A Research Note
Method for Selection of Lactic Acid Bacteria and Determination of Minimum Temperature for Meat Fermentations

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ABSTRACT

Arrhenius plots for the fermentation of dextrose in meat by Pediococcus acidilactici and Pediococcus pentosaceus showed discontinuities at 32 and 24°C, respectively. The Arrhenius energy of activation (Ea) of P. pentosaceus was 25% lower than that of P. acidilactici at temperatures above the discontinuity. The Ea of P. acidilactici and of P. pentosaceus increased about 2- and 3-fold, respectively, at temperatures below the discontinuity. The Ea can be used for selection of efficient starter culture strains. The temperature of discontinuity may be used to determine the lowest efficient temperature for lactic acid fermentation.

RESULTS AND DISCUSSION

It is generally accepted that the metabolic activities of bacteria decrease with decreasing temperature. At some critical temperature, processes associated with the cellular membrane deviate markedly from the expected leading to a metabolic disorder. At the critical temperature, a phase transition takes place in the lipid portion of the cellular membrane, i.e., a change from a liquid crystalline state to a solid gel state. The phase change disrupts enzymatic activity and metabolism, leading to malfunction of the membrane and the cell (4,9). One useful and simple tool to detect the occurrence of phase transitions in cell membranes has been the presentation of data relating the influence of temperature on reaction velocity of membrane-associated activities (such as enzyme systems, sugars and amino acids transport) as an Arrhenius plot (1,4,8). The existence of a discontinuity of two straight lines in the plot can be explained by the occurrence of a phase change of the cell’s membrane lipids (4,8,9). The energy of activation (Ea) of the activity under consideration can be obtained from the slope of the plot. The Ea may serve as an indicator of metabolic efficiency. This is the basis of the concept brought herein. There are relatively few reports in the literature relating the influence of temperatures and the fermentative activity of lactic acid bacteria (LAB) in food products to the Arrhenius concept. The objectives of this study were: (a) to examine the possibility of utilizing the relationship between temperature and fermentative activity of LAB (Arrhenius plot) in meat for the selection of efficient starter culture strains and (b) to determine the minimum efficient temperature for lactic acid fermentation.

MATERIALS AND METHODS

Frozen cultures of Pediococcus acidilactici (NRRL B5624) and Pediococcus pentosaceus (ATCC 1091) were prepared (2.2 X 10^10 and 1.6 X 10^10 CFU/ml, respectively) as described previously (6). A semi­dry sausage-like product was prepared by combining ground beef (chuck) and ground pork loin (2:1). The product (20% fat) was formulated to contain 0.0156% NaNO2, 3.0% NaCl, 2.0% dextrose and 0.6% Summer sausage spice mix. Separate 50-g meat samples were inoculated with each culture to provide a final population of 3.0 X 10^7 CFU/g meat. A control with no added culture was included. Each sample was tightly packed in a Whirl-Pak bag (7.6x12.7 cm) and fermented at 16, 21, 24, 27, 29, 32, 38, 41 and 43°C to obtain a final pH of 5.0. The pH was measured using an Altex digital pH meter (model 3560, Beckman Instruments). Measurements of pH were done at the beginning and at the end of the fermentation period using a slurry prepared by blending 20 g of meat with 60 ml of distilled water for 45 s. The pH values were used to obtain [H+] in moles according to the equation:

\[ \text{pH} = -\log [\text{H}^+] \]

The net [H+] (i.e., [H+] final - [H+] initial) was divided by the time (h) of fermentation to obtain the rate of dextrose fermentation. This rate at different temperatures was plotted as log rate vs. the reciprocal of the absolute temperature (T). The least regression analysis was used to find the best fit line. The graphical plot has a slope of \(-\text{Ea}/2.303\text{R}\) (J). The value of R, the gas constant, was 1.9872 cal/mole X K. Ea was determined from the slope of the graphical plot. The pH of the control (no culture added) did not change.

The Arrhenius plots of the fermentation of dextrose by P. acidilactici and P. pentosaceus showed discontinuities at 32 and 24°C, respectively (Fig. 1). Above the transition (discontinuity) temperature, the Ea of P. pentosaceus was 25% lower than that of P. acidilactici (8.5 vs. 11.4 kcal/mole). The Ea of P. acidilactici and of P. pentosaceus increased about 2- and 3-fold, respectively, below the discontinuity. This may be due to a more heterogenic lipid composition of the membrane of P. acidilactici than that of P. pentosaceus. As a result, the latter culture may have had a larger formation of solid gel domains during the phase transition resulting in a higher Ea than that of the former culture.
The temperature of the discontinuity on the Arrhenius plot can be used to determine the lowest efficient fermentation temperatures were 32 and 24°C for P. acidilactici and P. pentosaceus, respectively. These temperatures are used in commerce. At 24°C, it was more efficient than P. pentosaceus. P. acidilactici took 3.3 times longer to reduce the pH for a particular LAB.

This study has at least two practical implications. The Ea can be used for the selection of efficient fermentative species/strains of LAB. The lower the Ea, the more efficient is the species/strain under consideration. In this study, P. pentosaceus was more efficient than P. acidilactici at temperatures above the discontinuity. The temperature of the discontinuity on the Arrhenius plot can be used to determine the lowest efficient temperature of fermentation for a particular LAB. In this study, the lowest efficient fermentation temperatures were 32 and 24°C for P. acidilactici and P. pentosaceus, respectively. These temperatures are used in commerce. At 24°C, it took P. acidilactici 3.3 times longer to reduce the pH to 5.0 as compared to P. pentosaceus.

Homofermentative bacteria such as P. acidilactici and P. pentosaceus utilize the phosphoenolpyruvate (PEP):sugar phosphotransferase system (PTS) for the transport of dextrose into the cell (7). The Embden-Meyerhof-Parnas (EMP) pathway, by which dextrose is fermented, depends on the sugar transport system(s) for its substrate. On the other hand, the PEP:PTS depends on the EMP pathway for PEP required for transport (2). Thus, the EMP pathway and the PEP:PTS are interrelated. The sugar-specific protein of the PEP:PTS, enzyme II, is an integral component of the bacterial cellular membrane and is in association with phospholipids which are necessary for its activity (3,7). It is possible that the discontinuities in the Arrhenius plots (Fig. 1) may partially reflect the effect of temperature on the transport of dextrose into the cell. The metabolism of dextrose via the EMP pathway is reduced with a decrease in temperature and shows a discontinuity on an Arrhenius plot. Since EMP enzymes are soluble, this suggests dependence of EMP activity on some membrane event such as sugar transport. The phase transition that takes place in the lipid portion of the cellular membrane at temperatures below the discontinuity of the Arrhenius plot may affect the transport of the sugar via enzyme II. This protein is presumed to move or rotate within the membrane. The formation of solid gel domains during the phase transition of the membrane lipids decreases the mobility of this sugar carrier (5,9). This condition may lead to a decrease in the amount of functional enzyme II and to a higher Ea (5). The end result may be less sugar transported into the cell and slower glycolysis which is reflected by a lower fermentation rate. It was reported previously (5,8,10) that the discontinuities on the Arrhenius plot of glucose transport correspond to the liquid crystalline solid gel phase transition of Mycoplasma and Escherichia coli membrane lipids. Although results from studies of Mycoplasma and E. coli may not be related directly to Pedioococcus, they may serve to develop working hypothesis.

REFERENCES