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# EFFECT OF STREPTOMYCIN ON THE ASSIMILATION OF AMINO ACIDS BY *BACILLUS SUBTILIS*

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Studies on the utilization of amino acids by *B. subtilis* during growth in the presence of streptomycin indicate that the antibiotic greatly influences the rate of amino acid uptake by the organism. There is, however, no selective action in the ultimate utilization of amino acids. The variation in the rate of assimilation of amino acids from the broth as induced by streptomycin may account for the difference in the rate of growth of bacterial cells. The antibiotic causes the accumulation of lesser number of free amino acids as compared to those accumulated in the streptomycin-untreated cells.

Earlier investigations of amino acid metabolism by GALE (*1*) and TAYLOR (*2*) have shown that the Gram-positive cocci are able to assimilate certain amino acids and concentrate them in the free state in the internal environment before metabolism into protein. GALE (*3*), GALE *et al.* (*4*) and GALE and FOLKES (*5*) also studied the assimilation of amino acids in bacterial metabolism. The utilization of amino acids by *Bacillus subtilis* during its production of an antifungal antibiotic was studied by MAJUMDAR and BOSE (*6*). But no information is available on the amino acid assimilation by *B. subtilis* in the presence of streptomycin. It has been previously observed in course of studies on the effect of streptomycin on the metabolism and growth of *B. subtilis* that streptomycin greatly interferes with the glutamic acid metabolism (*7*). In the present work an effort has been made to study the effect of streptomycin on the assimilation of amino acids by *B. subtilis*.

## EXPERIMENTAL

### *Fermentation experiments*

For studying the effect of streptomycin on the utilization of amino acids by *B. subtilis* strain $B_3$, a producer of an antifungal antibiotic, mycobacillin

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(*8*), the concentration of the antibiotic (1.5$\mu$g/ml of the broth) was so chosen as to cause 50% inhibition of growth at 48 hr. The bacterial strain $B_3$ was grown under the stationary process in a medium consisting of the following: Casamino acids 6.94 g; glucose 10.0 g; $KH_2PO_4$ 0.5 g; $MgSO_4 \cdot 7H_2O$ 0.2 g; $MnSO_4 \cdot 4H_2O$ 0.001 g; $FeSO_4 \cdot 7H_2O$ 0.001 g; water 1,000 ml; pH 7.0.

In a typical experiment, a 250 ml Erlenmeyer flask containing 50 ml of the medium was inoculated with 0.5 ml of cell suspension and incubated at 30° for different periods. In preparing the cell suspension used as inoculum, cells were grown in 50 ml of the same medium in a 250 ml Erlenmeyer flask for 24 hr at 30° by shake culture process and then centrifuged asceptically at 0–4°, washed and suspended homogeneously in 50 ml of saline. Growth was determined by taking dry weights of cells after drying at 70° overnight.

*Preparation of samples for paper chromatographic analysis*

The cells, after different periods of incubation, were separated from the culture fluid by centrifugation at 4°, washed twice with distilled water to remove traces of medium and dried in vacuo at 0–4°. For preparing the samples for estimation of free amino acids of cells, a measured amount of dried cells was extracted with 80 per cent aqueous ethyl alcohol (*9*) at 30° for 24 hr. The extract after separation of cell debris was evaporated to dryness and the residue was taken up in 3 ml of 10% aqueous isopropyl alcohol solution. The extract was then analysed for individual amino acids by two-dimensional paper chromatography. Both the control (without streptomycin) and streptomycin-treated culture broths, after separation of cells, were chromatographed directly.

*Estimation of amino acids by paper chromatography*

The amino acid composition of different samples was determined by descending two-dimensional paper chromatography (*10*) using water-saturated phenol and *n*-butanol: acetic acid: water (4 : 1 : 1) as the first and second solvent systems, respectively. After development with 0.1% (w/v) ninhydrin solution in acetone, the coloured spots were identified and separately eluted with 6.0 ml of 75% (v/v) ethyl alcohol containing 5 mg $CuSO_4 \cdot 5H_2O$ per ml (*11*). The colour intensities were then measured in a Klett-Summerson photoelectric colorimeter. The amounts of amino acids present were obtained from the standard curve of individual amino acid chromatographed under identical conditions.

## RESULTS

The early stages of growth (0–24 hr) in the control experiment (without streptomycin) is marked by rapid utilization of amino acids from the medium and by 48 hr when the culture shows a peak value of growth (Table 1) there is complete disappearance of all the amino acids with the exception of glutamic

Table 1. Effect of streptomycin on the growth of *B. subtilis*.

| Period of fermentation (hr) | Cell wt. (mg/10 ml) in the presence of | |
|---|---|---|
| | No streptomycin | Streptomycin (1.5 μg/ml) |
| 0 | 0 | 0 |
| 24 | 17.15 | 1.81 |
| 48 | 30.21 | 14.12 |
| 72 | 28.91 | 22.20 |
| 96 | 25.51 | 20.61 |

acid, cystine and leucines (Table 2). The utilization of amino acids by the organism in the presence of streptomycin is rapid between the period 24–72 hr (Table 2). All the amino acids except cystine, glutamic acid, tyrosine and leucines disappear completely from the medium at 72 hr when maximum cellular growth is attained. Proline disappears completely from the medium at 48 hr of bacterial growth in the presence or absence of streptomycin, but reappears only in the streptomycin-treated broth at 96 hr of fermentation.

The results in Table 3 indicate that streptomycin greatly influences the composition of free amino acids in the cell. Concomitant with the uptake of amino acids from the medium, free amino acids of cells show an increase. In general, the concentration of free amino acids (except proline and arginine) of cells in the control experiment (Table 3) show maximum values at 24 hr and decrease slowly during the remaining period of fermentation. The maximum accumulation of proline and arginine is, however, observed on the second day of growth. The free amino acid pool in *B. subtilis* grown in the presence of streptomycin (Table 3) is found to consist mainly of cystine, alanine, glutamic acid, aspartic acid, threonine, histidine, valine, leucines and proline, of which the first two show the highest concentration at 72 hr and the remaining seven show maximum accumulation at 48 hr.

## DISCUSSION

It has been previously observed that streptomycin interferes with glutamic acid metabolism of *B. subtilis* (*7*). An effort was, therefore, made to study the effect of streptomycin on the rate of assimilation of different amino acids from fermentation broth and their subsequent accumulation as free amino acids of cells. Streptomycin has been found to show an adverse effect on the assimilation of amino acids during the initial stage of bacterial growth. Although the rate of uptake of amino acids by *B. subtilis* in the presence and absence of streptomycin is different, there is no selective action in their ultimate utilization which was otherwise than was observed by PROOM and WOIWOOD (*12*) while studying with another strain of *B. subtilis*. The variation

Table 2. Utilization of amino acids from the fermentation broth by *B. subtilis* during growth.

| Amino acids | Concentration of amino acids (mg/10 ml) in the broth | | | | |
|---|---|---|---|---|---|
| | 0 hr | 24 hr | 48 hr | 72 hr | 96 hr |
| Cystine | 4.43 | 3.61 (4.35)* | 2.64 (2.24) | 3.10 (2.0) | 2.56 (0.86) |
| Aspartic acid | 5.80 | 4.54 (5.56) | — (1.66) | — (−) | — (−) |
| Glutamic acid | 10.80 | 8.99 (10.57) | 1.2 (6.06) | 0.86 (0.94) | + (+) |
| Serine | 2.90 | 0.97 (2.73) | — (1.51) | — (−) | — (−) |
| Glycine | 0.88 | 0.636 (0.831) | — (0.402) | — (−) | — (−) |
| Lysine | 6.75 | 5.58 (6.63) | + (3.43) | — (−) | — (−) |
| Threonine | 2.10 | 1.47 (1.74) | + (−) | — (−) | — (−) |
| Arginine | 1.40 | — (0.88) | — (0.63) | — (−) | — (−) |
| Alanine | 2.61 | 1.56 (2.48) | — (1.10) | — (−) | — (−) |
| Histidine | 3.20 | 1.72 (2.80) | — (1.31) | — (−) | — (−) |
| Tyrosine | 1.90 | 0.99 (1.75) | — (1.19) | — (0.64) | — (−) |
| Valine | 2.62 | 1.62 (2.48) | + (1.36) | — (−) | — (−) |
| Methionine | 0.71 | 0.44 (0.65) | — (0.19) | — (−) | — (−) |
| Leucines | 7.20 | 6.12 (6.96) | 2.15 (3.19) | + (0.91) | — (−) |
| Proline | 3.16 | 2.44 (2.84) | — (−) | — (−) | — (1.22) |
| Phenylalanine | 3.20 | 2.47 (2.83) | — (−) | — (−) | — (−) |

+, trace; —, absent.

* Results within parentheses indicate the amount of amino acid in streptomycin-treated broth.

in the rate of utilization of amino acids from the broth as induced by streptomycin may be due to the difference in the rate of growth of bacterial cells. Streptomycin greatly influences the composition of free amino acids in the cell. The antibiotic causes the accumulation of lesser number of free amino acids as compared to those accumulated in cells grown without the antibiotic. The free amino acid pool in *B. subtilis* cells grown in the presence of strepto-

Table 3. Free amino acid composition of *B. subtilis* during growth.

| Amino acids | Free amino acids (mg) per 100 mg dry cells after incubation for | | | |
|---|---|---|---|---|
| | 24 hr | 48 hr | 72 hr | 96 hr |
| Cystine | 0.06 (+)* | 0.056 (0.044) | 0.12 (0.064) | 0.028 (0.030) |
| Aspartic acid | 0.084 (+) | 0.070 (0.036) | 0.042 (0.024) | 0.034 (0.006) |
| Glutamic acid | 0.874 (+) | 0.626 (0.892) | 0.352 (0.394) | 0.068 (0.32) |
| Serine | 0.074 (−) | 0.066 (+) | 0.048 (+) | 0.036 (−) |
| Lysine | 0.072 (+) | 0.064 (+) | 0.056 (+) | + (−) |
| Threonine | 0.052 (+) | 0.048 (0.042) | 0.036 (0.029) | + (0.032) |
| Alanine | 0.126 (+) | 0.078 (0.066) | 0.092 (0.088) | 0.050 (0.072) |
| Histidine | 0.046 (+) | 0.02 (0.042) | 0.068 (0.026) | − (−) |
| Arginine | 0.022 (+) | 0.034 (+) | 0.028 (+) | − (−) |
| Valine | 0.062 (+) | 0.046 (0.058) | 0.028 (0.032) | 0.020 (−) |
| Methionine | 0.052 (−) | 0.046 (−) | 0.028 (−) | 0.020 (−) |
| Leucines | 0.090 (−) | 0.080 (0.074) | 0.048 (0.062) | 0.024 (+) |
| Proline | − (−) | 0.386 (0.24) | 0.142 (0.166) | − (−) |

+, trace; −, absent.

* Results in parentheses indicate the amount of free amino acids in *B. subtilis* grown in the presence of streptomycin.

mycin is found to consist mainly of cystine, alanine, glutamic acid, aspartic acid, threonine, histidine, valine, leucines and proline, of which the first two show the highest accumulation at 72 hr and the last seven show maximum levels on the second day of growth. Moreover, the maximum levels of free amino acids (except glutamic acid) of cells cultivated in the absence of streptomycin were higher than those of cells grown with the antibiotic. The lower accumulation of amino acids in streptomycin-treated cells may be due to the interference of the antibiotic with either the growth and metabolic activity of the organism or the permeation processes of the cells (*13*).

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