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On the Growth Stimulating Activity and Utilization of Phosphopeptone and Phosphorylated Amino Acids on some *Lactobacilli*

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In recent experiments from this laboratory [1] it was found that phosphopeptone prepared from casein was a very active growth stimulator for *L. casei* and *L. delbrückii* cultivated in a complete synthetic medium. In the light of current information on the impermeability of cell membranes to most phosphorylated intermediates [2] we were interested in a more detailed investigation of the utilization and growth stimulating activity of phosphopeptone and some phosphorylated amino acids.

EXPERIMENTS AND RESULTS

The microbiological procedures have previously been described in detail [3,4]. The total volume of medium was 5 ml per tube. The original medium (OM) of Steele *et al.*[5] was also used in the modified form (MM) recently described [6]. The microorganisms were *L. casei* (7469), *L. mesenteroides* P—60 (8042), and *L. delbrückii* (9595). Phosphopeptone as a rather complicated mixture of phosphopeptides [7] was obtained through the courtesy of professor O. Mellander, prepared according to the method outlined by him [8]. Phosphoserine and phosphothreonine were crystallized samples prepared in this laboratory from casein [9-11]. A synthetic sample of phosphothreonine [12] was also used. Phosphoglycine was prepared according to Winnick and Scott [13] as the magnesium salt.

In Table 1 a comparison is made of the growth effects of phosphopeptone on the three microorganisms cultivated in the medium of Steele *et al.*[5]. When compared with the corresponding control series where phosphopeptone was not present in the medium it is quite evident that *L. casei* and *L. delbrückii* were stimulated to a conspicous growth and acid production even by the addition of 0.1 mg of phosphopeptone per ml of medium. *L. mesenteroides* was not influenced at all by these additions.

Table 1. The growth stimulating effect of phosphopeptone on some lactobacilli. Addition of 1 mg and 0.1 mg of phosphopeptone per ml of medium.

Microorganism	Growth after							
	12 hours		24 hours		48 hours		72 hours	
	1 mg	0.1 mg	1 mg	0.1 mg	1 mg	0.1 mg	1 mg	0.1 mg
L. delbrückii	67*	25*	335*	118*	415*	280*	415*; 10.5**	315*; 7.6**
Control	7		29		83		129 ; 2.1	
L. casei	92	15	265	125	350	275	350 ; 10.0	300 ; 8.1
Control	7		20		85		170 ; 3.0	
L. mesenteroides P-60	230	224	290	283	350	350	365 ; 7.7	360 ; 7.7
Control	223		270		330		340 ; 7.6	

* Given as scale readings on the Klett-Summerson photoelectric colorimeter. The readings 0 corresponds to 100 % transmission. The values are mean values from three tubes and have been corrected for the blank values.

** Given as ml of 0.1 *N* NaOH to titrate 5 ml of final solution. The values are mean values from three tubes and have been corrected for the blank titrations.

Experiments were next made to find out if the esterified phosphate of the peptide mixture could be utilized when the inorganic phosphate, which normally must be present in the medium, was excluded. The results from a typical series are given in Table 2. There seemed to be a slight effect with 1 mg samples of phosphopeptone on the growth of *L. casei*. Calculation shows that the original medium contains 12.5 mg of potassium phosphate per 5 ml of

Table 2. The growth stimulating effect of phosphopeptone on L. casei and L. mesenteroides P-60 when inorganic phosphate was excluded from the medium. Addition of 1 mg and 0.1 mg per ml of medium.

Microorganism	Growth after							
	12 hours		24 hours		48 hours		72 hours	
	1 mg	0.1 mg	1 mg	0.1 mg	1 mg	0.1 mg	1 mg	0.1 mg
L. casei	1*	1*	1*	1*	11*	7*	24*; 0.6**	8*; 0**
Control without any phosphate at all	0		1		6		9 ; 0	
L. mesenteroides P-60	2	2	2	3	5	5	7 ; 0	7 ; 0
Control without any phosphate at all	2		3		3		3 ; 0	

* Given as scale readings on the Klett-Summerson photoelectric colorimeter. The readings 0 corresponds to 100 % transmission. The values are mean values from three tubes and have been corrected for the blank values.

** Given as ml of 0.1 *N* NaOH to titrate 5 ml of final solution. The values are mean values from three tubes and have been corrected for the blank titrations.

medium which corresponds to 2.4 mg of phosphorus. From 5 mg of calcium phosphopeptone only 0.2 mg of phosphorus will be available. This inadequate supply may be one explanation to the low effect observed, another may be the absence of potassium in the medium. Both possibilities will be further investigated.

The growth stimulating effects of some phosphorylated amino acids when added to the original medium of Steele *et al.*[5] or to the corresponding improved medium [6] is demonstrated by the figures of Table 3. When compared with the corresponding controls phosphoserine seemed to have a slight growth stimulating effect. This medium probably contains too little serine since the growth effect disappeared in the improved medium. Here both phosphoserine and phosphothreonine had a slight growth-inhibiting activity. If this is a parallel to the recent find of azaserine as a growth inhibitor of tumours and microorganisms [14] can at present not be decided. Of interest is that phosphoglycine which contains nitrogen-bound phosphorus had a slight stimulating effect on the growth and lactic acid production after 24 hours of incubation. With regard to the instability of this compound [13] it was aseptically added to the sterilized medium. Phosphoglycine had no growth stimulating effect after 4 or 8 hours of incubation. From these experiments the conclusion can be drawn that phosphopeptone does not stimulate the growth of lactobacilli through a preliminary digestion in the medium with the formation of the free phosphorylated amino acids.

Since these amino acids did not stimulate growth it was of interest to test their ability to replace the corresponding unphosphorylated amino acids. *L. mesenteroides* P-60 was used as test organism. In Fig. 1 some typical results with phosphoglycine are given. The samples of phosphorylated amino

Table 3. The effects of some phosphorylated amino acids on the growth of L. c a s e i. Additions of 1 mg and 0.1 mg of amino acid per ml of medium.

Amino acid	Growth after							
	24 hours		48 hours		72 hours			
	1 mg	0.1 mg	1 mg	0.1 mg	1 mg		0.1 mg	
L-Phosphoserine [1]	14*	14*	74*	48*	159*;	2.8**	116*;	1.9**
DL-Phosphothreonine [1]	15	16	57	58	117 ;	1.9	118 ;	1.8
Phosphoglycine [1]	59	22	—	—	—	—	—	—
Control [1]	16		17		124 ;	2.1		
L-Phosphoserine [2]	20	14	152	138	252 ;	5.5	224 ;	5.1
DL-Phosphothreonine [2]	21	16	171	128	240 ;	5.4	220 ;	4.6
Control [2]	25		203		284 ;	5.9		

[1] Medium of Steele *et al.* (4).

[2] Improved medium for *L. casei* (5).

* Given as scale readings on the Klett-Summerson photoelectric colorimeter. The readings 0 corresponds to 100 % transmission. The values are mean values from three tubes and have been corrected for the blank values.

** Given as ml of 0.1 *N* NaOH to titrate 5 ml of final solution. The values are mean values from three tubes and have been corrected for the blank titrations.

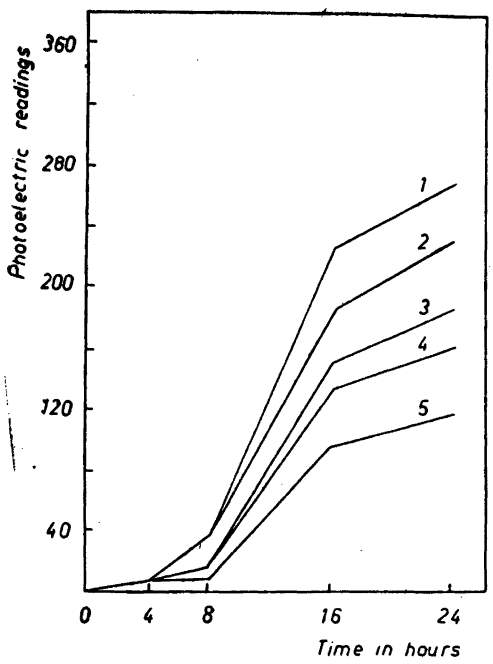

Fig. 1. Capacity of phosphorylated glycine to replace free glycine in the growth of L. mesenteroides P-60. 1. 20 μg of glycine. 2. 65 ug of Mg-phosphoglycine. 3. 5 μg of glycine. 4. 17 μg of Mg-phosphoglycine. 5. Control.

acids in this and the following experiments were calculated to yield the same amounts of unphosphorylated amino acids as the corresponding levels of free amino acids. From Fig. 1 it is evident that phosphoglycine is not utilized to the same degree as free glycine. The results with phosphoserine and phosphothreonine are given in Table 4. There is a striking difference between the utilization of phosphoserine and phosphothreonine. The first compound can

Table 4. The utilization of phosphoserine, phosphothreonine and the corresponding free amino acids by L. mesenteroides P-60. Incubation time 72 hours.

Amino acid	Lactic acid production by adding to the medium in μg			
	20	10	5	2.5
L-serine	11.5*	10.4*	9.5*	8.2
DL-serine (added in double amounts)	13.7	12.5	11.3	10.4
L-phosphoserine	11.8	10.7	10.0	8.8
L-threonine	9.4	5.2	2.8	0.9
DL-threonine (added in double amounts)	9.4	5.2	2.8	1.0
L-phosphothreonine	1.1	0.4	0.2	0.1

* Given as ml of 0.1 *N* NaOH to titrate 5 ml of final medium. The values are mean values from three tubes and have been corrected for the blank titrations.

replace serine as an essential amino acid for the growing microorganism while phosphothreonine can not be substituted for threonine. At present time it can not be decided if this dissimilarity originates in differences in cell permeability or in initial dephosphorylation of the two compounds in the medium. The series with racemic amino acids show as could be anticipated that *L. mesenteroides* can utilize D-serine but not D-threonine.

SUMMARY

Phosphopeptone is a very active growth stimulator for *L. casei* and *L. delbrückii* but not for *L. mesenteroides* P-60. This growth stimulating effect is not produced by the free phosphorylated amino acids of phosphopeptone. Phosphopeptone can to a certain degree be used in place of inorganic phosphate by *L. casei* but not by *L. mesenteroides*. When serine or threonine is excluded from the medium, the last mentioned microorganism can completely utilize phosphoserine for growth but only to a very slight degree phosphothreonine. Phosphoglycine, also, can not completely replace glycine for growth.

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