JOURNAL OF BACTERIOLOGY, July 1974, p. 220-227
Vol. 119, No. 1
Printed in U.S.A.
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# Homologous and Hybrid Complexes of Anthranilate Synthase from *Bacillus* Species

NANU PATEL, W. M. HOLMES, AND J. F. KANE

*Department of Microbiology, University of Tennessee Medical Units, Memphis, Tennessee 38163*

Received for publication 12 March 1974

The subunits of anthranilate synthase were separated and partially purified by Sephadex G-100 gel filtration from the following six species of *Bacillus*: *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus alvei*, *Bacillus coagulans*, *Bacillus pumilus*, and *Bacillus mascerans*. Our data suggest that the enzyme from *B. alvei* is unique among these species. First, the anthranilate synthase complexes are readily dissociated during gel filtration in the absence of glutamine into a large component (aminotransferase), subunit E, and a small component subunit X (glutamine-binding protein), whereas a higher salt concentration is required to dissociate the complex from *B. alvei*. Second, the aminotransferase activity from all six species is stimulated by glycerol and inhibited by tryptophan; however, only the large component from *B. alvei* is stimulated by 2-mercaptoethanol. Finally, the large component can be titrated with the small component to yield a complex which can utilize glutamine as a substrate (amidotransferase). The homologous complexes have an amidotransferase to aminotransferase ratio of 1.4 to 2.3, but the *B. alvei* complex has a ratio of 0.9. Except for complexes that involve the large component from *B. alvei*, hybrid complexes can be formed which have ratios as good as the homologous complexes. These data are consistent with the hypothesis that *B. alvei* is unique among the bacilli with respect to some enzymes in the aromatic amino acid biosynthetic pathway.

Anthranilate synthase is the first enzyme unique to the tryptophan biosynthetic pathway. In all microorganisms studied thus far, this enzyme is composed of two nonidentical subunits (22). The large component can catalyze the formation of anthranilate by using $NH_3$ as a substrate (aminotransferase). The small component has no anthranilate synthase activity by itself; rather, it serves as a glutamine-binding protein that binds with the large component to give a complex which can utilize either glutamine or $NH_3$ as a substrate (amidotransferase).

In *Bacillus subtilis* (12, 13), *Bacillus alvei* (7), *Bacillus pumilus* (7), *Acinetobacter calcoaceticus* (19), *Clostridium butyricum* (1), and some species of *Pseudomonas* (17), anthranilate synthase is a readily dissociated enzyme complex that is not associated with any other tryptophan biosynthetic enzyme. Since free subunits can be obtained by gel filtration, and since amidotransferase activity requires a complex composed of a large and small component, the activity of homologous and hybrid complexes can be readily studied (8, 16, 17).

Using this technique, we examined six species of *Bacillus*, namely, *B. subtilis*, *B. alvei*, *Bacillus coagulans*, *B. pumilus*, *Bacillus licheniformis*, and *Bacillus mascerans*, with the following objectives in mind: (i) to determine if a readily dissociable enzyme complex was characteristic of this genus; (ii) to examine the formation of hybrid complexes; (iii) to compare the anthranilate synthase from *B. alvei* with the enzyme from the other species of *Bacillus* since *B. alvei* is unique among the bacilli in many respects (2, 3, 7, 11).

## MATERIALS AND METHODS

**Bacterial strains.** The *B. subtilis* mutant, NP100, used in this study is a derivative of strain 168 and has been described elsewhere (14). The strains *B. licheniformis*, *B. coagulans*, and *B. mascerans* which were obtained from Roy A. Jensen have been utilized in the taxonomic study of 3-deoxy-D-arabino-heptulosonate-7-phosphate (DAHP) synthase (11). The *B. alvei* mutant TS22 is a tryptophan auxotroph and was obtained from Ralph DeMoss, University of Illinois at Urbana. The *B. pumilus* strain was obtained from Frank E. Young, University of Rochester.

**Growth conditions.** All strains were grown on minimal-salts glucose media (10) with the following

additional supplements for: *B. subtilis*, 50 μg of phenylalanine/ml; *B. alvei*, 0.1% casein hydrolysate, 50 μg of tryptophan per ml and 2.5 μg of thiamine per ml; *B. pumilus*, 1.0 μg of biotin per ml; *B. mascerans*, 1.0 μg of biotin per ml and 2.5 μg of thiamine per ml. All cultures were grown at 37 C except *B. alvei* which was grown at 32 C.

**Derepressed mutants.** As has been described, the *B. subtilis* mutant NP100 is constitutive for the tryptophan biosynthetic enzymes (14). To obtain higher yields of enzyme subunits, we isolated constitutive mutants from all strains except *B. alvei*. Approximately $10^8$ cells was plated on minimal salts glucose plates containing 5 μg of 5-fluorotryptophan per ml and the appropriate vitamins. The plates were incubated at 37 C for 24 to 48 h. Resistant clones were picked and purified by restreaking on 5-fluorotryptophan-supplemented media.

The 5-fluorotryptophan-resistant ($FT^R$) clones were examined for their ability to excrete tryptophan as follows. Approximately $10^8$ cells of a *B. subtilis* tryptophan auxotroph was spread on minimal salts glucose plates containing 0.1% casein hydrolysate. The $FT^R$ mutants were spotted on this lawn and after 24 h at 37 C, tryptophan excretors could be detected by the appearance of halo of growth around the $FT^R$ mutants.

Since *B. alvei* produces tryptophanase (5), it is resistant to tryptophan analogues. Therefore, the tryptophan mutant TS22 was grown as described above. The cells were harvested in late exponential phase, washed with minimal media, and resuspended in the media that lacked tryptophan. The cells were allowed to derepress for about 4 h after which time they were harvested.

**Preparation of cell extracts.** Cultures were harvested in late exponential phase of growth and pelleted by centrifugation at 8,000 × *g* for 15 min. The pellet was resuspended in 20 mM tris (hydroxymethyl)aminomethane (Tris) buffer, pH 7.5, which contained 6 mM 2-mercaptoethanol, 0.1 mM ethylenediaminetetraacetic acid (EDTA), and 30% glycerol. The cells were disrupted by sonic treatment for 1 to 3 min in a sonifier (Heat Systems-Ultrasonics, Inc., Plainview, N.Y.). The extracts were clarified by centrifugation at 15,000 × *g* for 15 min.

In all cases, the extracts were brought to 0.4 saturation with $(NH_4)_2SO_4$. After stirring for 10 min at room temperature the precipitate was removed by centrifugation at 15,000 × *g* for 30 min. The precipitate was discarded and the supernatant was brought to 0.8 saturation with $(NH_4)_2SO_4$ and stirred at room temperature for 10 min. After centrifugation, the precipitate was resuspended in a minimal volume of extract buffer. The recovery of amidotransferase and aminotransferase activities was 70 and 90%, respectively. The specific activity of anthranilate synthase was increased about threefold by this procedure.

**Gel filtration.** The extracts were applied to a Sephadex G-100 column (2.5 by 90 cm) equilibrated with the buffer described above. For extracts from *B. alvei* 0.3 M KCl was also included in this buffer. The column was calibrated with bovine serum albumin (67,000 daltons), ovalbumin (45,000 daltons), chymotrypsinogen A (45,000 daltons), and cytochrome *c* (12,400 daltons). The downward flow rate was 10 to 12 ml/h at 4 C, and 3-ml fractions were collected. Appropriate fractions containing subunit E (aminotransferase) and subunit X were pooled and concentrated on Amicon membranes PM30 and UM2, respectively. The protein concentration was estimated by the method of Lowry et al. (15) with bovine serum albumin as a standard.

**Anthranilate synthase assay.** Amidotransferase activity was determined fluorometrically (excitation wavelength, 313 nm; emission wavelength, 393 nm, both uncorrected), in 1.0 ml of reaction mixture containing 60 μmol of Tris buffer (pH 7.75), 10 μmol of MgCl2, 20 μmol of glutamine, 400 nmol of chorismate, and 3 μmol of EDTA. With the enzyme from *B. alvei*, 4 μmol of 2-mercaptoethanol were also included in the assay mixture.

Aminotransferase activity was determined in a 1.0-ml reaction mixture containing 60 μmol of Tris buffer (pH 8.6), 50 μmol of $NH_4Cl$, 10 μmol of $MgCl_2$, 400 nmol of chorismate, and 40% glycerol. With the enzyme from *B. alvei*, 4 μmol of 2-mercaptoethanol was also added.

One unit of enzyme activity is equal to 1 μmol of anthranilate formed per min.

**Complementation studies.** Increasing concentrations of the partially purified subunit X were added to a fixed level of aminotransferase and incubated in the presence of 20 μmol of L-glutamine and 3 μmol of EDTA in a final volume of 0.3 ml at room temperature for 3 min. When *B. alvei* subunits were used, 4 μmol of 2-mercaptoethanol were also included in the preincubation mixture. After incubation, the remaining reaction mixture was added and amidotransferase activity was followed continuously until a steady state velocity was reached.

Since the components were only partially purified, protein concentration may not be a valid representation of the amount of component present. Therefore, appropriate concentrations of subunit E were used to give comparable aminotransferase activities. The subunit E from each species was incubated with various concentrations of partially purified subunit X, and the amidotransferase activity of these EX mixtures was determined. The amidotransferase activity of the complex was plotted as a function of the concentration of subunit X, and the ratio of amidotransferase activity to aminotransferase activity at saturation was determined. This ratio was used for the quantitative expression of complementation between the homologous and heterologous components.

## RESULTS

**Dissociation of the EX complex.** The readily dissociable nature of the anthranilate synthase complex from *B. subtilis* after gel filtration has been described (12). The degree of dissociation, however, is a function of the concentration of the subunits as the data in Table 1 illustrate. As

the concentration of enzyme applied to the Sephadex G-100 column is increased the degree of dissociation decreases. This is reflected by an increase in the ratio of the amidotransferase to aminotransferase activity.

A similar observation was made with the enzyme from *B. pumilus*. The data in Fig. 1 illustrate the elution profile of the enzyme complex and free subunit X. In this experiment there was an 84% recovery of subunit E and a 90% recovery of subunit X as compared to a sample left at 4 C for a comparable period of time. Under these conditions only 80% of the enzyme complex dissociated (Fig. 1A). If the first peak containing free subunit E and some EX complex is pooled, concentrated, and subjected to a second gel filtration, complete dissociation of the subunits is observed (Fig. 1B). In contrast to a report by Hoch and Crawford (7), these data indicate that a free subunit X can be isolated from *B. pumilus*. Since subunit X is inactivated in the absence of EDTA and 2-mercaptoethanol (W. M. Holmes and J. F. Kane, manuscript in preparation), the absence of subunit X in their eluate fractions can be attributed to a lack of these two reagents in their column buffers (6).

TABLE 1. *Effects of subunit concentration on the elution of an EX complex during gel filtration*

| Amidotransfer act (mU) | Ratio of amidotransferase to aminotransferase act | |
|---|---|---|
| | Sample applied | EX complex |
| 2,016 | 1.6 | 0.02 |
| 19,900 | 1.4 | 0.13 |
| 110,000 | 1.7 | 0.33 |

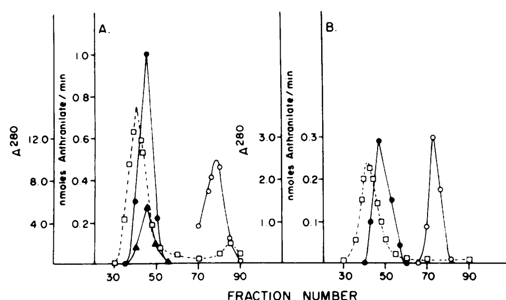


FIG. 1. *Sephadex G-100 gel filtration of EX complex from B. pumilus. (A) An extract of B. pumilus containing 252 mg of protein was applied to a Sephadex G-100 column (2.5 by 95 cm) equilibrated as described. The fractions were assayed as follows: absorbance at 280 nm (□); aminotransferase activity (●); amidotransferase activity (▲); amidotransferase activity in the presence of subunit E from B. subtilis (○). (B) Fractions 40 to 50 were pooled and concentrated, and 129 mg of protein was applied to the same column. The symbols are the same as those in (A).*

The anthranilate synthase complex from *B. licheniformis*, *B. coagulans*, and *B. mascerans* could also be readily dissociated by gel filtration. The enzyme complex from *B. alvei*, however, would not completely dissociate under these conditions, regardless of the concentration of enzyme complex applied to the column (Fig. 2A). Complete dissociation was observed if 0.3 M KCl was included in the column buffer (Fig. 2B). In this experiment there was a 74% recovery of subunit E and an 85% recovery of subunit X when compared to an enzyme preparation left at 4 C for a comparable period of time. The association of the EX complex (molecular mass 80,000 daltons) in the absence of glutamine is unique among the species tested.

The estimated molecular masses for the subunits of anthranilate synthase from these six species are shown in Table 2. The values of subunit E ranged from 58,000 to 80,000 daltons except for the component from *B. licheniformis* which was consistently larger than 100,000 daltons. The glutamine-binding proteins (subunit X) from all six species had molecular masses that range from 15,000 to 24,000 daltons.

**Stability of aminotransferase activity.** The data in Fig. 3 illustrate the effect of increasing concentrations of glycerol on the aminotransfer-

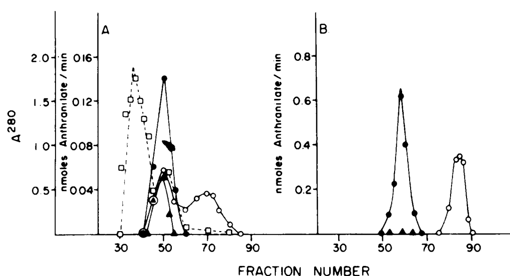


FIG. 2. *Sephadex G-100 gel filtration of EX complex from B. alvei. (A) An extract of B. alvei containing 46 mg of protein was applied to the top of a Sephadex G-100 column equilibrated with a buffer that did not contain 0.3 M KCl. The fractions were assayed as follows: absorbance at 280 nm (□); aminotransferase (●); amidotransferase (▲); amidotransferase activity in the presence of excess subunit E from B. alvei (○). (B) Fractions containing the EX aggregate (23 mg of protein/ml) from two different experiments were pooled, concentrated, and applied to the top of a Sephadex G-100 column equilibrated with buffer containing 0.3 M KCl. The fractions were assayed for aminotransferase activity (●), amidotransferase activity (▲), and amidotransferase activity in the presence of excess subunit E from B. alvei (○).*

TABLE 2. *Estimated molecular masses of subunits E and X from gel filtration on Sephadex G-100*

| Strain | Subunit E | Subunit X |
|---|---|---|
| *B. subtilis* | 67,000 | 20,000 |
| *B. pumilus* | 80,000 | 23,000 |
| *B. licheniformis* | >100,000 | 24,000 |
| *B. coagulans* | 80,000 | 18,000 |
| *B. mascerans* | 62,000 | 24,000 |
| *B. alvei* | 58,000 | 15,000 |

ase activity of *B. subtilis*. A similar stimulatory effect by glycerol was noted for all of the aminotransferases studied (Table 3). In the absence of glycerol the velocity of the aminotransferases were decreased but remained constant for at least 3 min. The only exception was the aminotransferase from *B. alvei* which lost all activity in about 3 min (Fig. 4). The addition of 2-mercaptoethanol reactivated the aminotransferase to a rate that was about one-half that observed in the presence of 40% glycerol. This reducing agent, which stimulated the aminotransferase activity from *B. alvei*, did not stimulate this activity from any of the other species of *Bacillus* tested.

**Inhibition of aminotransferase activity by tryptophan.** The tryptophan binding site for all anthranilate synthases examined appears to be on the aminotransferase component (9, 17, 19, 21). As expected, all six of these aminotransferases were inhibited by L-tryptophan (Fig. 5). Subunit E from *B. alvei*, *B. mascerans*, and *B. pumilus* appears to be less sensitive to tryptophan (200 μM gave 50% inhibition) than the subunit E from the other species (from 5 to 10 μM gave 50% inhibition). The possibility that the mutation to 5-fluorotryptophan resistance in *B. mascerans* and *B. pumilus* resulted in a decrease in feedback inhibition was examined. In crude extracts, the mutant enzyme from both strains was as sensitive to tryptophan inhibition as the wild-type enzyme (data not shown).

**Formation of homologous and hybrid complexes.** The data in Fig. 6 illustrate the velocity of the amidotransferase activity of the enzyme complexes as a function of the concentration of subunit X from *B. subtilis* (Fig. 6A) and *B. alvei* (Fig. 6B). With subunit X of *B. subtilis* all of the aminotransferases except that from *B. alvei* reach about the same velocity at saturation and have an amidotransferase to aminotransferase ratio of 1.0 to 2.1. The ratio in the hybrid complex formed between subunit E from *B. alvei* and subunit X from *B. subtilis* was only 0.5. In an analogous experiment the

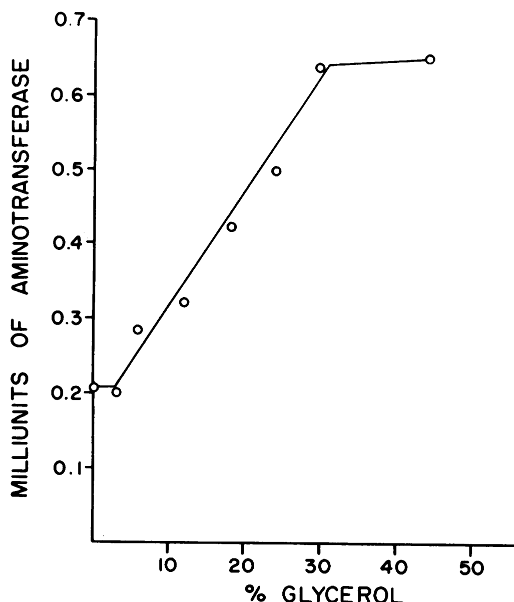


FIG. 3. *Effects of glycerol on the aminotransferase subunit from B. subtilis. The aminotransferase activity of subunit E was assayed as previously described except that increasing concentrations of glycerol were added to the reaction mixture. Units of activity are plotted as a function of the percent of glycerol present in the reaction vessel.*

TABLE 3. *Effect of 2-mercaptoethanol and glycerol on aminotransferase activity*

| Source | Aminotransferase (munits) | | | |
|---|---|---|---|---|
| | −Glycerol | | +40% Glycerol | |
| | −ME | +ME | −ME | +ME |
| *B. alvei* | 0.05 | 0.20 | 0.40 | 0.40 |
| *B. subtilis* | 0.10 | 0.10 | 0.42 | 0.38 |
| *B. coagulans* | 0.08 | 0.06 | 0.32 | 0.26 |
| *B. mascerans* | 0.12 | 0.08 | 0.30 | 0.24 |
| *B. pumilus* | 0.24 | 0.20 | 0.44 | 0.40 |
| *B. licheniformis* | 0.07 | 0.06 | 0.34 | 0.26 |

aminotransferases were titrated with the subunit X from *B alvei*. In this case the small component binds equally well with most of the aminotransferases. A list of the ratios of amidotransferase to aminotransferase activity for all of the complexes is shown in Table 4. It is apparent that homologous complex from *B. alvei* which has a ratio of 0.9 is not as efficient in its amidotransferase activity as the other homologous complexes which have ratios that range from 1.4 to 2.3. Since subunit X confers the glutamine-binding site to the complex, one would expect that this subunit would be the determining factor in the amidotransferase ac-

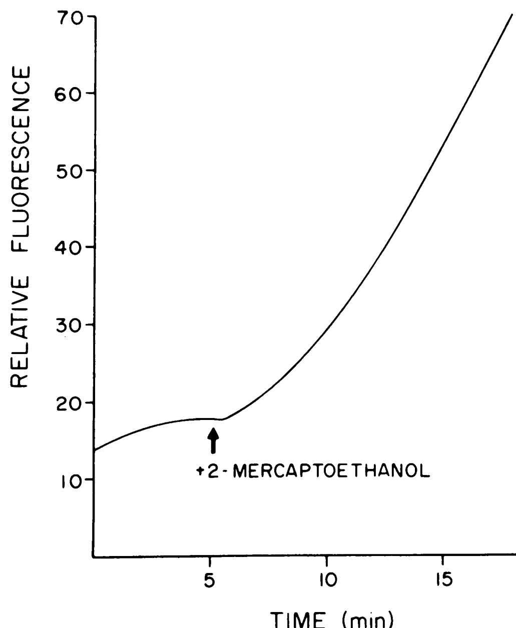


FIG. 4. *The aminotransferase activity of subunit E from B. alvei. The aminotransferase activity of subunit E was assayed in a reaction mixture that lacked glycerol. The reaction was initiated by the addition of subunit E, and at the time indicated 2-mercaptoethanol was added to a final concentration of 5 mM. The relative fluorescence of the product anthranilate is plotted as a function of time.*

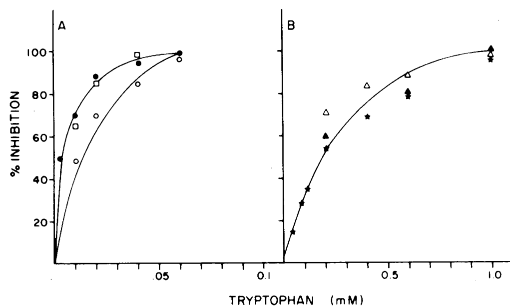


FIG. 5. *The inhibition of aminotransferase activity by tryptophan. The percent inhibition of amino transferase activity is plotted as a function of the concentration of L-tryptophan in the reaction mixture. The aminotransferases from the six species of Bacillus are indicated as follows: (A) B. subtilis (●); B. licheniformis (□); B. coagulans (O); (B) B. alvei (▲); B. mascerans (△); B. pumilus (★).*

tivity, and hybrid complexes would not be expected to be more efficient than homologous complexes. The most consistent observations are as follows. (i) Subunit X from *B. alvei* is about equally effective with all aminotransferases. (ii) Subunit X from all other species

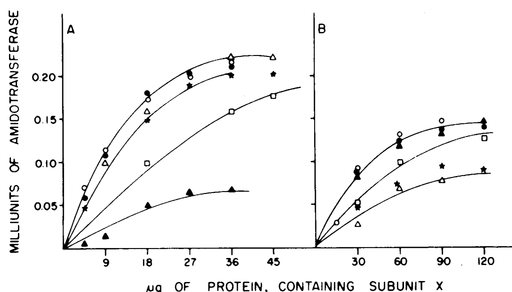


FIG. 6. *Titration of the aminotransferase subunits with the glutamine binding proteins from B. subtilis or B. alvei. (A) Increasing concentrations of subunit X from B. subtilis were added to a constant concentration of aminotransferase as follows: 0.10 mU from B. subtilis (●); 0.10 mU from B. licheniformis (O); 0.15 mU from B. alvei (▲), 0.13 mU from B. mascerans (△); 0.12 mU from B. coagulans (□); 0.13 mU from B. pumilus (★). Units of amidotransferase activity are plotted as a function of the concentration of protein containing subunit X. (B) Increasing concentrations of subunit X from B. alvei were added to a constant concentration of aminotransferase activity as follows: 0.16 mU from B. subtilis (●); 0.16 mU from B. licheniformis (O); 0.16 mU from B. alvei (▲); 0.17 mU from B. mascerans (△); 0.16 mU from B. coagulans (□); 0.14 mU from B. pumilus (★). The data are plotted as in (A).*

forms hybrid complexes of comparable efficiencies except for those involving subunit E from *B. alvei*. These latter hybrid complexes are at most 30% as effective as the homologous complexes.

## DISCUSSION

**Properties of the anthranilate synthase complexes.** The anthranilate synthase complex from at least six species of *Bacillus* is a readily dissociable enzyme complex composed of two nonidentical subunits. In contrast to this enzyme from *Salmonella typhimurium*, *Escherichia coli* and *Enterobacter aerogenes* (22), the glutamine-binding protein (subunit X) in these six species of *Bacillus* is not complexed with anthranilate-5-phosphoribosyl-pyrophosphate phosphoribosyl transferase (PR transferase) the second enzyme in the tryptophan biosynthetic pathway. This observation is based upon the observed molecular masses of the subunit X (from 15,000 to 24,000 daltons) and the reported molecular masses of PR transferase (45,000 to 57.000 daltons) from *B. subtilis*, *B. pumilus*, and *B. alvei* (7).

The anthranilate synthase complex from *B. alvei* appears to be unique in that complete dissociation of the EX complex was not observed during gel filtration in the absence of

TABLE 4. *Ratio of amidotransferase to aminotransferase activity*

| Source of subunit E | Source of subunit X | | | | | |
|---|---|---|---|---|---|---|
| | *B. subtilis* | *B. licheniformis* | *B. coagulans* | *B. pumilus* | *B. mascerans* | *B. alvei* |
| *B. subtilis* | *2.1* | 1.5 | 1.1 | 1.5 | 2.2 | 0.9 |
| *B. licheniformis* | 2.1 | *1.9* | 1.2 | 1.6 | 2.1 | 0.9 |
| *B. coagulans* | 1.0 | 1.5 | *1.4* | 1.5 | 1.5 | 0.8 |
| *B. pumilus* | 1.5 | | 0.8 | *1.6* | | 0.7 |
| *B. mascerans* | 1.7 | 1.5 | 1.0 | 1.1 | *2.3* | 0.5 |
| *B. alvei* | 0.5 | 0.4 | 0.4 | 0.3 | 0.4 | *0.9* |

glutamine unless the buffer contained 0.3 M KCl. Since none of the other five species demonstrated a requirement for an increased salt concentration to dissociate the complex, these data suggest that there is a quantitative difference in the stability of the EX complex from *B. alvei* as compared to the other EX complexes.

From these studies the EX complex from *B. alvei* has a molecular mass of 80,000 daltons. This value probably represents a weight average since some of the small component has been dissociated. Catena and DeMoss (2) reported that the EX complex was 90,000 daltons. Since their aggregate was isolated in the presence of 100 mM glutamine this may be a more accurate estimate than our value of 80,000 daltons. Hoch and Crawford (7) reported a value of 55,000 daltons for the aggregate; however, their elution profile after gel filtration illustrates a peak of aminotransferase activity with little if any amidotransferase activity. This trace amount of amidotransferase activity could represent some residual subunit X that is present in these fractions. We find that the isolated subunit E from *B. alvei* has a molecular mass of 58,000 daltons and suggest that the enzyme complex is an EX dimer and not an $E_2X_2$ tetramer as has been proposed (7).

The aminotransferase activity of subunit E from all species of *Bacillus* was increased in the presence of glycerol. This stimulating effect of glycerol has also been observed with aminotransferase (component I) from *S. typhimurium* (21) and *A. calcoaceticus* (19) and may reflect the instability of the aminotransferase components when they are not aggregated to their glutamine-binding proteins.

In the absence of glycerol, the rate of the aminotransferase activity of the various subunit E's was reduced by about two- to fourfold. Only the subunit E from *B. alvei*, however, was completely inactivated in 3 min. The addition of 2-mercaptoethanol led to a slow reactivation of the aminotransferase activity to a rate that was equivalent to the rate determined with 2-mercaptoethanol present at the beginning of the assay. This effect of 2-mercaptoethanol was not observed with the subunit E from any other species tested. These data suggest the presence of key sulfhydryl groups on the subunit E from *B. alvei* that are essential for aminotransferase activity and point to a fundamental difference between the large subunit from *B. alvei* and that found in the other species of *Bacillus*.

The aminotransferase subunit from all six species of *Bacillus* is inhibited by tryptophan, indicating the presence of the tryptophan binding site on this subunit. Under these assay conditions the large subunit from *B. alvei*, *B. mascerans*, and *B. pumilus* required 200 $\mu$M tryptophan to inhibit aminotransferase activity by 50%, whereas the aminotransferase from *B. subtilis*, *B. licheniformis*, and *B. coagulans* was inhibited 50% by 5 to 10 $\mu$M tryptophan. Catena and DeMoss (2) reported that the amidotransferase activity from *B. alvei* was inhibited 50% by 2.5 $\mu$M tryptophan. This apparent discrepancy may be related to an increased sensitivity of the EX complex as compared to the sensitivity of the free subunit E. A similar observation has been made with the anthranilate synthase from *Escherichia coli* (9). That is, the aminotransferase activity of the intact complex was more susceptible to inhibition by tryptophan than the isolated aminotransferase.

**Homologous and hybrid complexes.** The ratio of amidotransferase to aminotransferase activity in the homologous complexes at saturation was 1.4 to 2.3 for *B. subtilis*, *B. licheniformis*, *B. pumilus*, *B. mascerans*, and *B. coagulans*. For the most part, hybrid complexes formed between subunits from these species were as catalytically active as the homologous complexes.

In contrast, the anthranilate synthase complex from *B. alvei* never had a ratio that exceeded 1.0 under optimal assay conditions. Since the aminotransferase from this microorganism was the only one stimulated by 2-mercaptoethanol, it is not surprising that the hy-

brid complexes involving the subunit E from *B. alvei* were only 40 to 50% as effective as the homologous *B. alvei* complex.

The subunit X from *B. alvei*, however, does as well with the subunit E from *B. subtilis*, *B. coagulans*, and *B. pumilus* as it does with the subunit E from *B. alvei*. A similar observation has been made with heterologous complexes involving *Pseudomonas aeruginosa* and *B. subtilis* (16). That is, the glutamine-binding protein from *P. aeruginosa* formed a hybrid complex with the *B. subtilis* subunit E that was as effective as the homologous *B. subtilis* complex. However, the glutamine-binding protein from *B. subtilis* complexed with the aminotransferase subunit of *P. aeruginosa* to form a complex that was only 20% as efficient as the homologous *P. aeruginosa* complex (16). Since these enzyme complexes carry out the identical functions and since the catalytic sites must have been highly conserved through evolution, there are enough subtle differences to account for either formation of catalytically nonfunctional complexes or an inability to form a heterologous complex. The subunit E may be the key to complex formation because the two aminotransferase subunits that are most inefficient in heterologous complex formation are the subunits from *B. alvei* and *P. aeruginosa*. Both of these differ from the subunit of the other species of *Bacillus* in that the *B. alvei* component requires 2-mercaptoethanol for otpimal activity, whereas the *P. aeruginosa* component is not stimulated by glycerol (N. Patel, unpublished observations). Perhaps, the aminotransferase subunits from the other species of *Bacillus* are more flexible and can accomodate different types of subunit X.

Similar observations have been made with homologous and heterologous complexes of tryptophan synthase from *B. subtilis* (3, 4, 7). The activity of the $B_2$ subunit from *B. subtilis* is stimulated by the $\alpha$ subunit from *B. subtilis*, *B. pumilus*, *E. coli*, or *Pseudomonas putida*. There is, however, no stimulation of the $\alpha$ subunit from *B. subtilis* by the $B_2$ subunit from *E. coli* or *P. putida*. Thus, in *B. subtilis* the $B_2$ subunit of tryptophan synthase and the subunit E of anthranilate synthase may have less stringent requirements for forming hybrid complexes which are catalytically functional.

**Complementation as a taxonomic tool.** The various species of *Bacillus* have been divided into groups based upon their sporangial structure (20). *B. alvei* which is placed in the same sporangial class as *B. mascerans*, has certain unique characteristics that are not representative of its sporangial class. First, the DAHP synthase is not subject to sequential feedback inhibition (11). Second, it produces a constitutive tryptophanase (5). Third, the anthranilate synthase complex, although easily dissociated, requires a higher salt concentration to separate its components than do the anthranilate synthase complexes from other species of *Bacillus*. Finally, the aminotransferase subunit does not form hybrid complexes with the glutamine binding proteins from other species of *Bacillus* that are as efficient as the homologous complexes.

It does appear that the readily dissociable nature of the anthranilate synthase enzyme complex can be used as a reliable generic characteristic. In *B. subtilis* the rational for such a complex residues in the observation that subunit X is also involved in the biosynthesis of folic acid (12). Perhaps this too is a generic characteristic and may indicate the evolution of a glutamine-binding protein that is capable of functioning in various amidotransferase reactions. In any event, it appears that the study of structure, function and regulation patterns of anthranilate synthase will be a useful tool in the study of bacterial taxonomy.

#### ACKNOWLEDGMENTS

This research was supported by the National Science Foundation grant BO-38145 and by funds from the Research Corporation, Atlanta, Ga.

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