OBSERVATIONS ON THE METABOLISM OF PENTOSES IN *ESCHERICHIA COLI*

by

U. Z. LITTAUER* AND B. E. VOLCANI

The Weizmann Institute of Science, Rehovoth (Israel)

and

E. D. BERGMANN

Scientific Department, Israel Ministry of Defence (Israel)

It has been demonstrated that the oxidation or fermentation of pentoses by *E. coli*[20,4,6,1], *Aerobacter cloacae*[11] and *Lactobacillus pentosus*[21,22,24] are adaptive processes. The presence of pentose-isomerase in *E. coli*[8,9**] and other bacteria[29,18], tends to show that part of the adaptation to the sugar involves the conversion of the aldopentoses into the isomeric keto-sugars.

The present study deals with the effects of the media, more especially the presence of certain sugars in them, on the oxidation, phosphorylation and isomerization of pentoses by *E. coli*.

EXPERIMENTAL

Materials. The carbohydrates used were high grade commercial preparations. Xylulose was prepared according to the procedure of LEVENE AND TIPSON[25] from its monoacetone compound, kindly supplied by the laboratory of Prof. A. R. TODD, Cambridge. Its purity was assayed chromatographically, using phenol saturated with water as the solvent and spraying with aniline phthalate reagent[30]. Only one spot was observed, with a R_F value 0.59[34]. Adenosine triphosphate (ATP), barium salt, a product of Delta Chemical Works, was converted into the sodium salt before use. Triphosphopyridine nucleotide (TPN) (80%) was obtained from the Sigma Chemical Company. Phenazine methosulphate was prepared from phenazine, according to HILLEMANN[17].

Preparation of resting cells and extracts. Cultures of *E. coli* K-12 were maintained on 0.5% Difco yeast extract agar slants, with the addition of 0.2% of the corresponding sugar. Cells from an 8 hour slant culture were inoculated into a 4-liter Erlenmeyer flask containing 1.5 liters of either a mineral medium[4] with 0.2% of the various sugars or 0.5% Difco yeast extract with 0.05% of the various sugars. The cultures were aerated vigorously and incubated for 16 to 24 hours at 37°; the cells were harvested in a Sharples supercentrifuge and washed twice with chilled 0.9% KCl solution.

Cell-free extracts were prepared by grinding the cells with alumina powder (A-301, Aluminum Company of America), as described by MCILWAIN[27]. The ground cell paste was triturated with 5 ml cold 0.9% KCl solution per gram of original wet cells and then centrifuged in a Servall type SS-1 centrifuge at 23,000 × *g* for 30 minutes at 2°. The resulting clear viscous yellow solution was stored at —20°.

Manometric measurements. Oxygen uptake was measured by the conventional Warburg manometric techniques[33]. The phosphorylation of the various sugars was determined by the manometric method of COLOWICK AND KALCKAR[10].

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** The work on xylose-isomerase appeared after the present investigation had been completed.

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Pentose-isomerase. Enzymic activity was measured according to COHEN[8] using the cysteine carbazole colour reaction[13], with the Klett-Summerson colorimeter at 540 mμ (filter No. 54).

Pentose determination. The orcinol method of DRURY[11] was used and readings were taken at 670 mμ with the Beckman DU Model spectrophotometer.

RESULTS

Oxidation

Resting cells previously grown in a mineral medium were compared with cells grown in yeast extract medium, both media containing glucose or various pentoses as the carbohydrate source.

Mineral medium. Glucose-grown cells oxidized glucose rapidly, but oxidized L-arabinose and D-xylose only after a lag period of 40 and 80 minutes, respectively, as has also been reported by COHEN[4,6]. With D-ribose, the lag period lasted four hours, during which some oxidation occurred. Xylulose and D-arabinose were not attacked (Fig. 1A).

Cells grown on the various pentoses rapidly oxidized the corresponding pentoses and D-ribose, but D-xylose and L-arabinose only after a lag period. On D-arabinose, bacteria failed to grow.

Xylulose was oxidized only by xylose-grown cells, at a rate much lower than that of xylose (the oxygen consumption after 20 minutes was 118 μl for xylose, and 16 μl for xylulose) (Fig. 1B).

The inability of glucose-grown cells to oxidize xylulose, though they oxidized xylose, was rather unexpected. Also, when xylulose was added to resting cells after the oxidation of xylose was just completed, no further oxidation occurred; xylulose (1.5 μM) was not oxidized when incubated together with xylose (1.5 μM) or glucose (1.5 μM).

Yeast extract medium. As the oxidation of a pentose in a mineral medium appeared to depend entirely upon the presence of that sugar in the medium, it was of interest to determine whether this correlation is altered by growing the cells in a richer medium, such as yeast extract, containing preformed metabolites.

Resting cells obtained from a medium containing 0.5% yeast extract oxidized ribose immediately, but L-arabinose, D-xylose and D-xylulose were oxidized only after a prolonged lag period (Fig. 1C). Resting cells obtained from a medium containing 0.5% yeast extract *and 0.05% glucose* oxidized ribose and D-xylulose immediately, and L-arabinose and D-xylose only after prolonged lag periods. D-Arabinose was not oxidized under any conditions (Fig. 1D).

Oxidation by cell-free extracts. In order to determine whether the fact that xylose-grown cells oxidized xylulose more slowly than xylose is due to a permeability barrier, experiments were carried out with cell-free extracts. Contrary to the results with resting cells, extracts obtained from xylose-grown cells oxidized xylulose at a higher rate than xylose (Table I). The extracts, kept for several days at —20°, partially lost the ability to oxidize xylose, while xylulose-oxidation remained practically unaffected.

Since glucose-grown cells oxidized xylose rapidly after adaptation, but failed to attack xylulose even after adaptation to xylose, it was interesting to examine the ability of *extracts* of such cells to oxidize xylulose. The cells were washed and divided into three portions of 1 g each. From the first, a cell-free extract was prepared, and

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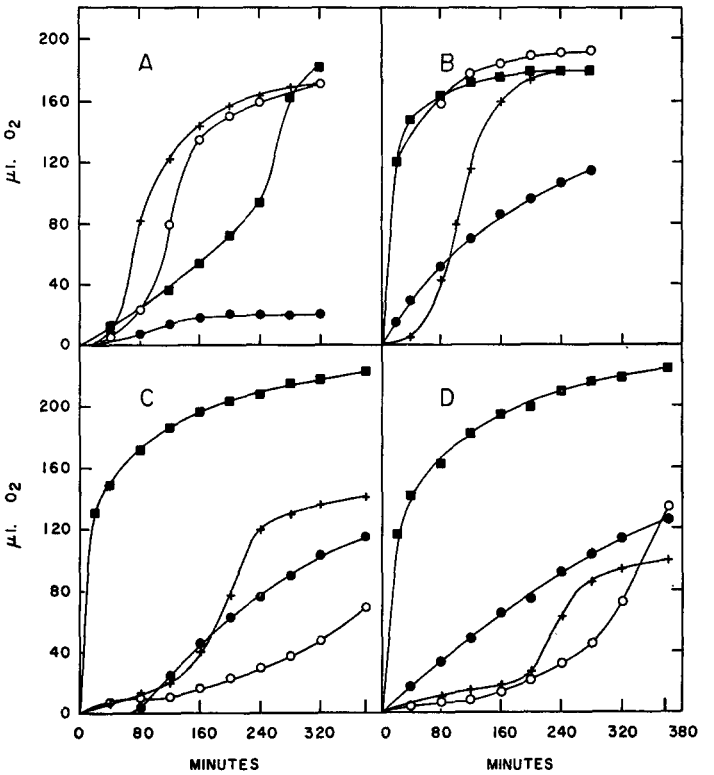

Fig. 1. Oxidation of pentoses by cells grown on various media. Each flask contained, in the main well, 1.1 ml of 0.1 *M* K-Na phosphate buffer pH 6.8, and 0.5 ml bacterial suspension (25 mg wet cells); in the side arm 0.2 ml of the substrate solution (3 μM). The center well received 0.2 ml 20% KOH. Final volume 2.0 ml; gas phase air; temperature 30°. (+——+) L-Arabinose, (■——■) D-ribose, (○——○) D-xylose, (●——●) D-xylulose. A. Mineral medium + glucose. B. Mineral medium + xylose. C. Yeast extract. D. Yeast extract + glucose. Endogenous values subtracted from each curve.

the oxidation of xylose and of xylulose was determined. The second was suspended in 72 ml 0.1 *M* phosphate buffer pH 6.8 and 120 μM xylose in a final volume of 80 ml. The third, in a similar suspension but without xylose, served as a control. The mixtures were shaken in a 250 ml Erlenmeyer flask, at 30° for 4 hours, the time required—according to independent experiments in Warburg vessels—for the complete oxidation of xylose. After collection, the cells were washed twice with 40 ml cold 0.9% KCl solution and extracts were made. Samples of the various preparations were taken for manometric determination of the rate of xylose oxidation.

Table I shows that after adaptation to xylose the extract oxidized xylulose to a considerable extent, while xylose was almost unaffected; it was oxidized, although slowly, when the enzyme concentration was doubled. This very low rate of xylose oxidation was not unexpected since in resting cells the Q_{O_2} for xylose in the course of the adaptation to the sugars had been small.

Pentose-isomerase. The presence of pentose-isomerase was established in extracts obtained from cells grown in mineral media containing various sugars. Table II demonstrates the specificity of the isomerase to the pentose in which the cells were grown. No isomerase, however, was found in ribose- and glucose-grown cells.

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TABLE I

OXIDATION OF XYLOSE AND XYLULOSE BY RESTING CELLS AND EXTRACTS, AND FORMATION OF ISOMERASE

Cells grown on	*Substrate*	Q_{O_2}*	*Oxidation by extracts* $\mu l\ O_2$**		*Xylose-isomerase*** G.R.
			60 min	*120 min*	
Xylose	Xylose	354	49	102	82
	Xylulose	48	76	108	
Glucose	Xylose	0	10	8	7
	Xylulose	19	4	9	
Glucose, incubated with xylose	Xylose	112	5	5	32
	Xylulose	14	23	48	
Glucose, incubated without xylose	Xylose	0	11	12	5
	Xylulose	0	9	14	

* Oxygen consumption of resting cells calculated per 25 mg wet cells.

** Each Warburg flask contained in the main compartment 0.5 ml extract (equivalent to 100 mg wet cells), 0.1 ml (0.1 mg) TPN and 0.1 ml $MgCl_2 \cdot 6H_2O$ (0.06 *M*). One side arm contained 0.3 mg phenazine methosulphate in 0.2 ml of tris buffer pH 7.0; in the second side arm: 0.1 ml Na-ATP (9 μM) and 0.6 ml (9 μM) substrate. The center well contained 0.2 ml 20% KOH. Final volume 2.0 ml; gas phase air; temperature 30°.

*** Values given as corrected galvanometer readings (*cf.* Table II).

From all figures, the endogenous values have been subtracted.

TABLE II

PENTOSE-ISOMERASES IN EXTRACTS FROM CELLS GROWN ON VARIOUS SUGARS

Substrate	*Sugar in media**			
	D-*Xylose*	D-*Ribose*	L-*Arabinose*	*Glucose*
D-Xylose	82	2	0	7
D-Ribose	5	0	0	4
L-Arabinose	4	0	52	6
D-Arabinose	5	3	4	6

* Mineral media was used.

Each test tube contained: 0.25 ml extract (equivalent to 50 mg wet cells); 6 μM substrate, 0.6 ml 0.05 *M* tris buffer pH 7.5; final volume 2.0 ml. Incubation temperature 30°. Aliquots of 0.1 ml were taken at 0 and 60 min and added to 0.9 ml 0.1 *N* HCl. Values given as galvanometer readings were determined by subtracting the reading of the endogenous product from the complete system, and correcting for similar differences at zero time.

Phosphorylation

The phosphorylation of xylose by *L. pentosus* has been investigated by LAMPEN[23,24] and MITSUHASI AND LAMPEN[29]. In *E. coli*, phosphorylation studies have so far been confined to D-arabinose and D-ribose[5,7*]. It was therefore of interest to follow up in this organism the phosphorylation of xylose and xylulose and to see whether it is also governed by adaptation to the sugar and correlated with the oxidation of the pentoses.

* Phosphorylation of ribose was reported very recently, also, by HEALD AND LONG[16] and LONG[26].

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Mineral media. D-Ribose and D-xylulose were phosphorylated at a very slow rate, by extracts of glucose-grown cells, while D-xylose, L-arabinose and D-arabinose were not attacked at all. The extracts of xylose-grown cells phosphorylated xylose, xylulose, ribose and glucose; those of D-ribose-grown cells phosphorylated glucose and ribose, but none of the other sugars (Fig. 2).

Yeast extract media. Active extracts for phosphorylation experiments were obtained from cells of 22-hour old cultures. Extracts of cells grown on yeast extract medium without sugar, phosphorylated D-ribose rapidly, while xylulose was only very slowly attacked; D-xylose, L-arabinose and D-arabinose were inert. When 0.05% glucose was added to the medium, the cell-free extracts phosphorylated glucose and ribose and, to a considerable extent, xylulose. D-Xylose and L-arabinose were again not affected (Fig. 2).

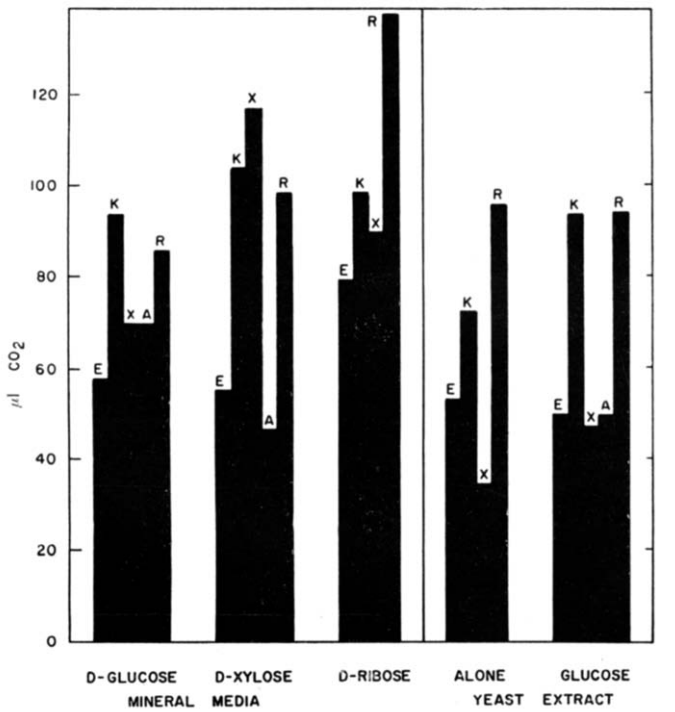

Fig. 2. Phosphorylation of pentoses by extracts of cells grown on mineral and yeast extract media. Each flask contained, in the main well, 0.15 ml bacterial extract (equivalent to 30 mg wet cells); 0.4 ml of 0.08 *M* $NaHCO_3$; 0.1 ml of 0.4 *M* KF; 0.1 ml of 0.06 *M* $MgCl_2.6H_2O$ and 0.2 ml substrate (3 μM). The side arm received 9 μM ATP in 0.4 ml of 0.02 *M* $NaHCO_3$. Final volume 2.0 ml; gas phase 95% N_2 + 5% CO_2; temperature 30°; incubation time 120 min. E = endogenous; K = D-xylulose; A = L-arabinose; R = D-ribose; X = D-xylose.

DISCUSSION

It has been shown by COHEN[4,6] that the oxidation and fermentation of pentoses by *E. coli* are strictly specific; they are dependent on the presence of the particular sugar in the medium. Glucose-grown cells attack the pentoses only after a lag period,

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in the course of which adaptation occurs. Similar findings have been reported for *Aerobacter cloacae*[11].

The present study of the oxidation of pentoses by *E. coli*, shows that cells are also induced to oxidize ribose when grown on D-xylose and L-arabinose. In the case of ribose, therefore, the specificity is not as strict as for the other pentoses.

The composition of the medium, also, has an effect on the formation of the enzymic pattern of the bacterial cell (see, for similar observations in the case of acetate metabolism in *E. coli*[32]). For example, the oxidation of ribose by cells harvested from yeast extract media is peculiar to *E. coli*. Other microorganisms, such as *L. pentosus*[21], *Aerobacter cloacae*[11], and *Streptomyces coelicolor*[3] are unable to oxidize ribose when grown in the presence of yeast extract, although they can do so when grown in the presence of ribose. Furthermore, the length of the lag period for the adaptation to oxidize D-xylose and L-arabinose varied with the composition of the medium: it was shorter in a mineral than in a yeast extract medium.

The behaviour of xylulose is even more peculiar. In a mineral medium, only xylose-grown cells oxidized xylulose, while cells grown on glucose or other pentoses failed completely to do so, even though they oxidized xylose after a lag period. On the other hand, cells harvested from a medium containing only yeast extract oxidized xylulose after a lag period, but oxidized it immediately when a very small amount (0.05%) of glucose was added to this medium. Perhaps, under these conditions, the bacteria form xylulose from glucose *via* a sugar acid (5-keto-D-mannonic acid) and thus become adapted to D-xylulose. Several such pathways have been reported, though not in bacterial systems[15,31].

According to LAMPEN[22], the first steps in the fermentation of D-xylose by *L. pentosus*, are represented by the following scheme:

$$\text{xylose} \rightleftharpoons \text{xylulose} \longrightarrow \text{pentose phosphate} \rightarrow C_2 + C_3\text{-phosphate}.$$

Xylose-5-phosphate is not attacked by the bacterial extracts[21]; it cannot, therefore, be an intermediate of this sequence. A similar pathway has also been observed in *Pseudomonas hydrophila*[18,19].

The present experiments with *E. coli* show that intact cells, previously grown in a mineral medium with xylose, are only partly permeable to xylulose; when the barrier of the cell membrane is removed, xylulose is oxidized by the extracts much faster than xylose. The high rate of xylulose-oxidation indicates that in *E. coli*, too, this compound may be an intermediate in the degradation of xylose. That the first step in xylose degradation might be isomerization to xylulose is in accordance with the observed adaptive formation of xylose-isomerase both by xylose-grown cells and by resting cells, which had been adapted to xylose. In contradistinction to *L. pentosus*, extracts of *E. coli* degrade xylose-5-phosphate[2]; in the latter case, therefore, phosphorylation may occur before isomerization. On the whole, however, the pathway of xylose (and xylulose) degradation appears to be parallel in both organisms.

Our knowledge of the course of L-arabinose degradation is less complete. The observation that extracts of L-arabinose-grown cells contain a specific isomerase for L-arabinose, tends to show that in this case, too, the formation of the corresponding keto-isomer may precede degradation*. The complexity of this degradation is indicated by the formation of D-phosphoglyceric acid from L-arabinose[1]. This can mean

* Dr. J. O. LAMPEN kindly informed us of analogous observations in the case of *L. pentosus*.

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that at the stage of glyceraldehyde-3-phosphate or of 3-phosphoglyceric acid itself, an equilibrium exists with a substance, lacking an asymmetric carbon atom, which is re-converted (or further converted, as the case may be) in a stereospecific manner. An indication of such a possibility has been reported recently in the case of *Azotobacter vinelandii*[28], in which the transformation of glyceraldehyde-3-phosphate to pyruvic acid through the action of triose phosphate-dehydrogenase and enolase takes place.

In the case of ribose no isomerase could be found, thus indicating that ribose may be directly phosphorylated to a ribose phosphate. Ribose is exceptional in another respect as well; it is phosphorylated by cells grown on other pentoses. This is also the case with *L. pentosus*[23].

It should be pointed out that the ability of the bacterial extracts to transfer the terminal phosphate group from ATP to the various sugars is paralleled by their ability to oxidize these sugars, thus indicating a close relationship between both processes[12].

SUMMARY

The oxidation, phosphorylation, and isomerization of pentoses by *Escherichia coli* are reported.

1. Resting cells grown on mineral media containing pentoses, rapidly oxidize the corresponding pentoses and D-ribose, but oxidize D-xylose and L-arabinose only after a lag period. Xylulose is slowly oxidized only by xylose-grown cells.

2. Cells grown on yeast extract oxidize ribose rapidly and L-arabinose, D-xylose and D-xylulose only after a lag period. Cells from a yeast-extract glucose medium immediately oxidize xylulose as well.

3. Extracts from xylose-grown cells oxidize xylulose at a higher rate than xylose itself. The behavior of xylulose appears, therefore, to depend on permeability conditions.

4. Xylose is converted to xylulose; L-arabinose is isomerized before it is degraded. *E. coli* does not contain a specific ribose-isomerase.

5. Extracts of cells grown on a mineral medium containing glucose phosphorylate, although slowly, only D-ribose and D-xylulose; extracts of xylose-grown cells phosphorylate xylose, xylulose, ribose and glucose while those of ribose-grown cells phosphorylate ribose and glucose. Extracts of yeast-extract-grown cells phosphorylate ribose rapidly, and xylulose very slowly, whilst those growing in a yeast-extract-glucose medium rapidly phosphorylate xylulose also.

RÉSUMÉ

Les auteurs ont étudié l'oxydation, la phosphorylation et l'isomérisation des pentoses par *Escherichia coli*.

1. Des cellules non proliférantes, obtenues sur des milieux minéraux renfermant des pentoses, oxydent rapidement les pentoses correspondants et le D-ribose, mais n'oxydent le D-xylose et le L-arabinose qu'après une période de latence. Le xylulose n'est oxydé que par des cellules cultivées en présence de xylose, mais lentement.

2. Des cellules cultivées sur un extrait de levure oxydent rapidement le ribose et le L-arabinose, le D-xylose et le D-xylulose seulement après un temps de latence. Des cellules obtenues sur un milieu renfermant du glucose et de l'extrait de levure oxydent immédiatement le xylulose également bien.

3. Des extraits de cellules cultivées en présence de xylose oxydent le xylulose plus vite que le xylose lui-même. Le comportement du xylulose semble donc dépendre de conditions de perméabilité.

4. Le xylose est transformé en xylulose; le L-arabinose est isomérisé avant d'être dégradé. *E. coli* ne renferme pas de ribose-isomérase spécifique.

5. Des extraits de cellules cultivées sur un milieu minéral renfermant du glucose ne phosphorylent, lentement d'ailleurs, que le D-ribose et le D-xylulose; des extraits de cellules cultivées en présence de xylose phosphorylent le xylose, le xylulose, le ribose et le glucose, tandis que des extraits de cellules cultivées en présence de ribose phosphorylent le ribose et le glucose. Des extraits de cellules cultivées en présence d'extrait de levure phosphorylent rapidement le ribose et très lentement le xylulose, tandis que des extraits de cellules cultivées en présence de glucose et d'extrait de levure phosphorylent rapidement également le xylulose.

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ZUSAMMENFASSUNG

Es wird über die Oxydation, Phosphorylation und Isomerisierung von Pentosen durch *Escherichia coli* berichtet.

1. Auf anorganischem, pentosehaltigem Medium gezüchtete Zellen im Wachstumstillstand oxydieren schnell die entsprechenden Pentosen und D-Ribose, während D-Xylose und L-Arabinose nur nach einer Latenzperiode oxydiert werden. Xylulose wird nur durch auf Xylose-Medium gezüchtete Zellen und dann auch nur langsam oxydiert.

2. Ribose wird schnell, L-Arabinose, D-Xylose, sowie D-Xylulose werden nur nach einer Latenzperiode von auf Hefeextrakt gezüchteten Zellen oxydiert. Auf glukosehaltigem Hefeextraktmedium gezüchtete Zellen oxydieren auch Xylulose, und zwar sofort.

3. Extrakte aus auf Xylose-Medium gezüchteten Zellen oxydieren Xylulose mit grösserer Geschwindigkeit als Xylose selbst. Das Verhalten gegenüber Xylulose scheint also von Durchlässigkeitsbedingungen abzuhängen.

4. Xylose wird in Xylulose umgewandelt; L-Arabinose wird vor Abbau isomerisiert. *E. coli* enthält keine spezifische Ribose-Isomerase.

5. Extrakte aus auf anorganischem, glukosehaltigem Medium gezüchteten Zellen phosphorylieren, wenn auch langsam, nur D-Ribose und D-Xylulose. Extrakte aus auf Xylose gezüchteten Zellen phosphorylieren Xylose, Xylulose, Ribose und Glukose, während Extrakte aus auf Ribose gezüchteten Zellen Ribose und Glukose phosphorylieren. Ribose wird schnell, Xylulose jedoch sehr langsam von Extrakten aus auf Hefeextrakten gezüchteten Zellen phosphoryliert, während auf glukosehaltigen Hefeextrakten gezüchtete Zellen Extrakte ergeben, welche auch Xylulose schnell phosphorylieren.

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