A Microorganism Exhibiting a Growth Requirement for Peptides[1]

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IN SEVERAL RECENT PUBLICATIONS (*2, 3, 4*), data have been presented concerning the relative growth-promoting action of amino acids and peptides for appropriate mutant strains of *Escherichia coli*. In the case of one mutant, a *prolineless* strain, better growth was obtained in a medium containing L-proline peptides than in a medium containing an equimolar concentration of L-proline (*3*). While this enhanced growth-promoting activity of the proline peptides may be due to their direct incorporation into the bacterial proteins, other possible explanations cannot be excluded. Thus, it may be that, in the course of the bacterial growth, a portion of the free proline is converted to products that are not growth factors for the mutant, and that this conversion is prevented by the linkage of the proline residue in a peptide. Also, the possibility must be considered that the presence of proline in peptide linkage aids more directly in the incorporation of this amino acid in the bacterial proteins, perhaps by "transpeptidation" reactions. Whatever the correct explanation turns out to be in the case of the *prolineless* mutant, it may be expected that the closer study of this and other bacterial strains that exhibit characteristic growth requirements for peptides of well-defined structure will throw valuable light on the metabolism of peptides in living systems.

In the present communication, the authors wish to report preliminary studies on a microorganism which, under specific experimental conditions, grows in the presence of the dipeptide L-leucylglycine, but does not grow in the presence of a mixture of L-leucine and glycine, and grows only poorly in the presence of L-leucine. This microorganism was obtained from an unsterilized solution of L-leucylglycine in 0.9-percent NaCl which had been kept at room temperature for several weeks.[2] When a loopful of the turbid dipeptide solution was plated out on nutrient agar, only a

TABLE 1

GROWTH RESPONSE OF BACTERIAL STRAIN SF

Test compound	Growth* in saline			Growth* in saline-glucose		
	48 hr	72 hr	96 hr	48 hr	72 hr	96 hr
L-Leucine	0.009	0.020	0.026	0.032	0.027	0.018
D-Leucine	—	—	—	—	—	—
DL-Leucine	—	—	—	—	—	—
L-Leucylglycine	0.085	0.122	0.146	0.069	0.127	0.130
L-Leucine + glycine	—	—	—	—	—	—
D-Leucylglycine	0.004	0.004	0.004	—	0.002	0.006
L-Leucylglycylglycine				0.032	0.071	0.131
L-Leucylglycine + glycine	—	—	—	—	—	—
Glycyl-L-leucine	—	—	0.002	0.018	0.023	0.027
Glycyl-L-leucine + glycine	—	—	—	—	—	—
Acetyl-L-leucine†	—	—	—	—	—	—
L-Phenylalanylglycine				—	—	—
L-Phenylalanine	—	—	—	0.017	0.021	0.015
L-Phenylalanine + glycine				—	—	—
Glycylglycine	—	—	—	—	—	—
Glycine	—	—	—	—	—	—
L-Asparagine	—	—	—			
L-Alanine	—	0.062	0.080	0.032	0.041	0.042
L-Glutamic acid†	0.052	0.067	0.072	0.029	0.039	0.044
L-Isoleucine	—	—	—	—	0.012	0.018
L-Valine	0.148	0.213	0.228	0.110	0.098	0.069
NH_4Cl†				0.012	0.014	0.009
NH_4Cl† + glycine	—	—	—	—	—	—

* The extent of bacterial growth is recorded as the optical density of the culture, where density = 2 − log galvanometer reading. A dash indicates the absence of measurable growth.

† Neutralized with NaOH before use.

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single type of colony appeared after 24 hours at 30°. A subculture of one of these colonies served as the source of the organisms used in the tests reported below. For convenience, the isolated bacterial strain will be termed "SF."

Examination of the isolate showed it to be a short, nonmotile rod which is Gram-negative and occurs singly or in pairs in Gram-stained preparations.[3] In nutrient broth containing glucose, lactose, or sucrose, it does not cause the formation of acid or gas; after 3 days, such test solutions have a pH of 8 or higher. It turns litmus milk alkaline and liquefies gelatin. The organism has been tentatively classified, therefore, as a member of the genus *Alcaligenes*.

The ability of strain SF to grow in saline solutions containing a variety of amino acids or peptides is shown in Table 1. In performing the tests, Evelyn colorimeter tubes, containing 8 cc of the test medium, were incubated at 30°, and the extent of bacterial growth was measured at intervals in an Evelyn colorimeter with filter No. 540. As is indicated in the table, one series of tests was conducted in the presence of amino acids or peptides as the sole source of carbon and nitrogen in the medium, while in a second series glucose was added as an additional potential source of carbon. When 0.9-percent NaCl alone was used as the medium, the concentration of each test substance was 0.1 M; when saline was supplemented with glucose (0.17 M), the concentration of the test substances was lowered to 0.05 M. The inocula for the tests consisted of a drop of an aqueous suspension of cells taken from a peptone-yeast extract-agar slant which had been incubated for 24 hours at 30°. Under these conditions, visible growth was not evident until 24 hours after inoculation, and significant readings with the Evelyn colorimeter were not obtained until 36–48 hours after inoculation.

The data in Table 1 show that strain SF grew equally well when L-leucylglycine was present in the medium in combination with glucose or when the peptide represented the sole carbon source for growth. No growth was noted when L-leucine and glycine were present, and the presence of L-leucine promoted the growth only slightly. It was observed that, in every case in which it was tested, glycine exerted an inhibitory effect upon the growth of strain SF. It would appear, however, that glycine is not bactericidal, since the same number of viable cells could be recovered, after 5 days' incubation, from a medium containing glycine as from a medium containing DL-leucine, in neither of which was there any visible growth. The complete failure of strain SF to grow in the presence of DL-leucine suggests that the D-isomer may act as a growth inhibitor. It is also of interest that, with D-leucylglycine, there was noted only slight growth, which may be due to the presence of a small amount of the L-form of the peptide in the commercial preparation used for these experiments.

L-Leucylglycylglycine produced a growth response similar to that noted with L-leucylglycine. Glycyl-L-leucine and acetyl-L-leucine, however, showed little, if any, growth-promoting activity. It would appear, therefore, that significant growth of strain SF in the presence of leucine peptides is favored only when the L-leucine residue is present at the amino end of an unsubstituted peptide. When the L leucine residue in L-leucylglycine was replaced by that of L-phenylalanine or of glycine, the resulting L-phenylalanylglycine or glycylglycine was inactive in promoting bacterial growth. Although further studies of the specificity of the peptide requirements of strain SF are necessary, it may be concluded from the above results that the presence in the medium of a dipeptide *per se* does not satisfy the growth requirement.

The data in Table 1 show that the organism does not have a specific requirement for leucyl peptides since L-alanine and L-glutamic acid permitted fair growth and L-valine served as a better growth factor than even L-leucylglycine. On the other hand, L-asparagine did not promote bacterial growth, and a medium containing NaCl, NH_4Cl, and glucose gave only a slight growth response. However, other tests in which the basal medium usually employed by us for *Escherichia coli* (i.e., a mixture of inorganic salts, including NH_4Cl and NH_4NO_3, glucose, and a small amount of asparagine (*1*) was used, showed that strain SF is capable of growth when glucose is the source of carbon, and ammonia and nitrate are the principal sources of nitrogen. The addition of 0.1 mM of glycine per cc of this medium completely inhibited the growth of strain SF, while a concentration of 0.001 M glycine retarded growth appreciably.

[3] The authors are indebted to Dr. C. F. Robinow for his valuable advice in the characterization of the bacterial strain.

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