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Catabolic pathways of glucose in *Bacillus circulans* var. *alkalophilus*

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Abstract Enzymes and the metabolic pathways of glucose catabolism of *Bacillus circulans* var. *alkalophilus* were studied. The metabolism of the microbe was mixed acid fermentative yielding mainly acetic and formic acids as end products from glucose. It was estimated that *B. circulans* var. *alkalophilus* partitions 90%–93% of the carbon from glucose into the Embden-Meyerhof-Parnas (EMP) pathway and 7%–10% into the hexose monophosphate (HMP) and Entner-Doudoroff (ED) pathways. Rather low activities of glucose dehydrogenase and gluconokinase appeared in the early logarithmic and late stationary phases, whereas NADH oxidase was markedly high. This result can be explained by a demand to reduce NADH to NAD^+ for the EMP pathway; when acetic and formic acids are produced, no NADH is regenerated to NAD^+, which is required in the early steps of EMP and HMP pathways. A small percentage (1.6%–2.4%) of the total CO_2 was formed from (6-C) of glucose, which means that the tricarboxylic acid cycle was functional but its contribution was insignificant. Large differences do not seem to exist between alkaliphilic and neutrophilic bacilli in the use of glucose pathways.

Key words Alkaliphile · Glucose metabolism · *Bacillus circulans* var. *alkalophilus* · Enzyme activities

Introduction

The alkaline environment provides drastic consequences for the cell, such as effects on its pH balance, the positive or negative charge on the outer cell membrane of bacteria, and penetration of nutrients and toxic metabolites into the cell through the cell membrane. High pH also affects the ionic and colloidal state of nutrients. Many important ions such as Fe^{2+}, Ca^{2+}, Mg^{2+}, and Mn^{2+} may become insoluble and precipitate as carbonates, hydroxides, or phosphates (Langworthy 1978). Some common nutrients, such as certain vitamins and amino acids, are chemically reactive at high pH (Mäkelä et al. 1988a). An organism can survive in alkaline conditions only by maintaining its internal pH (pH_i) more acid than the external pH (Krulwich and Guffanti 1983). The need for active mechanisms for controlling pH_i is now generally accepted. An intracellular buffering capacity could contribute by reducing fluctuations of pH_i (Kroll 1990).

Alkaliphilic bacteria are able to acidify the cytoplasm, although ionic pumps move protons out of the cell and increase the pH_i (Guffanti et al. 1978). This mechanism has been the basis for studying special characteristics of the cell membrane (Krulwich and Guffanti 1983; Krulwich 1986). The control mechanisms of alkaliphilic and neutrophilic bacteria as well as those of their nonalkaliphilic mutants were compared, and it was deduced that the cell membranes of alkaliphilic bacteria contain special antiporter systems which transport protons into the cell at the same time as cations are transported out of the cell (Krulwich et al. 1979, 1982; Mandel et al. 1980). A Na^+/H^+ antiporter is of special importance in acidification of the cytoplasm (Guffanti et al. 1980). Some neutrophilic bacteria such as *Escherichia coli* (Zilberstein et al. 1982) have the Na^+/H^+ antiporter whereas a nonalkaliphilic mutant of alkaliphilic *Bacillus* does not (Mandel et al. 1980).

Certain aspects of the physiology of alkaliphiles have been well studied, but some bases of their metabolism are poorly known. For example, the intermediary sugar metabolism has not been studied. However, it may have a special importance in the pH homeostasis of alkaliphiles for providing protons through respiration (Paavilainen et al. 1995). The present study was conducted to investigate whether the glucose metabolism of *B. circulans* var. *alkalophilus* deviates from that of other bacilli.

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Materials and methods

Bacterial cultivations

Bacillus circulans var. *alkalophilus*, ATCC 21783, was cultivated in the basal carbonate medium, pH 10.3, developed by Horikoshi (1971). Precultivations were performed in 5 ml of the medium in Falcon tubes. Sterile medium was inoculated with 1 ml of frozen culture containing 10% of glycerol stored at −70 °C. A 150-ml portion of the basal medium was inoculated with the preculture and cultivated in 500-ml Erlenmeyer flasks on a shaker for 3 days at 37 °C. The cultivations (200 ml) were inoculated with 10 ml of the medium from the 150-ml step. Cultivations were carried out on a gyratory shaker at 37 °C or in a thermostated cultivation chamber containing a one-dimensional shaker. Microbes were grown in vessels inside the chamber including aeration by shaking. Samples (5 ml) were taken from the vessels periodically with a multichannel peristaltic pump to a fraction collector in a refrigerator for storage and to stop the growth of the bacteria (Mäkelä et al. 1988b).

Analyses

The absorbance at 600 nm (1-cm cuvette) was employed to follow the bacterial growth. The pH of cell-free supernatant was measured immediately after removal of the cells by centrifugation. Protein concentration was measured using the method of Lowry et al. (1951) or its modifications (Schacterle and Pollack 1973; Alam 1992) with bovine serum albumin as the standard. Reducing sugars were analyzed with 3,5-dinitrosalicylate (DNS) by the method of Sumner and Somers (1949).

Determination of organic acids

Organic acids were analyzed from culture samples by high-performance liquid chromatographic (HPLC) and gas chromatographic (GC) methods as described earlier (Paavilainen and Korpela 1993). An Aminex HPX-87H organic acid analysis column (Bio-Rad, Richmond, CA, USA) with 4 mM H_2SO_4 as the mobile phase was used in HPLC. The acids were analyzed in diethyl ether–sodium hydroxide extracts of culture samples (Paavilainen and Korpela 1993). In GC, a fused-silica capillary column (HNU-Nordion, Helsinki, Finland) was used. Volatile acids were analyzed from diethyl ether extracts of samples. Nonvolatile acids were derivatized with methanol, and methyl esters were extracted with chloroform (Paavilainen and Korpela 1993). Controls were from noninoculated media.

Enzyme assays

The bacterial cells in 5 ml of cultivation medium were harvested by centrifugation at 10000 *g* for 20 min. Cells were washed once with 1 ml of 0.1 M Na_2HPO_4, 10 mM KCl, pH 8.0, and resuspended in 1 ml of the same buffer. The cell suspension (1 ml) was sonicated on ice using an MSE ultrasonic disintegrator (100 watt model). Cell lysates were centrifuged (10000 *g*, 30 min) to remove cell debris. During the preparation of cell lysates, buffers and cells were kept at +0 °C. The lysates were stored at −20 °C if their enzyme activities were not measured immediately. Hexokinase (glucokinase) was determined using a modification of the method of Easterby and O'Brien (1973). The assay mixture contained 50 mM Tris/HCl, pH 8.0, 2.7 mM glucose, 9 mM ATP, 0.9 mM NADP, 34 mM $MgCl_2$, 1 U glucose-6-phosphate dehydrogenase, and 100 µl of enzyme sample in 1 ml. The reaction was started by the addition of the sample. Glucose-6-phosphate isomerase was determined as described by Noltman (1964) and fructose bisphosphate aldolase as by Rutter and Hunsley (1966). Phosphofructokinase was measured according to the method of Furuya and Uyeda (1981). Glucose dehydrogenase and glucose-6-phosphate dehydrogenase activities were measured by the method of Kobayashi and Horikoshi (1980). In glucose-6-phosphate dehydrogenase measurement, 10 mM glucose-6-phosphate was used in the final solution.

6-Phosphogluconate dehydrogenase was assayed as described by Pontremoli and Grazi (1966). Gluconokinase was measured as described by Laughon and Krieg (1974). The enzymes of the Entner-Doudoroff (ED) pathway, 6-phosphogluconate dehydratase and phospho-2-keto-3-deoxy-gluconate aldolase, were measured together (Wood 1971) because 2-keto-3-deoxy-6-phosphogluconate was not commercially available. Muscle lactate dehydrogenase was applied in the test to observe the enzymic activity. NAD(P)H oxidase backgrounds were measured under the same conditions as the corresponding enzyme without the substrate. One unit of enzymic activity is defined here as the amount of each enzyme that consumes/forms 1 µmol of substrate/product in 1 min under the assay conditions at 25 °C.

Studies with radioactive glucose

In the studies of catabolism with ^{14}C-labeled glucose, bacterial cells were grown to the late logarithmic phase ($A_{600\,nm}$ = 3-4), to the point when practically all glucose in the medium was consumed (Fig. 1). Four 10-ml samples of the broth were transferred into four 100-ml Erlenmeyer flasks and 10 µl of 50 mM glucose was added to each flask. To three of the four flasks was added 10 µCi (370 kBq) of [U-^{14}C]glucose, [1-^{14}C]glucose, or [6-^{14}C] glucose. The cultivation medium was then incubated for 1 h at 37 °C. In other experiments, the radioactive sugars were added to the growth medium at the beginning of cultivation. The total volume in radioactive cultivations was 50 ml when 5 µCi (184 kBq) labeled glucose was added. Samples (5 ml) were taken at the logarithmic and stationary phases.

The method by Goldman and Blumenthal (1963) was used for recovery of $^{14}CO_2$. Each cultivation flask had a glass

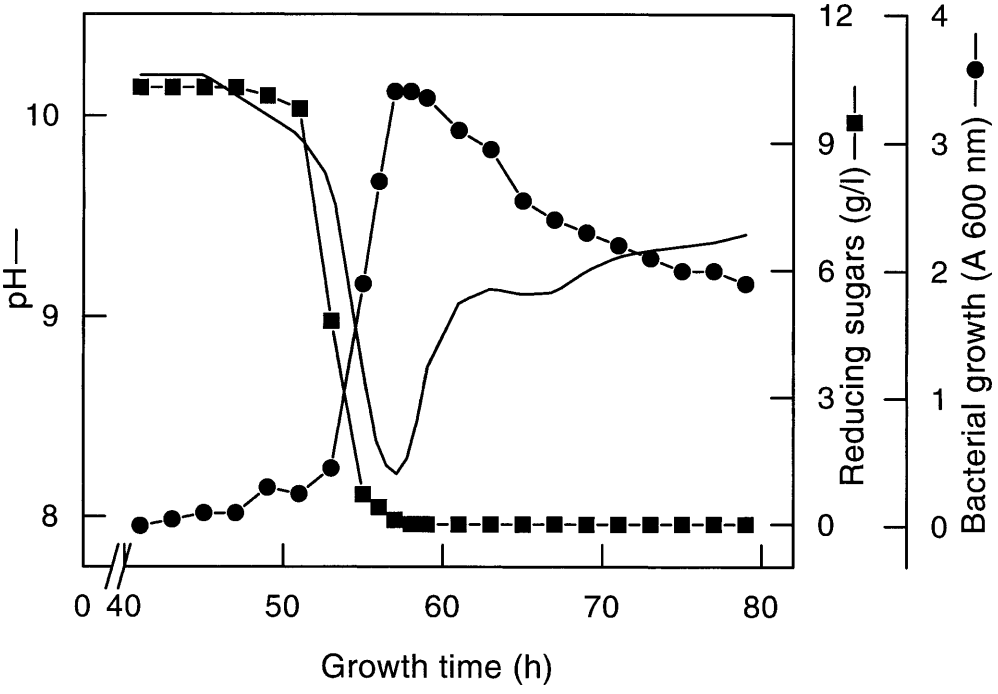

Fig. 1. Growth dynamics of *Bacillus circulans* var. *alkalophilus* in the basal carbonate medium at pH 10.3. The parameters measured were pH (*line*), reducing sugars (*squares*), and bacterial growth (*circles*)

vessel containing 200 µl of 2.5 M NaOH attached beneath the stopper. The glass vessel was changed each time when two culture samples (2 × 2 ml) were taken. One milliliter of 2 M citrate buffer, pH 5.0, was added to the culture sample (2 ml) and the reaction mixture was stored overnight at 4 °C to ensure that released CO_2 was completely trapped into NaOH. Acids formed in the culture medium were analyzed and separated by HPLC from another 2-ml sample (Paavilainen and Korpela 1993). The radioactivity in the acids and in CO_2 was determined by liquid scintillation counting (LKB 1210 Ultrobeta Scintillation Liquid Counter; Wallac Oy, Turku, Finland). The scintillation liquid contained 4 g 2,5-diphenyloxazol (PPO) and 100 mg 1,4-bid-2-(5-phenyloxazol) benzene (POPOP) in 250 ml of ethanol and 750 ml of toluene; 2 ml of the liquid was added to 100 µl of NaOH trap solution or to the sample. In addition, 700 µl of Triton X-100 was applied to the samples. The scintillation liquid, containing POPOP, POP, and Triton-X 100, formed a homogenous solution with all samples and was more reproducible than commercial Lumagel. The background activities were measured from culture samples grown without radioactive sugar.

The relative participation of the glucose pathways was calculated according to the distribution of radiolabeled carbon ([1-^{14}C] and [6-^{14}C]) in acetic acid (Dawes 1980). The following equation was used to calculate the fraction of glucose catablolized via the HMP and ED pathways:

$$\frac{[1-{}^{14}\text{C}]\text{Acetic acid}}{[6-{}^{14}\text{C}]\text{Acetic acid}} = \frac{1-(\text{HMP}+\text{ED})}{1+2(\text{HMP}+\text{ED})}$$

The fraction of glucose catabolized via the EMP pathway was calculated by 1 − (HMP + ED).

The values are shown as percentages in Table 4, which is shown later in this article.

Results

Activities of enzymes catabolizing glucose

The enzymes illustrated in Fig. 2 were assayed during the growth of *B. circulans* var. *alkalophilus*. Two maxima of specific activity of hexokinase appeared: the first one simultaneous with maximal cell density and the second occurred when the pH of the medium was increasing after its sharp drop (see Fig. 1). Phosphofructokinase and fructose bisphosphate aldolase of the EMP pathway also had maximum values in the growth maximum, whereas glucosephosphate isomerase had the highest activity before the stationary phase. Glucose-6-phosphate dehydrogenase appeared at the beginning of the logarithmic growth. The activity increased smoothly up to the stationary phase and was fairly stable until the dead phase. 6-Phosphogluconate dehydrogenase, the enzyme of the HMP pathway, also occurred in the logarithmic growth phase, but had its maximum value after the growth maximum.

Glucosephosphate isomerase of the EMP pathway appeared at the very beginning of the growth, whereas the other enzymes of the EMP and HMP pathways were in the logarithmic growth phase. In addition, glucosephosphate isomerase had another activity maximum in the stationary phase. Activities of the enzymes of the ED pathway, 6-phosphogluconate dehydratase and phospho-2-keto-3-deoxy-gluconate aldolase, increased until the growth maximum but decreased thereafter. *B. circulans* var. *alkalophilus* also showed small activities of glucose

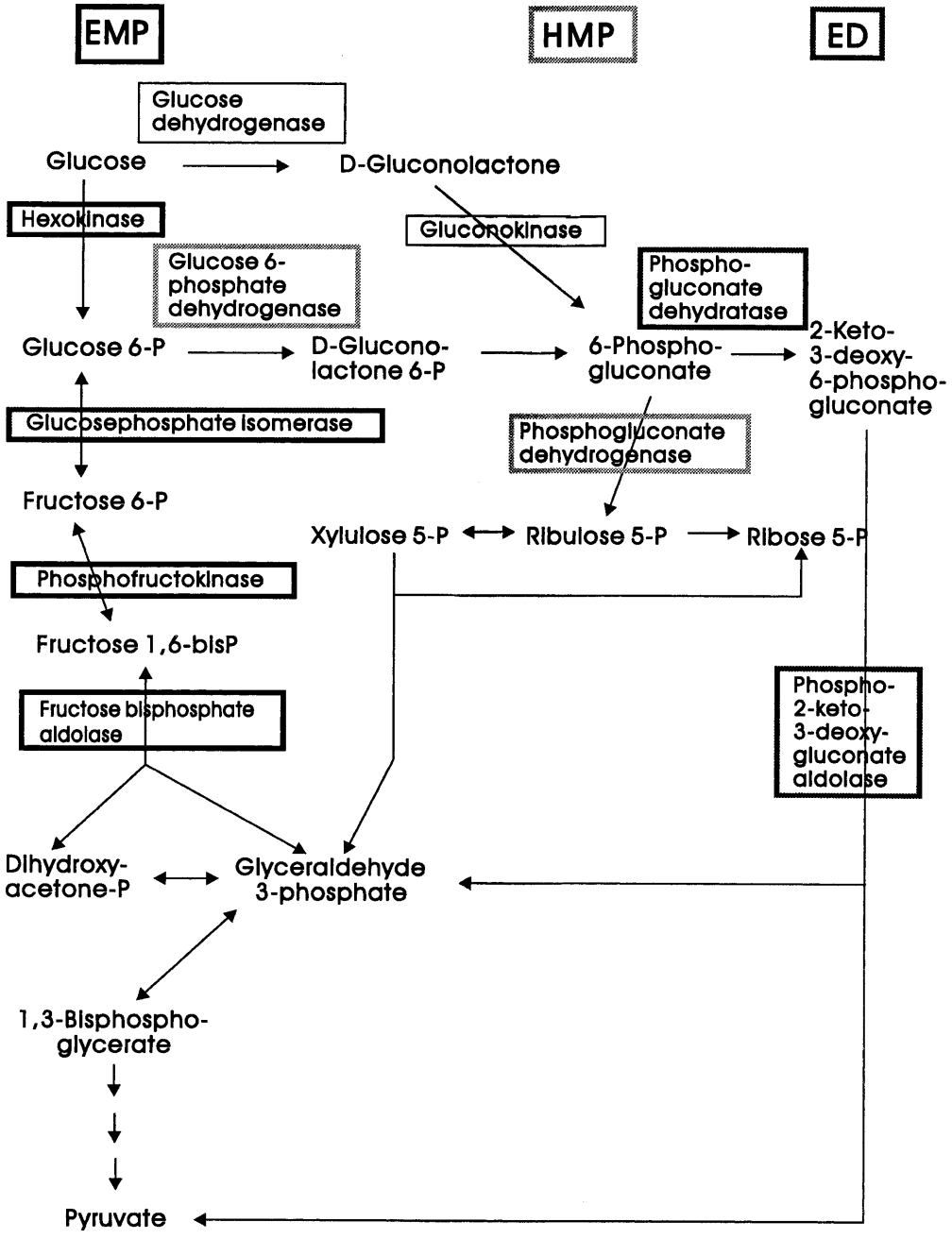

Fig. 2. Simplified presentation of interlinkages of three major catabolic pathways of glucose: EMP (Embden-Meyerhof-Parnas), HMP (hexose monophosphate), and ED (Entner-Doudoroff). Activities of the *boxed* enzyme were studied

Table 1. Specific activities of key enzymes catabolizing glucose in *Bacillus circulans* var. *alkalophilus* in the logarithmic growth phase

Enzyme	Specific activity (nmol $min^{-1} mg^{-1}$ protein)
Phosphofructokinase	11
Fructose bisphosphate aldolase	41
Glucose-6-phosphate dehydrogenase	41
6-Phosphogluconate dehydrogenase	44
6-Phosphogluconate dehydratase and phospho-2-keto-3-deoxy-gluconate aldolase	11
NADH oxidase	260

NADH oxidase activity was measured under the same conditions as fructose bisphosphate aldolase activity (Rutter and Hunsley 1966)

dehydrogenase and gluconokinase, and a high activity of NADH oxidase occurred throughout the bacterial growth (Table 1).

6-Phosphofructokinase, fructose bisphosphate aldolase, and the enzymes of the ED pathway showed similar patterns when the bacterium was cultivated in either glucose or starch medium. Hexokinase was more active in the stationary phase in the glucose than in the starch medium. Activities of glucose-6-phosphate dehydrogenase and of the enzymes of the HMP pathway were highest at the end of the logarithmic growth phase in the starch medium, whereas the maximal activities appeared in the stationary phase in the glucose medium.

Distribution of radioactivity in the end products from glucose

The relative proportions of the catabolic pathways were calculated from radioactive acetic acid produced in the culture medium. The radioactive glucose was added at the beginning of the growth phase. This method yielded more signal in the end products because bacteria had more time to use glucose in the medium than if the glucose were added later. The distribution of radioactivity from differently labeled glucose to the end products is shown in Table 2.

Radioactivity was transformed from [6-^{14}C]glucose and [1-^{14}C]glucose to acetic acid. In the logarithmic growth phase the difference between [1-^{14}C] and [6-^{14}C] was 1.3 fold while at the end the difference decreased slightly (to 1.2 fold). The respective difference for formic acid was 7 fold in the logarithmic growth phase, but in the stationary phase the distribution was 1.3 fold. Succinic acid had about equal amounts of radioactivity from [1-^{14}C]glucose and [6-^{14}C]glucose in the logarithmic growth phase, but in the stationary phase 2.5-fold more radioactivity was transformed from [1-^{14}C] than from [6-^{14}C]. Pyruvic acid had 1.2-fold more radioactivity from [6-^{14}C] than from [1-^{14}C] in the logarithmic growth phase and about 4-fold more radioactivity from [1-^{14}C] in the stationary phase. In the logarithmic growth phase, 8-fold more carbon was transformed from [1-^{14}C]glucose to CO_2 than from [6-^{14}C]glucose, but in the stationary phase the transformation of carbon from [1-^{14}C] to CO_2 was 4-fold compared to [6-^{14}C] (Table 3). The amounts of $^{14}CO_2$ formed from [1-^{14}C]- or from [6-^{14}C]glucose were 13.6% and 1.6%, respectively, as compared to $^{14}CO_2$ produced from [U-^{14}C]glucose in the logarithmic phase. In the stationary phase the amount of $^{14}CO_2$ formed from [1-^{14}C] decreased to 9.6% while that from [6-^{14}C] increased little (to 2.4%; Table 3).

The relative participation of the pathways of glucose catabolism was calculated according to the distribution of radiolabeled carbons [1-^{14}C] and [6-^{14}C] in acetic acid (Dawes 1980). It was concluded that *B. circulans* var. *alkalophilus* uses the EMP, HMP, and ED pathways. In the logarithmic growth phase the bacterium used 90% of the EMP pathway and 10% of the HMP and ED pathways; in the stationary phase, the contribution of the HMP and ED pathways decreased slightly (Table 4). The percentage participation of the catabolic pathways calculated on the ratio of the yields of ^{14}C in acetic acid was in agreement with the distribution of radiolabeled carbon in CO_2. Only 1.6%–2.4% of the total CO_2 formed from [6-^{14}C]glucose, showing that the contribution of the tricarboxylic acid (TCA) cycle was small. When [6-$^{14}CO_2$] was excluded from [1-$^{14}CO_2$] the remaining [1-$^{14}CO_2$] reflected the catabolic rates of the HMP and ED pathways. In the logarithmic and stationary growth phases, the amount of the remaining [1-$^{14}CO_2$] was 12% and 7.2%, respectively (see Table 3), which is in accordance with the values of 10% and 7% for the HMP and ED pathways together, calculated on the ratio of the radioactive acetic acid (Table 4).

Table 2. Distribution of ^{14}C radioactivity in organic acids (counts from radioactive atoms per minute per milliliter of the cultivation medium [CPM/ml]

End product	[U-^{14}C]		[1-^{14}C]		[6-^{14}C]	
	Log phase	Stationary phase	Log phase	Stationary phase	Log phase	Stationary phase
Acetic acid	26481	39396	32378	42909	43113	52747
Formic acid	3825	3072	741	4156	5298	5287
Succinic acid	2520	4287	3646	6052	3444	2455
Pyruvic acid	72	498	2271	3177	2720	820

Samples were taken at pH 9.0 in the logarithmic growth phase and at pH 9.5 in the stationary phase. [U-^{14}C] means the uniform labeling of glucose; [1-^{14}C] and [6-^{14}C] mean that ^{14}C locates at the position number 1 or number 6 in the glucose molecule, respectively

Table 3. Percentage values of $^{14}CO_2$ yields from radioactively labeled glucose by *B. circulans* var. *alkalophilus*

Growth phase	CO_2 from [1-^{14}C]-glucose (CPM/ml)	CO_2 from [6-^{14}C]-glucose (CPM/ml)	CO_2 from [U-^{14}C]-glucose (CPM/ml)	CO_2 from [1-^{14}C]-glucose (%)	CO_2 from [6-^{14}C]-glucose (%)	CO_2 from [1-^{14}C]-[6-^{14}C]-glucose(%)
Logarithmic	457	54	562	13.6	1.6	12
Stationary	865	216	1506	9.6	2.4	7.2

The bacterium was cultivated in the medium containing [1-^{14}C], [6-^{14}C], or uniformly labeled glucose. The radioactivity is shown as counts per min/ml (CPM/ml) of the cultivation medium. The proportion of $^{14}CO_2$ formed from [1-^{14}C]glucose or from [6-^{14}C]glucose compared to $^{14}CO_2$ formed from [U-^{14}C]glucose was calculated according to the following equation:

$$\frac{100\% \times {}^{14}CO_2 \text{ produced from [1 or 6-}{}^{14}\text{C]glucose}}{6 \times {}^{14}CO_2 \text{ produced from [U-}{}^{14}\text{C]glucose}}$$

Table 4. Estimates of the pathways of glucose catabolism in *Bacillus circulans* var. *alkalophilus* based on [^{14}C]acetic acid accumulated in the growth medium

Growth phase	Acetic acid from [1-^{14}C]-glucose (CPM/ml)	Acetic acid from [6-^{14}C]-glucose (CPM/ml)	Glucose used by EMP (%)	Glucose used by HMP + ED (%)
Logarithmic	32378	43113	90	10
Stationary	42909	52747	93	7

The proportions of glucose catabolism were calculated as shown in Materials and methods

Discussion

Bacillus circulans var. *alkalophilus* was mixed acid fermentative under all the conditions tested. Cultivations were carried out in microaerobic conditions that have also been used in other studies (Nakamura and Horikoshi 1976; Vandamme et al. 1984; Mäkelä et al. 1990).

Glucose dehydrogenase and gluconokinase exist in spores of *Bacillus* strains (Fujita et al. 1977; Otani et al. 1986, 1987; Sano et al. 1988). In *B. circulans* var. *alkalophilus*, glucose dehydrogenase activity appeared in the early logarithmic and the late stationary phases. Gluconokinase activity was highest during the logarithmic growth. However, the specific activities of these enzymes were quite low compared to the other enzymes studied.

NADH oxidase activity was markedly high in *B. circulans* var. *alkalophilus*. The NADH oxidase was found by the method described in Table 1, but not by the method of Kobayashi and Horikoshi (1980). The high NADH oxidase activity could be explained by a demand of the bacterium to reduce NADH to NAD^+ for the EMP pathway. *B. circulans* var. *alkalophilus* produced mainly acetic and formic acid and only very small amounts of lactic acid (Paavilainen et al. 1994). When acetic and formic acids are formed, no NADH is regenerated to NAD^+ as in the production of lactic acid and ethanol. However, NAD^+ is required in the early steps of the EMP and HMP pathways. Thus, most probably *B. circulans* var. *alkalophilus* uses NADH oxidase for these early stages of glucose metabolism.

Based on the measured enzyme activities, *B. circulans* var. *alkalophilus* employed the EMP, HMP, and ED pathways for glucose catabolism and, in addition, glucose dehydrogenase. Glucose dehydrogenase was detected during the main growth phase although it is usually a spore-specific enzyme. The transformation of [1-C] from glucose to acetic acid proved the existence of the EMP pathway, because through other pathways [1-C] flows to tricarboxylic acid cycle or forms CO_2. Also, HMP or HMP and ED pathways together were simultaneously active, because more radioactive CO_2 was formed from [1-^{14}C]glucose than from [6-^{14}C]glucose. 6-Phosphogluconate dehydrogenase of the HMP pathway was active in enzyme assays and could explain the conversion of [1-C] from glucose to CO_2. The enzymes of the ED pathway had lower activities in *B. circulans* var. *alkalophilus*. *Bacillus megaterium* spores show a slight activity of the ED pathway if both enzymes of this pathway, 6-phosphogluconate dehydratase and phospho-2-keto-3-deoxy-gluconate aldolase, are measured together (Shay and Vary 1978). However, by the direct measurement of phospho-2-keto-3-deoxy-gluconate aldolase, no activity appears in *B. megaterium* spores (Shay and Vary 1978). Possibly the ED pathway of *B. circulans* var. *alkalophilus* was active for some period during the growth.

Only a small percentage (1.6%–2.4%) of total CO_2 was formed from [6-C] of glucose, which means that the TCA cycle was functional but its contribution was insignificant. The glyoxylate bypass is not functional because the bacterium can not use acetic acid (Paavilainen et al. 1994, 1995).

EMP is the main pathway for glucose catabolism in *Bacillus* (Goldman and Blumenthal 1963, 1964; Pepper and Costilow 1964; Hill et al. 1967; Weimer 1984). Growth conditions have an influence on the relative proportions of the glucose pathways in bacilli. *B. subtilis* Marburg C4 cells metabolize glucose slightly more through the EMP pathway in anaerobic than in aerobic conditions. The nitrogen source has no remarkable effect on the catabolic pathway of glucose in *B. subtilis* (Goldman and Blumenthal 1963), but affects this pathway somewhat in *B. thuringiensis*. When the bacteria grow in glucose-yeast extract salts medium, the cells catabolize almost 100% of glucose by the EMP pathway. If the cells are grown in glucose-glutamate salts medium, *B. thuringiensis* catabolizes 5% of glucose through the HMP cycle and the TCA cycle is inactivated (Nickerson et al. 1974). In other bacilli glucose and glutamate together repress enzymes of the TCA cycle (Hanson et al. 1964). In another study (Bulla et al. 1970), glucose was catabolized less (84%) by EMP in *B. thuringiensis* than has been reported by Nickerson (Nickerson et al. 1974).

Bacillus cereus exploits 98% of the EMP and only 2% of the HMP pathway in each phase of growth (Goldman and Blumenthal 1964). High temperature favors the EMP whereas at lower temperatures the breakdown of glucose through HMP is preferred (Smith et al. 1952; Chung et al. 1976). The contribution of these pathways resembles glucose catabolism in *Bacillus stearothermophilus*, using the pathways 96% and 4%, respectively (Hill et al. 1967). Also, *Bacillus alvei*, *B. lentomorbus, B. popilliae* (Bulla et al. 1970), and *B. macerans* (Weimer 1984) cells catabolize glucose mainly by the EMP route. During the logarithmic growth phase the proportion of the EMP pathway in bacilli ranged from 75% and 94% (Table 5), proving that the main pathway is EMP although growth conditions slightly change

Table 5. The portions of Embden-Meyerhof-Parnas (EMP) and hexose monophosphate (HMP) pathways of eight *Bacillus* strains in aerobic or microaerobic conditions

Organism	Portion (%)		Reference
	EMP	HMP	
B. subtilis Marburg C4	60–70	30–40	a
B. cereus var. *terminalis*	98	2	b
B. thuringiensis (18 strains)	>99	<1	c
B. stearothermophilus (rough variant)	96	4	d
B. alvei NRRL B-348	94	6	e
B. lentimorbus NRRL B-2522	85	15	e
B. popilliae NRRL B-2309-P	75	25	e
B. circulans var. *alkalophilus* ATCC 21783	90	<10	f

References: a, Goldman and Blumenthal (1963); b, Goldman and Blumenthal (1964); c, Nickerson et al. 1974; d, Hill et al. 1967; e, Bulla et al. 1970; f, in the logarithmic phase, this study

the proportions between the EMP and HMP routes. The estimations of catabolic pathways of glucose in *B. circulans* var. *alkalophilus*, EMP 90%–93% and HMP and ED together at 7%–10%, resembled the glucose catabolism in neutrophilic bacilli (Table 5). Thus, although alkaliphiles prefer high pH for growth, their basic metabolism seems to resemble that of neutrophiles.

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