered in this medium.

Decoyinine treatment of *B. subtilis* cultures growing exponentially in either glucose or citrate minimal medium containing amino acids induced histidase expression only if the *B. subtilis* strain contained the *hutR4* mutation. A similar result was observed when the effect of decoyinine treatment on the synthesis of α-amylase, an enzyme whose expression is activated during stationary phase in nutrient sporulation medium, was examined. α-Amylase expression was only induced by decoyinine in strains containing a *cis*-acting mutation in the *amyE* promoter region which causes α-amylase expression to be resistant to catabolite repression (24). One interpretation of these results is that decoyinine treatment is unable to overcome a block in gene expression mediated by catabolite repression. If so, we are unable to explain why decoyinine treatment does not activate histidase synthesis in wild-type cultures grown in amino acid medium containing citrate, a carbon compound which does not by itself cause catabolite repression of histidase synthesis. One possible explanation is that the combination of citrate and amino acids leads to accumulation of sufficient metabolic intermediates to induce catabolite repression of *hut* expression.

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