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Altered Nutritional Requirements Associated with Mutations Affecting the Structure of Ribonucleic Acid Polymerase in *Lactobacillus casei*

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Rifampin-resistant mutants were isolated from *Lactobacillus casei* S1 and examined for possible simultaneous alteration in nutritional properties. Among the 36 mutants obtained either spontaneously or after mutagenesis with 2-aminopurine, 22 were found to be altered with respect to the specific growth requirements. The majority (20 of 22) of the latter mutants were shown to require L-glutamine in addition to the nutrients required by the parental strain for maximal growth, whereas the remaining mutants had apparently lost the requirement for L-aspartate. Further studies with one of the glutamine-requiring mutants revealed that the rifampin resistance of this strain is due to the resistance of ribonucleic acid polymerase itself and that a single mutation is responsible for both rifampin resistance and the glutamine requirement. These results strongly indicate that a structural alteration of the ribonucleic acid polymerase caused by the rifampin resistance mutation somehow affected glutamine metabolism, possibly through change in selective transcription of the genes involved.

A class of antibiotics that specifically inhibits the deoxyribonucleic acid (DNA)-dependent ribonucleic acid (RNA) polymerase has recently been employed as a means of studying various aspects of transcription in bacteria. Genetic and biochemical analyses of bacterial mutants that are resistant to rifamycins, streptovaricins, and streptolydigin have provided important information on the structure, function, and formation of RNA polymerase, which plays a central role in transcription of the bacterial and the viral genome (for reviews see references 1 and 11).

In the course of investigations on nutritional requirements in *Lactobacillus casei* (6), it became apparent that a specific requirement for most amino acids or vitamins could be lost by single-step mutations. Furthermore, the parental strain acquired the capacity to produce a specific enzyme, tryptophan synthetase, as a result of a single mutation. These results strongly indicate that only minor defects in the structure of DNA, such as single base substitutions rather than deletions, are responsible for the inability of the parental wild-type *L. casei* to synthesize each of the nutrients required. To understand further the mechanisms underlying growth requirements of these highly heterotrophic bacteria, we examined a possible change in nutritional properties as a result of structural alteration of the RNA polymerase by rifampin resistance mutations. It was also hoped that analysis of such mutants, if they exist, would contribute to our general understanding of the role of RNA polymerase in transcriptional control in bacteria.

As will be shown in the present paper, about half of the mutants that produce rifampin-resistant RNA polymerase are indeed altered with respect to their growth factor requirements. At least in one case studied in detail, evidence suggests that a single mutation is responsible for both structural alteration of RNA polymerase and a newly acquired growth requirement for L-glutamine.

MATERIALS AND METHODS

Bacterial strains. The *L. casei* wild type used in this study was strain S1 of the Yakult Institute for Microbiological Research (6). This strain requires 12 amino acids (arginine, aspartic acid, glutamic acid, isoleucine, leucine, lysine, methionine, serine, threonine, tryptophan, tyrosine, and valine) and four vitamins (riboflavin, pyridoxal, pantothenic acid, and nicotinic acid) for maximal growth. In addition, several nutrients including cystine, phenylalanine, folic acid, adenine, uracil, and xanthine were found to stimulate growth of this organism significantly.

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Media. Natural medium consisted of 10 g of polypeptone (Wako Drug Co.), 10 g of yeast extract (Difco Laboratories, Inc.), 10 g of sodium acetate, and 20 g of D-glucose per liter. Basal medium was a synthetic glucose-salts medium supplemented with all of the nutrients required by *L. casei* S1 (6). The pH of all media was adjusted to 7.2. Solid media contained 1.5% agar (Hakko Agar Co.).

Isolation of the mutants. Cells of wild-type S1 were inoculated at 10^8/ml into a number of tubes containing natural medium with or without 2-aminopurine (50 μg/ml). After incubation for 24 h at 37 C, cells of each tube were plated on natural agar medium containing 10 μg of rifampin per ml. Colonies that appeared after 3 days were picked (one from each plate to insure the independence of mutations) and purified by repeated single-colony isolations on the same medium.

Growth experiments. In most experiments, test tubes (18 by 1.8 cm) with or without a side arm were filled with 10 ml of medium and inoculated with about 10^6 cells per ml. Cultures were incubated by standing at 37 C without aeration, and optical density was determined by using a Klett-Summerson colorimeter with a no. 54 filter at appropriate time intervals or at the end of the experiment. Nutritional requirements, sugar fermentation properties, and drug sensitivity were also examined by streak tests on appropriate agar media.

Preparation of cell extracts. Cultures were grown in natural medium at 37 C for 24 h, and cells were harvested, washed, and suspended in buffer A [tris-(hydroxymethyl)aminomethane (Tris)-hydrochloride (pH 7.8) at 4 C, 10 mM; β-mercaptoethanol, 10 mM; glycerol, 10%; $MgCl_2$, 10 mM; ethylenediaminetetraacetate, 0.1 mM]. After treatment in a Kaijo ultrasonic oscillator (29 kc) for 5 min, disrupted cells were centrifuged at 10,000 × *g* for 20 min, and the resulting supernatant fluid was used as a crude RNA polymerase preparation.

Extracts used for the assay of glutamine synthetase activity were also prepared by the same procedure, except that the buffer contained 20 mM Tris-hydrochloride (pH 7.8), 10 mM β-mercaptoethanol, and 6 mM magnesium acetate, and sonic treatment was conducted in a Raytheon sonic oscillator for 30 min at 10 kc/s.

Assay for RNA polymerase. RNA polymerase activity was assayed by the method employed for the homologous enzyme of *Escherichia coli* (4). The reaction mixture (0.25 ml) contained the following (micromoles): Tris-hydrochloride (pH 7.8 at 4 C), 30; $MgCl_2$, 1.25; $MnSO_4$, 0.5; β-mercaptoethanol, 1.25; phosphoenolpyruvate, 0.5; pyruvate kinase, 5 μg; adenosine 5'-triphosphate (ATP), uridine 5'-triphosphate, guanosine 5'-triphosphate, and cytidine 5'-triphosphate, 0.04 each; [^{3}H]ATP, 0.2 μCi; calf thymus DNA, 10 μg; and enzyme solution, 0.15 ml. After incubation for 20 min at 37 C, the reaction was stopped by addition of 5% trichloroacetic acid at 4 C. The acid-insoluble precipitates formed were washed twice with 2% trichloroacetic acid and were dissolved in 0.5 ml of 5% NH_4OH. The radioactivity was determined in a Beckman scintillation counter.

Assay for glutamine synthetase. Glutamine synthetase activity was determined by the method of Ravel et al. (8). The reaction mixture (1 ml) contained the following (micromoles): L-glutamate, 40; Tris, 100; $MnCl_2$, 12; disodium ATP, 8; hydroxylamine hydrochloride, 400; and enzyme, 0.3 ml. The final pH was brought to 5.5. After incubation at 37 C for 20 min, the reaction was stopped by the addition of 3 ml of ferric chloride reagent. The precipitates were removed by centrifugation, and the optical density of the supernatants at 540 nm was determined in a Zeiss spectrophotometer. The amount of γ-glutamylhydroxamic acid formed was then calculated from a standard curve obtained with an authentic sample.

Protein determination. Protein was determined by the method of Lowry et al. (5).

RESULTS

Isolation of rifampin-resistant mutants. The effect of rifampin on wild-type *L. casei* S1 was first examined in liquid natural medium containing various concentrations of the drug. Rifampin at concentrations of 0.2 μg/ml or higher completely inhibits growth under these conditions (Fig. 1). Such a high sensitivity to the antibiotic has previously been found with other gram-positive bacteria (10, 15). Rifampin-

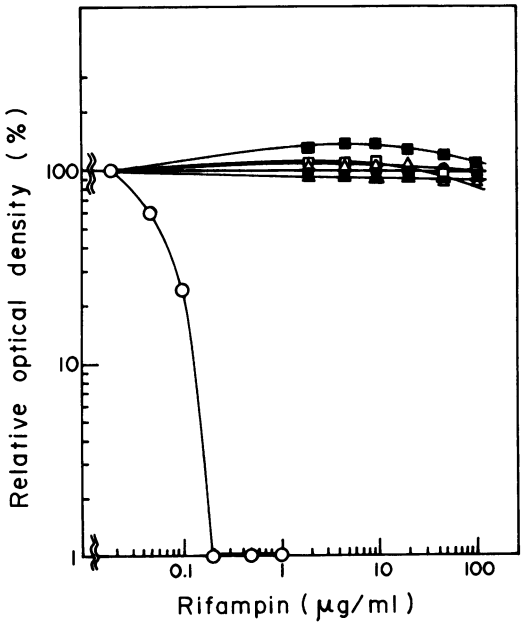

FIG. 1. *Effect of rifampin on the growth of wild-type L. casei and several rifampin-resistant mutants. An overnight culture in natural medium was diluted 100-fold by the same medium but containing rifampin at the concentrations indicated, and each was incubated at 37 C without aeration. Optical density was determined after 24 h, and the values are presented as a percentage of the control without rifampin. Symbols:* ○, *wild type (rif$^+$);* ●, *rif-1;* △, *rif-2;* ▲, *rif-3;* □, *rif-4;* ■, *rif-5.*

resistant mutants were then selected by plating cells of the parental strain on natural agar medium containing 10 μg of the drug per ml. Altogether 36 independent mutants were isolated either spontaneously or after mutagenesis with 2-aminopurine. The response of some of these mutants to rifampin in liquid medium is also shown in Fig. 1. It is apparent that all of the mutants tested can grow in the presence of as high as 100 μg of the antibiotic per ml. As is shown below, these mutants produce RNA polymerase with increased resistance to rifampin, indicating that the drug resistance observed is indeed due to a structural alteration of the RNA polymerase. The frequency of rifampin-resistant mutants obtained spontaneously under these conditions was estimated to be about 5×10^{-8} for several independent cultures.

Nutritional properties of the mutants. We examined the rifampin-resistant mutants obtained for their ability to grow on basal medium to discover possible simultaneous alteration in nutritional properties. Unexpectedly, more than half of the mutants tested (20 of 36) failed to grow on this medium, suggesting that they had acquired an additional requirement for some compound(s) that is present in the natural medium but not in the basal medium employed (type II mutants, Table 1).

The same mutants were also tested for their capacity to grow on a set of basal media each lacking one of the nutrients required by the parental organism. As expected, most of the mutants failed to grow on any of these incomplete basal media. However, two of them exhibited almost normal growth on the medium lacking aspartic acid (type III mutants, Table 1). These aspartate-independent mutants were found to retain the characteristic requirements for all other nutrients tested.

Glutamine requirement of the type II mutants. A series of experiments was carried out to identify the compound required by the type II mutants. Among the various nutrients tested, including amino acids, vitamins, purines, and pyrimidines, L-glutamine was found to be most effective in supporting growth of these mutants. This was true with any of the 20 independent mutants examined. The typical results obtained for some representative mutants are shown in Table 2. In addition to glutamine, L-proline was partially effective, and glycine seemed to stimulate growth of some mutants only slightly. L-Asparagine, L-alanine, and L-histidine were virtually without effect. The quantitative growth response of some of the mutants to L-glutamine is illustrated in Fig. 2A. L-Glutamine at concentrations of 10 μg/ml or higher was required to support maximal growth of these mutants as judged by optical density measurements; the growth rate was much reduced in the absence of glutamine, although appreciable growth was obtained after prolonged incubation (Fig. 2B). It appears likely,

TABLE 1. *Nutritional properties of rifampin-resistant mutants of L. casei S1*[a]

Type	Growth on:			No. of mutants obtained	
	Natural medium	Basal medium	Basal medium lacking aspartate	Spontaneous	2-Aminopurine induced
Parent	+[b]	+	−		
Mutant I	+	+	−	10	4
Mutant II	+	−	−	17	3
Mutant III	+	+	+	2	0

[a] Each mutant, as well as the parental strain, was grown in natural medium overnight at 37 C. Cells were harvested, washed twice in saline, and streaked on natural or basal agar medium as indicated. Although a set of basal media, each lacking one of the amino acids or vitamins required by the parental strain, was tested, only the results obtained with medium lacking aspartate are shown.

[b] +, Normal growth; −, little or no growth.

TABLE 2. *Growth requirement of the type II rifampin-resistant mutants*[a]

Addition to basal medium	Optical density (% of control)					
	Wild type	*rif-1*[b]	*rif-2*[c]	*rif-3*[c]	*rif-4*[c]	*rif-5*[b]
None	100	8	6	18	10	8
L-Alanine	89	10	13	6	8	0
L-Asparagine	66	8	5	0	4	0
L-Glutamine	92	70	68	90	90	96
Glycine	90	7	6	28	14	30
L-Histidine	103	7	10	12	10	14
L-Proline	98	28	31	50	46	52

[a] Basal media supplemented with each amino acid as indicated were inoculated with washed-cell suspensions (about 10^6 cells per ml). Alanine, glycine, histidine, and proline were added at 100 μg/ml, whereas asparagine and glutamine were used at 400 μg/ml. The glutamine used in this and all subsequent experiments was sterilized before being dissolved in sterile water. Cultures were incubated at 37 C, and optical density was read after 24 h. The values are presented as those relative to the value for the wild type grown in basal medium (indicated by underline).

[b] Spontaneous mutants.

[c] 2-Aminopurine-induced mutants.

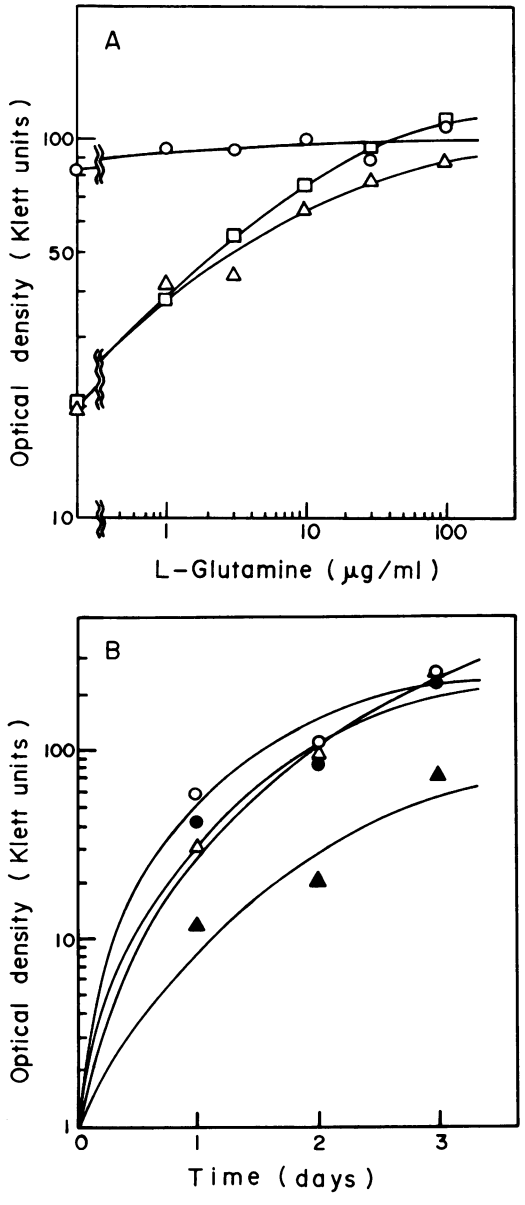

FIG. 2. *Effect of L-glutamine on the growth of type II rifampin-resistant mutants. A late log-phase culture was harvested, and cells were washed twice in saline and used to inoculate (at about 10^6 cells per ml) into basal medium containing varying concentrations of L-glutamine. Cultures were incubated at 37 C, and the optical density was read at intervals of 24 h. (A) Growth in the presence of varying concentrations of L-glutamine: O, wild type (rif⁺); Δ, mutant rif-2; □, mutant rif-7. Optical densities at 48 h of incubation are presented. (B) Time course of growth in the presence (open symbols) or absence (closed symbols) of L-glutamine at 100 μg/ml. Circles represent the wild type (rif⁺), whereas triangles represent mutant rif-2.*

therefore, that the metabolism or transport of L-glutamine is somehow affected in these mutants.

Sugar fermentation properties of the type II mutants. Possible alteration in sugar fermentation properties was then investigated with the type II mutants, since it had been shown in *E. coli* that glutamine synthesis and fermentation of certain sugars are controlled by a common mechanism involving cyclic adenosine 3',5'-monophosphate (7, 9). Among the 20 mutants examined, 9 appeared to be defective in utilizing maltose or mannose or both as the sole carbon source (Table 3). The frequent occurrence of simultaneous changes in rifampin resistance and metabolic properties such as glutamine requirement and sugar fermentation capacity suggests the interesting possibility that structural alteration of RNA polymerase is primarily responsible for the observed pleiotropic effects of these mutations.

Glutamine-independent revertants. To substantiate further the relationship between rifampin resistance and alteration of nutritional and fermentation properties of the mutants, glutamine-independent revertants were isolated from the type II mutants and examined for possible simultaneous changes in rifampin resistance. In the first experiment, 20 revertants obtained spontaneously from each mutant were tested, but none of them was sensitive to rifampin as determined by streak tests on natural agar medium containing 100 μg of the drug per ml. When tested in liquid medium, however, the growth of some revertants was partially inhibited by rifampin at 100 μg/ml (data not shown). In the second experiment, 120 spontaneous revertants were isolated independ-

TABLE 3. *Sugar fermentation properties of the type II rifampin-resistant mutants*[a]

Type	Growth on:				No. of mutants	
	Glucose	Galactose	Maltose	Mannose	Spontaneous	2-Aminopurine induced
Parent	+[b]	+	+	+		
Mutant IIA	+	+	+	+	11	0
Mutant IIB	+	+	−	+	4	3
Mutant IIC	+	+	−	−	2	0

[a] Overnight cultures grown in natural medium were harvested, and the cells were washed twice in saline and streaked on basal medium containing 1% sugar, as indicated, as the sole carbon source. Results were read after 3 days of incubation at 37 C.

[b] +, Normal growth; −, little or no growth.

ently from one of the type IIB mutants that cannot use maltose as a carbon source (*rif-2*). The average frequency of glutamine-independent revertants in a culture of this mutant was about 5×10^{-8}. Among the revertants thus obtained, about one-third were slightly sensitive to rifampin by tests in liquid medium, and at least two (no. 82 and 83) were clearly sensitive even by streak tests. The lack of a requirement for L-glutamine (or L-proline) with the latter revertants is shown in Fig. 3, although both growth rates and final yields appeared to be somewhat reduced as compared with that of the wild-type strain. Also, the revertants exhibited definite sensitivity to rifampin, although the sensitivity was less striking than that of the wild-type (Fig. 4). On the other hand, they seemed to remain incapable of utilizing maltose, like the parental mutant (data not shown). The latter result is probably related to the only partial recovery of rifampin sensitivity observed with these revertants. All of these results may be taken to indicate that a single mutation is responsible for both rifampin resistance and the glutamine requirement, at least in the case of mutant *rif-2*.

Altered RNA polymerase in mutant rif-2 and its revertants. To determine whether the changes in sensitivity of the *rif-2* mutant and its revertants to rifampin as studied in vivo reflect altered properties of RNA polymerase or altered permeability to the drug, the direct effect of rifampin on the polymerase was examined in vitro. Crude extracts were prepared and enzyme activity was determined by measuring incorporation of [^{3}H]ATP into an acid-insoluble fraction. The reaction continued linearly for about 20 min under the conditions employed. It was found that RNA synthesis catalyzed by the mutant enzyme is resistant to inhibition by rifampin at 100 μg/ml, in contrast to the reaction catalyzed by the wild-type enzyme, which was inhibited significantly even at 1 μg/ml (Fig. 5). This is in good agreement with the results obtained in vivo (Fig. 1). The result also implies that most of the [^{3}H]ATP incorporated in the present system is actually due to the DNA-dependent RNA polymerase. Figure 5 also

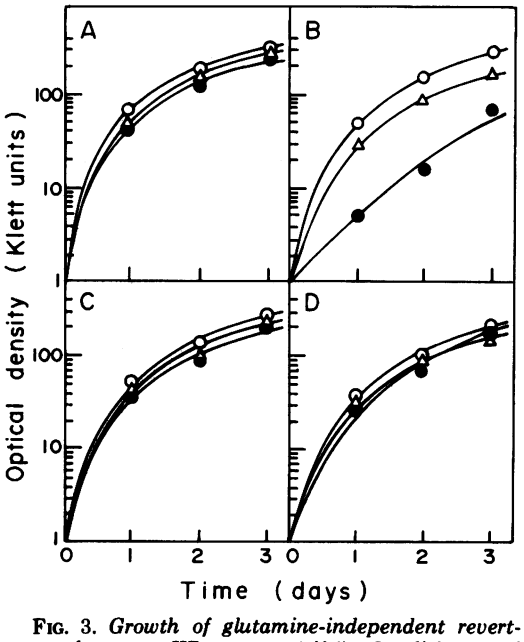

FIG. 3. *Growth of glutamine-independent revertants of a type IIB mutant (rif-2). Conditions and procedures are as described in the legend of Fig. 2. Cultures were grown in basal medium with or without 100 μg of L-glutamine or L-proline per ml at 37 C, and the optical density was determined at the times indicated. (A) Wild type, (B) mutant rif-2, (C) revertant no. 82, (D) revertant no. 83. Symbols: ●, basal medium without supplement; ○, with L-glutamine; Δ, with L-proline.*

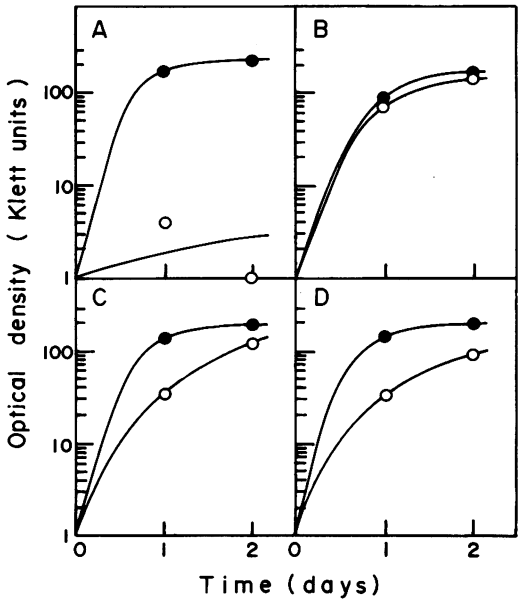

FIG. 4. *Rifampin sensitivity of glutamine-independent revertants of type IIB mutant rif-2. Overnight cultures grown in natural medium were diluted 100-fold in the same medium with (○) or without (●) rifampin (100 μg/ml). Cultures were incubated at 37 C, and the optical density was read at the times indicated. (A) Wild type, (B) mutant rif-2, (C) revertant no. 82, (D) revertant no. 83.*

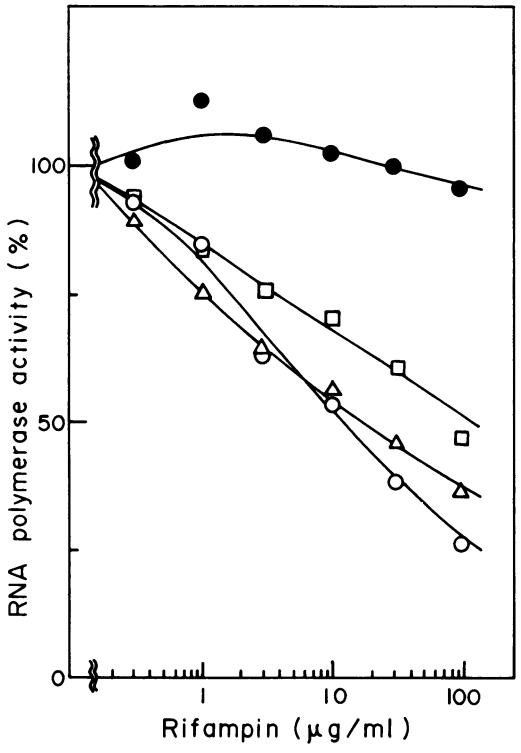

FIG. 5. *Effect of rifampin on RNA synthesis in vitro. Crude extracts were prepared and used for in vitro RNA synthesis as described in the text. Rifampin was added to the enzyme before the latter was mixed with other reagents of the reaction mixture. RNA polymerase activities are shown as a percentage of the control without drug for each enzyme preparation employed. Symbols:* ○, *wild type;* ●, *mutant rif-2;* △, *revertant no. 82;* □, *revertant no. 83.*

shows that the enzymes from the two revertants are almost as sensitive to the drug as is the wild-type enzyme. These results clearly demonstrate that both the rifampin resistance of mutant *rif-2* and the rifampin sensitivity of the revertants reflect an alteration of RNA polymerase rather than changes in permeability to the drug. Taken together with other evidence, it seems apparent that the growth requirement for L-glutamine exhibited by this mutant is correlated with, and perhaps caused by, the production of altered RNA polymerase as a result of the *rif-2* mutation.

Glutamine synthetase activity in the rif-2 mutant. As an approach to the basis for the L-glutamine requirement in the *rif-2* mutant, glutamine synthetase activity in the crude extract was determined, since this enzyme is presumably responsible for glutamine synthesis in *L. casei* as in *E. coli*. By using the assay system for *L. arabinosus* enzyme, about equal activities were found in extracts from both the mutant and the wild-type strains; about 1.5 μmol of the product (γ-glutamylhydroxamic acid) was formed per mg of protein under the conditions described in Materials and Methods. In addition, treatment of the extract with snake venom phosphodiesterase failed to activate the enzyme significantly, indicating that no appreciable amounts of adenylylated forms of glutamine synthetase are present in *L. casei* as in most other gram-positive bacteria tested (14). The glutamine-independent revertants tested (no. 82 and 83) also exhibited quantitatively similar activities. Thus, it seems unlikely, although it is not impossible, that the L-glutamine requirement of this mutant is due to reduced synthesis or activity of glutamine synthetase per se.

DISCUSSION

The data presented in this paper strongly indicate that a single mutation is responsible for both rifampin resistance and the growth requirement for L-glutamine in the case of mutant *rif-2*. Since rifampin resistance of the mutant was found to be due to an alteration of RNA polymerase itself, it may be concluded that the structural alteration of the RNA polymerase somehow affected glutamine metabolism in this mutant. This probably applies to other glutamine-requiring, rifampin-resistant (type II) mutants as well, although further analysis of revertants obtained from mutants other than *rif-2* may be required to establish this point.

As to the mechanism for the glutamine requirement in the type II mutants, several possibilities are suggested. The requirement might be brought about by reduced synthesis of glutamine, increased breakdown of glutamine, reduced permeability to glutamate, excessive leakage of glutamine out of the cell, or by increased need of glutamine for protein synthesis or for one or more biosynthetic reactions as the amino nitrogen donor. Available evidence does not permit us to distinguish among these various possibilities at the present time. Although the normal level of glutamine synthetase activity seems to be found in the mutant strain tested (*rif-2*), this by no means excludes the possibility that glutamine synthesis is the reaction primarily affected by the polymerase mutation. Obviously, further experiments are needed to elucidate the mechanism underlying

the specific requirement for glutamine in these mutants.

In the absence of experimental evidence, little can be said about a possible link between mutational alteration of the RNA polymerase and defects in glutamine metabolism in the type II mutants. It is quite possible that a structural alteration of the polymerase directly or indirectly affects selective transcription of the genes related to glutamine metabolism. In this connection, some of the rifampin-resistant mutants obtained in other bacteria have been shown to be defective in growth at high temperature (16), sporulation (13), capacity to support growth of bacteriophage (12), or in the ability to be lysogenized with a temperate phage (2, 3). However, direct evidence that a mutation affects selective transcription of specific operons is lacking in any of these or other mutants that produce altered RNA polymerase.

Many rifampin-resistant mutants obtained from *E. coli*, for example, are slow growers as compared with the wild type, particularly in minimal medium containing only the nutrients required by the parental organism. Their growth is often stimulated greatly by the addition of Casamino Acids or yeast extract to the medium. However, to our knowledge, there has been no report of rifampin-resistant or other RNA polymerase mutants that require a specific nutrient for growth. Furthermore, it seems rather surprising that more than half of the spontaneous as well as mutagen-induced rifampin-resistant mutants obtained from the present strain of *L. casei* belong to this class. We cannot offer an adequate explanation for this finding, but it is not inconceivable that a part of the structure of the RNA polymerase directly involved in rifampin binding is critical in regulating transcription of the genes related to glutamine metabolism in these bacteria. At any event, further work on these and other mutations affecting both the RNA polymerase structure and nutritional requirements may provide useful information on the nature of transcriptional control of specific metabolic pathways in this heterotrophic bacteria. It would also be interesting to determine whether similar *E. coli* mutants might be found with which genetic and enzymatic analyses can be performed in much greater detail than is possible at present with *L. casei*.

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LITERATURE CITED

1. Burgess, R. R. 1971. RNA polymerase. Annu. Rev. Biochem. **40**:711-740.
2. Grodzicker, T., R. R. Arditti, and H. Eisen. 1972. Establishment of repression by lambdoid phage in catabolite activator protein and adenylate cyclase mutants of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **69**:366-370.
3. Hong, J., G. R. Smith, and B. N. Ames. 1971. Adenosine 3':5'-cyclic monophosphate concentration in the bacterial host regulates the viral decision between lysogeny and lysis. Proc. Natl. Acad. Sci. U.S.A. **68**:2258-2262.
4. Iwakura, Y., A. Ishihama, and T. Yura. 1973. RNA polymerase mutants of *Escherichia coli*. II. Streptolydigin resistance and its relation to rifampicin resistance. Mol. Gen. Genet. **121**:181-196.
5. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265-275.
6. Morishita, T., T. Fukada, M. Shirota, and T. Yura. 1974. Genetic basis of nutritional requirements in *Lactobacillus casei*. J. Bacteriol. **120**:1078-1084.
7. Prusiner, S., R. E. Miller, and R. C. Valentine. 1972. Adenosine 3':5'-cyclic monophosphate control of the enzymes of glutamine metabolism in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **69**:2922-2926.
8. Ravel, J. M., J. S. Humphreys, and W. Shive. 1965. Control of glutamine synthesis in *Lactobacillus arabinosus*. Arch. Biochem. Biophys. **111**:720-726.
9. Rickenberg, H. V. 1974. Cyclic AMP in prokaryotes. Annu. Rev. Microbiol. **28**:353-369.
10. Riva, S., and L. G. Silvestri. 1972. Rifamycins: a general view. Annu. Rev. Microbiol. **26**:199-224.
11. Sethi, V. S. 1971. Structure and function of DNA-dependent RNA polymerase. Progr. Biophys. Mol. Biol. **23**:67-101.
12. Snyder, L. R. 1972. An RNA polymerase mutant of *Escherichia coli* defective in the T4 viral transcription program. Virology **50**:396-403.
13. Sonenshein, A. L., and R. Losick. 1970. RNA polymerase mutants blocked in sporulation. Nature (London) **227**:906-909.
14. Tronick, S. R., J. E. Ciardi, and E. R. Stadtman. 1973. Comparative biochemical and immunological studies of bacterial glutamine synthetases. J. Bacteriol. **115**:858-868.
15. Wehril, W., and M. Staehelin. 1971. Actions of the rifamycins. Bacteriol. Rev. **35**:290-309.
16. Yura, T., K. Igarashi, and K. Masukata. 1970. Temperature-sensitive RNA polymerase mutants of *Escherichia coli*, p. 71-89. *In* L. Silvestri (ed.), Proceedings of the First International Lepetit Colloquium on RNA polymerase and transcription. North-Holland Publishing Co., Amsterdam.