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BIOENERGETICS AND CATION PERMEABILITY OF MEMBRANES OF EXTREMOPHILES

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The ion and particularly the proton permeability of cytoplasmic membranes play a crucial roles in the bioenergetics of micro-organisms. A basic postulate of the chemi-osmotic hypothesis is that the proton permeability of cytoplasmic membranes is low so that proton pumping systems in these membranes can effectively build up a high proton motive force To test the validity of this hypothesis in organisms that grow under extreme conditions membrane veiscles were isolated from psychrophilic, mesophilic, thermophilic and hyperthermophilic bacteria and archaea and also from halophilic archaea. The proton and the sodium-ion permeabilities were measured in these membrane vesicles and in liposomes prepared from lipids isolated from these vesicles.

In all membranes the proton and sodium-ion permeability increased with the temperature. Membranes from psychrophilic and mesophilic bacteria and from mesophilic , (hyper)thermophilic and halophilic archaea had about the same proton permeability at the temperatures at which the organisms were grown. These observations suggested that micro-organism are capable of adjusting the lipid composition of their membranes in such a way that the permeability of protons at the respective growth temperatures was constant. This *homeo-proton permeability theory* was confirmed by studies in membranes of *Bacillus subtilis* grown at different temperatures.

Thermophilic bacteria are an exception. These organisms are unable to maintain a constant proton permeability at high temperatures of growth. At their optimum growth temperature their membranes are extremely leaky for protons and these organisms cannot maintain a proton motive force. The sodium-ion permeability was found to be very similar and a factor 3 lower than the proton permeability in all membranes studied and to increase with temperatures in all membranes in the same way. Thermophilic *bacteria* rely on this low sodium-ion permeability to generate a *sodium-motive* force which is subsequently used to drive energy requiring membrane bound processes.

Van de Vossenberg et al. (1999) La Recherche 317:54-56.; van de Vossenberg et al.(1998) Extremophiles 2:163-170.

MALTOSE AND FRUCTOSE METABOLISM OF *LACTOBACILLUS SANFRANCISCENSIS* UNDER HIGH PRESSURE CONDITIONS

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Introduction. *Lactobacillus sanfranciscensis* is a heterofermentative lactic acid bacterium with industrial application in sourdough fermentations. Heterolactic fermentation of maltose by *L. sanfranciscensis* results in production of lactate and ethanol. In the presence of fructose as co-substrate, lactate and acetate are the major end products and fructose is reduced to mannitol (Stolz et al., 1995). We studied the influence of high pressure on the maltose metabolism of *L. sanfranciscensis* in the presence or absence of fructose at pressures ranging from 0.1 to 300 MPa. Substrate consumption and product formation was determined by HPLC.

Maltose and fructose metabolism under high pressure. *L. sanfranciscensis* LTH2581 tolerates pressures of 0.1 to 150 MPa without significant loss of viability. In the absence of fructose, lactate and ethanol were the sole products of maltose metabolism at any pressure. Metabolism was inhibited at 200 MPa. In the presence of fructose, application of 50 MPa did not affect maltose uptake, the amount and composition of metabolites remained unaltered. Incubation at 100, 130, and 150 MPa resulted in a decrease of maltose consumption by 67%, 75% and 82%, respectively. The molar ratio of lactate produced to maltose consumed was unchanged even by pressures of 100 - 150 MPa. Incubation at 100 - 150 MPa resulted in formation of lactate and acetate only, ethanol formation was not observed. This results conforms with the preferential production of acetate observed also at comparable maltose turnover levels at ambient pressure in the presence of fructose. Whereas *L. sanfranciscensis* treated at 100 MPa exhibited normal metabolic activity after pressurization, pressure treatment at 150 MPa results in an inhibition of maltose metabolism up to 3 h post-treatment. Application of pressure of 200 MPa reduced viable cell counts by 2 log cycles and metabolic activity was reduced to less than 10% of the activity of the control.

Isotope distribution in metabolites formed under high pressure. To elucidate mechanisms of pressure induced effects on carbohydrate metabolism, the ratio of ^{12}C / ^{13}C isotopes in maltose and ethanol was measured by IRMS and GC-c IRMS, respectively. The isotope ratio analysis of carbon in ethanol produced by *L. sanfranciscensis* from maltose revealed that incomplete maltose conversion resulted in an enrichment of ^{12}C in the ethanol due to a kinetic isotope effect. Preliminary results suggest that this kinetic isotope effect is enhanced by metabolism under high pressure conditions with the same maltose turnover levels.

Stolz, P. et al. (1995) Z. Lebensm. Unters. Forsch. 201:402-410.