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Effect of Glycine-Peptides on the Growth of *Leuconostoc mesenteroides*

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In general, the effect of peptides on the growth of microorganisms is relatively little known so far. Nevertheless, many proofs have been obtained showing that, at least in the microbiological determination of amino acids, peptides are noteworthy growth factors, for in certain cases they can replace the amino acid to be determined.

As early as 1943 Kuiken *et al.*[1], using *Lactobacillus arabinosus* 17—5 as a test organism, found that DL-leucylglycine and glycyl-L-leucine possess 80—100 % of the activity of leucine. Later, Schweigert [2] noted with the same organism that DL-leucylglycylglycine, glycyl-DL-leucylglycine and glycyl-glycyl-DL-leucine have a leucine-activity ranging from 30 to 90 % depending on peptide. On the contrary, Hegsted [3] failed to demonstrate any valine-activity with *L. arabinosus* in three different isomers of benzoylvalylvaline. The investigations of Stokes *et al.*[4] with *Streptococcus faecalis* and of Lewis and Olcott [5] with *L. arabinosus* 17—5 also suggest that the peptides are generally inactive if one component in them is an acid other than amino acid.

Apart from these few separate observations, only Ågren [6,7], Krehl and Fruton [8] have examined in detail the effect of synthetic peptides on the growth of lactic acid bacteria and Simmonds *et al.*[9–12] on the growth of *Escherichia coli*.

According to the studies of Ågren [6] with *Str. faecalis* R, *L. delbrückii* LD5, and *L. casei*, these test organisms are able to utilize dipeptide-valine and dipeptide or tripeptide-leucine. However, the activity of these peptides depends decidedly on the position of valine and leucine in the peptide. Krehl and Fruton [8] also arrived at similar results with *L. arabinosus* 17—5 and *Str. faecalis* R when examining the activity of di- and tri-peptides. Likewise, the later studies by Ågren [7] with 10 different strains of lactic acid bacteria and

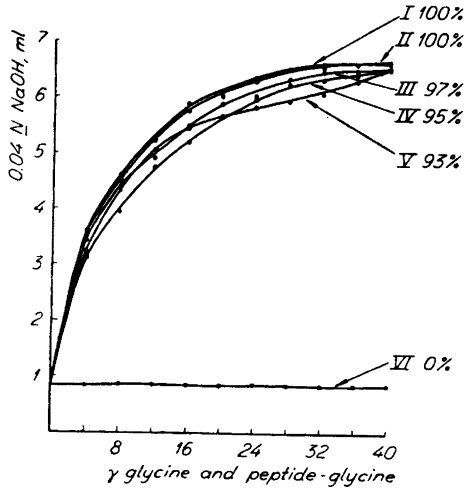

Fig. 1. The effect of glycine peptides on the growth of Leuconostoc mesenteroides P-60.

I glycyl-L-leucine
II glycine
III glycyl-glycine
IV DL-alanyl-glycine
V DL-leucyl-glycine
VI hippuryl-glycine and sarcosine

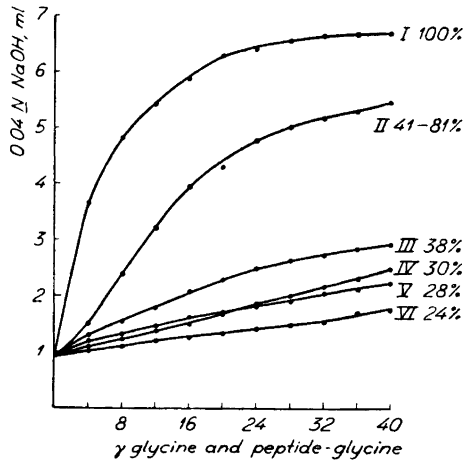

Fig. 2. The effect of glycine peptides on the growth of Leuconostoc mesenteroides P-60.

I glycine
II glycyl-L-tyrosine methylester
III triglycine ethylester
IV pentaglycine ethylester
V glycyl-DL-phenylalanine methylester
VI glutathione

8 leucine-peptides support the concept that the activity of peptides depends on the position of the indispensable amino acid in the peptide as well as on the order of other amino acids in respect to this amino acid. According to Ågren the activity of peptides may vary depending on the composition of the basal medium.

Simmonds *et al.* [9–12] report that mutants of *E. coli*, too, are able to utilize peptides in addition to amino acids. The activity of peptides in these bacteria, as well as in lactic acid bacteria, depends on the position of the indispensable amino acid in the peptide. Of special interest is the exceptional activity of proline-peptides which is greater than that of the equimolar free proline.

The present paper deals with the effect of 10 different glycine-peptides and sarcosine on the growth of *Leuconostoc mesenteroides* P-60 (obtained from Prof. E. E. Snell, University of Wisconsin, U. S. A.).

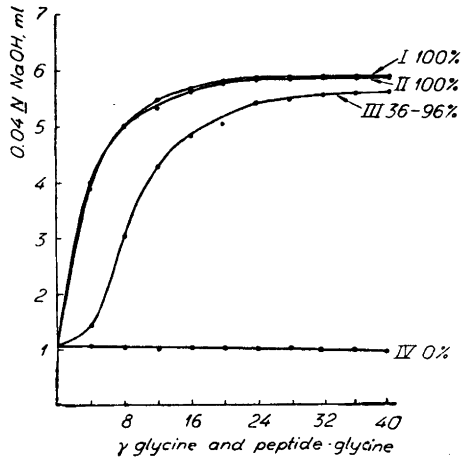

Fig. 3. The influence of glycine, benzoylglycine, benzoylglycylglycine and benzoic acid on the growth of Leuconostoc mesenteroides P-60.

Compound	Amounts of unbound and peptide-glycine in γ *											Glycine-activity %
	0	4	8	12	16	20	24	28	32	36	40	
	0.04 *N* NaOH in ml											
Benzoic acid + glycine	1.08	3.90 97.1	5.01 100.0	5.50 104.5	5.64 100.0	5.77 98.8	5.85 100.0	5.91 101.0	5.93 99.9	5.95 100.7	5.94 100.1	100
Glycine	1.08	4.02	5.01	5.30	5.64	5.84	5.85	5.85	5.94	5.90	5.93	100
Hippuric acid	1.09	1.43 35.7	3.03 60.5	4.30 81.2	4.84 85.8	5.03 86.2	5.46 93.3	5.54 94.7	5.60 94.2	5.65 95.8	5.67 95.6	36—96
Benzoic acid	1.08	1.07	1.04	1.00	1.03	1.01	1.01	1.04	1.00	1.01	1.00	0

* The amounts of benzoic acid were respectively in γ: 0, 5.5, 11.0, 16.5, 22.0, 27.5, 33.0, 38.5, 44.0, 49.5, 55.0.

EXPERIMENTAL

The experimental technique introduced by Henderson and Snell[13] was chiefly employed in the activity determinations. The quantity of DL-alanine was one fifth of the alanine concentration of the original basal medium. The investigation comprised 5 series of experiments grown at different times. Each series included standard solutions of glycine, the glycine concentration of which was increased by 4 gammas from 0 to 40 gammas, and different peptide solutions, in which the peptide-glycine concentration corresponded to the quantity of equimolar free standard glycine (the quantity of glycylglycine was, however, the same as that of glycine). The duration of

growth was 72 hours in each series and the growth temperature 37° C. Titrations were carried out with 0.04 *N* NaOH electrometrically by using quinhydrone electrode.

The results are presented by curves in Figs. 1—3.

RESULTS

It can be seen from the results that the glycine-activity of DL-alanylglycine, glycylglycine, glycyl-L-leucine, DL-leucylglycine, and glycylglycine has been 100 % or a little less. The position of glycine in the dipeptides has not much affected the activity. On the other hand, the glycine-activity of glycyl-DL-phenylalaninemethylester, glycylglycylglycine-ethylester and glycylglycyl-glycylglycylglycine-ethylester has only about one third of the activity of free equimolar glycine. Glycyl-L-tyrosinemethylester behaves in a very interesting way — its glycine activity rises from 41 to 81, while the peptide-glycine concentration rises from 4 to 40 gammas. A corresponding rise is detectable also with benzoylglycine (hippuric acid), thus — contrary to former findings — peptide can be active, although one component in it is an acid other than amino acid. With all other peptides, instead, the peptide-glycine activity is fairly independent of the concentration of peptide. The lowest activity, a little more than 20 %, was noted with glutathione glycine. Benzoylglycylglycine was entirely inactive and so was sarcosine, too. Benzoyl-glycylglycine and benzoic acid had no inhibitory action on the glycine activity.

SUMMARY

The suitability of different glycine peptides for the glycine nutrition of *Leuconostoc mesenteroides* P-60 has been examined. Most of the peptides studied possessed a distinct or a very strong glycine-activity. The mechanism of the growth effect of glycine-peptides will be discussed in another paper.

We wish to express our cordial thanks to Prof. Dr. E. E. Snell for kindly sending at our disposal the pure cultures of lactic acid bacteria and the necessary reagents for the basal medium.

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