SUGAR TRANSPORT IN A PSYCHROPHILIC YEAST

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ABSTRACT

CIRILLO, VINCENT P. (Seton Hall College of Medicine and Dentistry, Jersey City, N.J.), PETER O. WILKINS, AND JOSEPH ANTON. Sugar transport in a psychrophilic yeast. J. Bacteriol. **86**:1259–1264. 1963.—The mechanism and temperature characteristic for sugar transport were compared in a psychrophilic and a mesophilic yeast. Between 0 and 10 C, glucose utilization, glucosamine accumulation, and sorbose transport showed a very high temperature characteristic in the mesophile (μ = 50,000) compared with the psychrophile (μ = 12,000). Hexokinase activity in cell-free extracts from both yeasts, however, showed the same low temperature characteristic (μ = 15,000). Although the temperature characteristic for sugar transport was markedly different for the mesophile and the psychrophile, sugar transport in both yeasts met the criteria for carrier-mediated facilitated diffusion.

Baxter and Gibbons (1962), in a study comparing a psychrophilic with a mesophilic *Candida*, reported that the rate of sugar transport in the psychrophile is virtually independent of temperature from 0 to 30 C. Sugar transport by the mesophile, however, showed an expected high temperature characteristic. These conclusions were based on the uptake of the poorly metabolized amino sugar, glucosamine. The unusual temperature independence claimed for sugar transport by the psychrophile (*Candida* No. 5), if confirmed, should support the suggestion by Ingraham and Bailey (1959) that the minimal temperature for growth and metabolism of an organism might be related to the effect of temperature on cell permeability. Although the experiments to be reported do not confirm the temperature independence of sugar transport in *Candida* No. 5, they do demonstrate that sugar transport in this psychrophile is *less* affected by temperature, especially between 0 and 10 C, than in a mesophilic baker's yeast.

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MATERIALS AND METHODS

Preparation of yeast cells. The psychrophile *Candida* No. 5, originally isolated from refrigerated grape juice (Lawrence, Wilson, and Pederson, 1959), was kindly provided by N. E. Gibbons. The cells were grown in 100 ml of medium in 500-ml flasks on a rotary water-bath shaker at 10 C. Transfers were made at 48-hr intervals by use of a 10% (v/v) inoculum. The medium consisted of 1.0% Tryptone, 0.3% yeast extract (both from Difco), 2% glucose, and 0.4% KH_2PO_4 (both from Merck, Sharp and Dohme, Rahway, N.J.), and was adjusted to pH 7.0.

Saccharomyces cerevisiae was obtained as 1-lb cakes of compressed baker's yeast (Anheuser-Busch, Inc.).

Both yeasts were washed by repeated centrifugation in distilled water at room temperature. The volume of the centrifuged yeast was determined after 5 min of centrifugation at 3000 $\times$ *g*, and a 10% (v/v) suspension was prepared in distilled water. In most experiments, 1-ml portions of this 10% suspension were placed in 12-ml centrifuge tubes, representing 0.1 ml of packed yeast per tube.

Sugar-transport procedure. Experiments to determine sugar uptake were initiated by adding 1 ml of sugar solution to the centrifuge tubes containing 1 ml of 10% yeast suspension and rapidly mixing the suspension. The sugar solutions and the cell suspensions were equilibrated to the desired temperature before mixing. The incubations were carried out in water-bath shakers in stoppered 12-ml centrifuge tubes kept at an acute angle to insure violent agitation. At the end of the experimental period, the tubes were centrifuged at 3000 $\times$ *g* for 1 min at 4 C.

The cells were then washed with three 5-ml portions of ice-cold 10^{-3} M uranyl nitrate solution at pH 4.0.

The use of uranyl nitrate solution for washing in place of 0.9% NaCl used in sugar-transport studies with baker's yeast (Cirillo, 1962) was introduced to prevent sugar loss from the psychrophile during the washing procedure. Although this procedure is not necessary in the case of baker's yeast, both yeasts were washed with uranyl nitrate. The use of uranyl nitrate in sugar-transport studies is being reported separately (Cirillo and Wilkins, J. Bacteriol., *in press*).

The washed cells were suspended in 5 ml of distilled water and placed in a boiling-water bath for 20 min. After cooling, the extracts were separated from the cells by centrifugation, diluted appropriately, and analyzed.

Chemical analysis. Glucosamine was determined by the Nelson method (Neish, 1952); sorbose was determined by the cysteine hydrochloride method of Dische and Devi (1960). Glucose was measured with a Glucostat reagent (Worthington Biochemical Corp., Freehold, N.J.).

The concentration of unphosphorylated sugars was determined by analysis of extracts treated successively with 0.5 volume of 4.7% $Ba(OH)_2 \cdot 8H_2O$ and 0.5 volume of 5.3% $ZnSO_4 \cdot 7H_2O$ to remove phosphorylated sugars by precipitation. Tests with mixtures of glucose, glucose-6-phosphate, glucosamine, and glucosamine-6-phosphate confirmed that this procedure efficiently and selectively removed the phosphorylated derivatives from the mixtures.

Glucosamine and glucosamine-6-phosphate in cell extracts were identified by one-dimensional paper chromatography according to the method of Bandurski and Axelrod (1951).

Hexokinase assay. Cell-free extracts were prepared by grinding 30 ml of cell suspension with 40 g of glass beads in a homogenizer (Virtis Co. Inc., Gardiner, N.Y.) for 15 min. The homogenates were clarified by centrifugation at 3000 × *g* for 10 min and stored in the deep freeze. Hexokinase activity was measured according to the procedure of Sols and de la Fuente (1961).

Calculation of intracellular sugar. Intracellular concentration of glucosamine and sorbose was calculated on the basis that intracellular water accounts for 47% of the packed volume of centrifuged yeast (Cirillo, 1962).

Temperature relationships. The temperature characteristic, μ, was calculated from the equation:

$$\mu = \frac{(\log_{10} V_2/V_1)\, 2.303R}{\frac{1}{T_1} - \frac{1}{T_2}}$$

where V_1 and V_2 are the reaction velocities at the absolute temperatures T_1 and T_2, respectively; R is the gas constant (1.98 cal per degree per mole); 2.303 is the constant for conversion from $\log_e$ to $\log_{10}$; and the dimensions of μ are calories per mole.

The temperature coefficient, Q_{10}, is the ratio of velocities at two temperatures 10 degrees apart. Q_{10} over the range from 0 to 20 C was calculated from the formula:

$$Q_{10} = \sqrt{\frac{V \text{ at } 20\text{ C}}{V \text{ at } 0\text{ C}}}$$

RESULTS

Growth experiments confirmed that *Candida* No. 5 is an obligative psychrophile, as found both by Lawrence et al. (1959) and by Baxter and Gibbons (1962). Growth occurs over the range from 0 to 20 C with a maximum at 10 C but has never been observed at room temperature or above, thereby satisfying the criteria of Ingraham and Stokes (1959) for classification as an obligative psychrophile.

Glucose utilization. The rate of glucose utilization by aerated cell suspensions of *Candida* No. 5 and baker's yeast was measured from 0 to 30 C. The effect of temperature on the rate of glucose utilization is shown for each yeast in an Arrhenius plot in Fig. 1. The following differences between

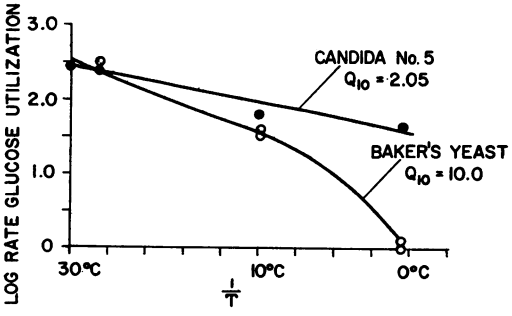

FIG. 1. *Arrhenius plot of the rate of glucose utilization against the reciprocal of the absolute temperature. The corresponding centigrade temperatures are shown. The Q_{10} was calculated from the rates at 0 and 20 C.*

Candida and baker's yeast are seen. The temperature characteristic for the psychrophile is constant from 0 to 30 C, whereas the temperature characteristic for baker's yeast varies from 0 to 10 C and from 10 to 30 C. The temperature characteristic over the entire range for the psychrophile is 12,500; for baker's yeast, it is 50,000 between 0 and 10 C and 17,000 between 10 and 30 C.

Analogous results for the influence of temperature on glucose metabolism in mesophiles and psychrophiles were described for bacteria by Brown (1957) and Ingraham and Bailey (1959). The latter authors found, however, that the differences observed in whole cells were not apparent in ruptured-cell preparations. More importantly, disruption of the cell lowered the temperature characteristic of the mesophile but left that of the psychrophile relatively unchanged.

Hexokinase activity. To determine whether cell rupture would eliminate the difference in temperature characteristic between the two yeasts, hexokinase activity was measured as a function of temperature in cell-free preparations. An Arrhenius plot of these data (Fig. 2) shows that the difference in temperature characteristic is eliminated. Furthermore, the temperature characteristic for hexokinase activity for both yeasts is 15,000; therefore, cell rupture has lowered the temperature characteristic of the mesophile.

Glucosamine accumulation. The fact that the temperature characteristic for glucose utilization

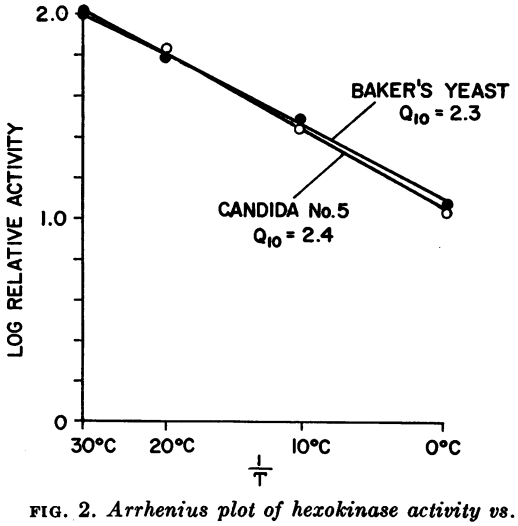

FIG. 2. *Arrhenius plot of hexokinase activity vs. temperature.* Q_{10} *was calculated as for Fig. 1.*

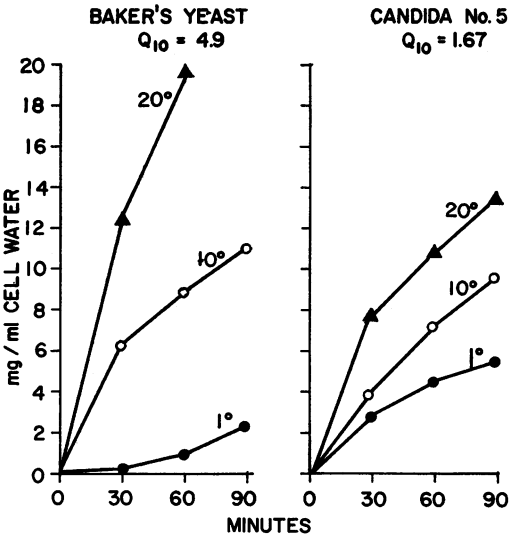

FIG. 3. *Glucosamine uptake.* Q_{10} *was calculated as for Fig. 1.*

by whole cells and cell-free preparations is the same for the psychrophile, whereas a marked difference exists between the temperature characteristic for whole cells and extracts of the mesophile, suggests that permeability may be a factor. This, in fact, was the conclusion reached by Baxter and Gibbons (1962), based on the influence of temperature on glucosamine accumulation by *Candida* No. 5 and a mesophile, *C. lipolytica.*

The influence of temperature on glucosamine accumulation in *Candida* No. 5 and baker's yeast is shown in Fig. 3.

Although the independence of temperature for glucosamine accumulation for *Candida* No. 5 was not confirmed, glucosamine accumulation by *Candida* No. 5 was found to be *less* affected by temperature than was that by baker's yeast. An Arrhenius plot of those data results in curves for glucosamine accumulation which are almost identical to those obtained for glucose utilization (Fig. 1). The μ values for *Candida* and baker's yeast between 0 and 10 C are 9000 and 52,000, respectively.

Baxter and Gibbons (1962) chose glucosamine as a model for sugar transport on the basis that this sugar is accumulated by baker's yeast. However, most of the glucosamine accumulated by baker's yeast is, in fact, glucosamine phosphate (Burger and Hejmova, 1961). This now has been confirmed for glucosamine accumulation

by both *Candida* No. 5 and baker's yeast (Fig. 4). In Fig. 4, total intracellular reducing sugar is contrasted with intracellular barium-soluble sugar. The barium-insoluble sugar was confirmed to be glucosamine phosphate by paper chromatography.

Sorbose transport. A more desirable model for sugar transport would be a sugar which is metabolically inert. L-Sorbose is a metabolically inert sugar which has been used extensively in transport studies in baker's yeast (Cirillo 1961*a*, *b*, 1962). Sorbose was also found to be transported by *Candida* No. 5 and is recovered from cell extracts exclusively as the free, nonphosphorylated sugar. The effect of temperature on sorbose transport in *Candida* and baker's yeast is shown in Fig. 5.

The temperature characteristic for sorbose transport is similar to those for glucose utilization and glucosamine accumulation; between 0 and 10 C, μ is 12,000 for the psychrophile and 49,000 for baker's yeast.

Mechanism of sugar transport in Candida No. 5. It is quite clear that the temperature characteristic for sugar transport at low temperatures for the psychrophilic yeast is significantly lower than that of baker's yeast, and that it is essentially the same as that of hexokinase activity in cell-free preparations (Table 1).

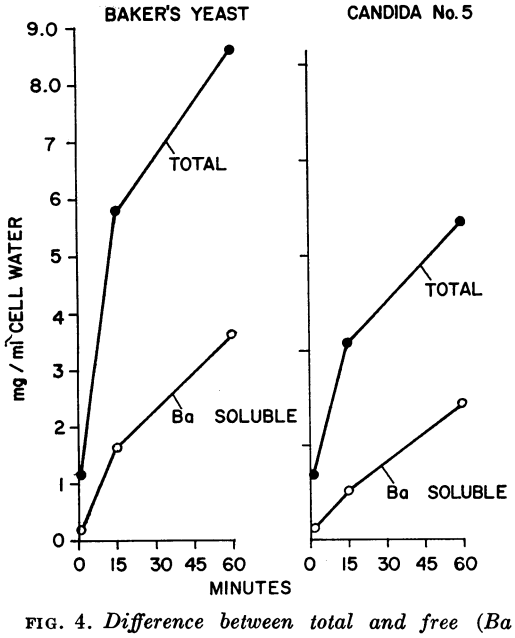

FIG. 4. *Difference between total and free (Ba soluble) glucosamine content.*

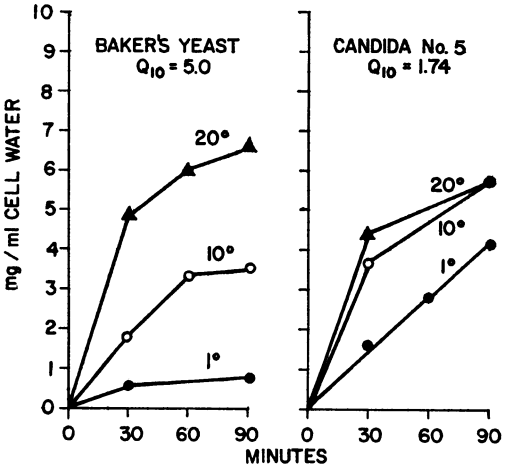

FIG. 5. *Sorbose transport.*

TABLE 1. *Temperature characteristic for sugar utilization and transport by Candida No. 5 and Saccharomyces cerevisiae between 0 and 10 C*

Reaction	Temp characteristic (μ)	
	Candida No. 5	*S. cerevisiae*
Glucose utilization	13,000	50,000
Glucosamine accumulation	9000	52,000
Sorbose transport	12,000	49,000
Hexokinase activity	16,000	14,000

To assess the significance of this difference, it is necessary to determine whether there is a basic difference in the mechanism of sugar transport in the two yeasts.

Sugar transport in baker's yeast (Cirillo 1961*a*, 1962) is a carrier-mediated facilitated diffusion characterized by saturation kinetics, competitive inhibition for transport between penetrating sugars (i.e., glucose and sorbose), and the phenomenon of counterflow; that is, the addition of glucose to sorbose-equilibrated cells induces the uphill efflux of sorbose from the cell. All of these characteristics have been demonstrated with *Candida* No. 5. The uptake of the nonphosphorylated L-sorbose does not occur against a concentration difference; in the steady state, the intracellular sorbose concentration is equal to that of the external medium. However, when the initial rate of sorbose transport is measured at increasing external concentrations, saturation kinetics are observed (Table 2). Treatment of these data according to the method of Lineweaver and

Burk (Umbreit, Burris, and Stauffer, 1957) gives a K_m for sorbose transport of 2.0%.

Sorbose transport is competitively inhibited by glucose (Fig. 6). In Fig. 6, the reciprocal of the initial rate of sorbose transport at two concentrations is plotted against the glucose concentration. The curves extended beyond the ordinate cross before they reach the negative abscissa. This observation confirms that glucose inhibition is competitive and the K_I of glucose inhibition is given by the point of intersection of the two curves (Umbreit et al., 1957). Finally, glucose induces counterflow of both sorbose and glucosamine (Fig. 7). In each case, the addition of glucose to the equilibrated cells resulted in the movement of the internal sugar out of the cell against a concentration difference.

TABLE 2. *Saturation of sorbose transport in Candida No. 5 by high external concentrations of sorbose at 30 C*

External sorbose concn (mg/ml)	Rate of sorbose transport (mg per ml of cell water per hr)
10	21.2
25	58.0
50	88.4
100	86.4
150	97.2

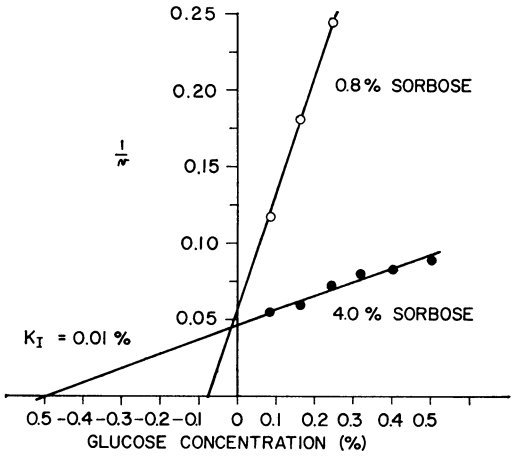

FIG. 6. *Competitive inhibition of sorbose transport by glucose in Candida No. 5. The reciprocal of the initial rate of sorbose transport at 0.8 and 4.0% sorbose (ordinate) is plotted against the glucose concentration (abscissa). The point of intersection of the two curves to the left of the ordinate represents K_I of glucose.*

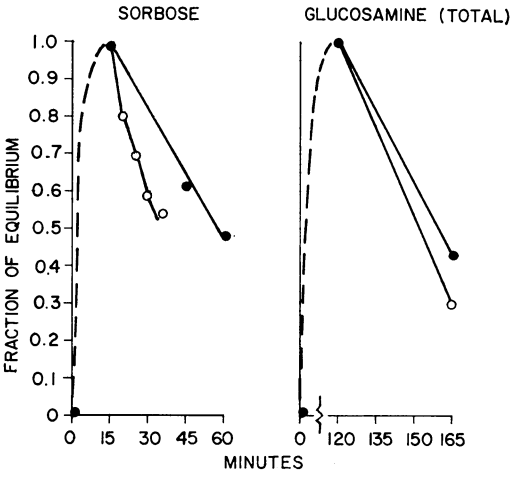

FIG. 7. *Sorbose and glucosamine counterflow induced by glucose in Candida No. 5. The cells were equilibrated with 1% sorbose and glucosamine for 15 min and 120 min, respectively, when 1% of glucose in the same concentration of sorbose or glucosamine was added. The levels of intracellular sugar after glucose addition are expressed as the fraction of the intracellular level when glucose was added.*

Candida No. 5, then, transports sugars by a carrier-mediated, facilitated diffusion system similar to that described for baker's yeast (Cirillo, 1961*a*, 1962).

Discussion

Sugar transport in the psychrophilic *Candida* is affected less by low temperature than is that of the mesophile, baker's yeast. It is quite probable that the marked difference between the temperature characteristic of glucose metabolism in these two yeasts is a result of a difference in the temperature characteristic for glucose transport preceding its intracellular metabolism.

Since the metabolism of sugar transport in the psychrophile does not seem to be unique except for its low temperature characteristic, one can only speculate about the basis of the difference between a psychrophile and a mesophile. One possibility was suggested by Kates and Baxter (1962), who found a higher concentration of unsaturated fatty acids in the phospholipids of *Candida* No. 5 than in a mesophilic yeast when both were grown at 10 C. The point in carrier-mediated transport which would be most affected by temperature can only be speculated upon, but the nature of membrane lipids theoretically

could be expected to affect the temperature characteristic of the diffusion of the hypothetical mobile carrier involved in sugar transport. However, it is equally possible that the temperature characteristic of sugar transport is determined more by sugar-carrier association or dissociation (Sen and Widdas, 1962). A difference in the nature of phospholipids could also affect the temperature characteristic of sugar-phospholipid complex formation as has recently been studied with phospholipids from erythrocyte ghosts by LeFevre (1963), although the nature of this association and its role in facilitated diffusion are still obscure.

Finally, on the basis of the correlation of the temperature characteristic of growth and sugar transport, it is tempting to conclude that the minimal growth temperature of an organism is determined by the temperature characteristic of its sugar-transport system. However, the recent experiments of Ng, Ingraham, and Marr (1962) and Marr and Ingraham (1962) on the influence of temperature and nutrition on growth rate and lipid composition in *Escherichia coli* do not support this conclusion, at least in *E. coli*.

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