RELATIONSHIP BETWEEN BIOLOGICAL AND PHYSICAL PROPERTIES OF DRY YEAST CELLS

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Physical and biological studies of microbial cells dried to varied extents would seem to provide information of interest concerning the properties and functions of water in native cells. As mentioned in the previous paper (1), an excellent series of sample for studies of this kind can be readily prepared from dried baker's yeast commercially available as active dry yeast. Using these samples humidified to various water contents, several physical measurements were made by the present authors with special regard to the properties of the cell water (2).

In order to make comparative studies on their physical properties and biological activities, biological experiments are needed on the same series of dry yeast. It is known that the heat resistance of dry yeast is markedly affected by the cell moisture content, but no systematic data seems to be available in the literature. Dry yeast cells lose their viability when rehydrated with a cold medium, the so-called "cold shock effect", but no explanation has been presented so far for this phenomenon.

Two groups of experiments were designed in the present work: one is concerned with the heat resistance of the dry yeast and the other with the cold medium effect mentioned above. The results of the former show the dependence of the heat resistance upon the cell moisture in a quantitative manner, giving apparent activation energies for this inactivation process, while the latter shows that the cold medium effect can be prevented by preheating as well as by prehumidifying. Those properties of dry yeasts with varied moisture content will be discussed in terms of a domain map presented to describe physical states of the cells.

MATERIALS AND METHODS

Test organism. Active dry yeast, commercially available from the Oriental Yeast Co., Ltd. was used as the starting material for the present preparation. The active dry yeast had a spherical pellet structure with

diameters ranging from 0.5 to 1.3 mm. After sieving the pellet fractions of medium size (0.7-1.0 mm) were used. This fraction had a water content of 7-8% and its viability was found to be invariably above 90%.

A series of samples with various water contents between 4-25% were prepared by placing the active dry yeast in desiccator hygrostats which contain a large amount of sulfuric acid solution at proper concentrations. After standing for more than 24 hr at 30° in atmosphere of desired humidity, the yeast cells were found to attain an equilibrium moisture. To obtain a sample with a water content less than 4%, the active dry yeast was dried in vacuum at room temperature.

The samples thus prepared showed little hysteresis in their biological and physical properties, provided due precautions were taken during the preparation procedure. All treatment procedures must be made slowly in isothermal ways, humidification being achieved through the water vapor phase.

The cell water content was measured as weight decrease after heating for 5 hr at 105° and is expressed in weight percentage on the wet base.

Heat inactivation experiment. (50 ± 0.5) mg of yeast cells with various moisture contents were weighed into a glass tube 2 mm ID and 50 mm length, which was then sealed and placed for 60 min in a water bath at a given temperature. Since the volume of the air space was small as compared to that occupied by the cells in the sealed tube, the cell moisture was assumed to be approximately constant during the heating process, the amount of evaporated water being neglected.

The glass tube was then taken out and opened after cooling in chilled water for several minutes. The heated yeast was hydrated with 1.5 ml water at $30 \pm 0.5^\circ$ and stained with a 0.01% alcoholic solution of methylene blue. The stained fraction was counted with a hemocytometer under the microscope. The methylene blue values were given as differences between the values of the stained fractions obtained with the heated yeast and those with the untreated yeast used as a control.

Rehydration experiment. 30 mg dry weight of yeasts of various moisture contents were added to 100 ml of water in a beaker placed in a thermostat regulated at given temperature. After the pellet structure of the samples disappeared upon standing for 10-20 min, the suspension was slowly stirred and aliquots of 1 ml were taken to be used for methylene blue tests and colony count tests. Released substances were evaluated with the centrifuged supernatant by measurement of optical densities at $260 m\mu$ and by chemical analysis of carbohydrates using the anthrone reagent.

RESULTS AND DISCUSSION

Heat inactivation

Survival curves for heating were studied by use of the methylene blue

Fig. 1. Heat inactivation curves for dry yeast.

Killed fractions were estimated with the methylene blue staining method. At temperatures below 70°, the inactivation could not be observed during the period of the test.

Fig. 2. Heat resistance of dry yeast as function of water content.

The temperatures at which 50% of the cells were killed after 30 min heating are shown for dry yeasts of various water contents.

staining as described above. The results were in agreement with those obtained by the colony counting method. An example of survival curves for yeast with a water content of 12.7% is shown in Fig. 1, where the ordinate represents the percent fractions of stained cells and the abscissa the time of heating at respective temperatures.

The temperatures at which 50% of the yeast cells were stained after 30 min heating were taken as a measure for the heat resistance of the yeast cells with specified water contents. The heat resistance thus obtained was plotted against the water content as shown in Fig. 2. The heat resistance increases rapidly as the moisture decreases in the range below 20%.

By assuming the killing process shown in Fig. 1 as a reaction of the first order and taking the initial tangents of the curves as the expression for the velocity constants, the apparent activation energy (ΔE) for this process is obtained by the Arrhenius plot as shown in Fig. 3. The values, i.e., 80 kcal/mole for wet cells and 60 kcal/mole for dry cells, may correspond to the activation energies for the rate determining step in each complex process.

Rehydration experiment

The activity of rehydrated dry yeast depends upon the temperature

Fig. 3. Temperature dependence of the inactivation rate constant.

Arrhenius plots are shown by assuming first order kinetics for the following samples with various water contents:

—●— 4.0%, —○— 7.0%, —△— 12.3%,
—□— 23.9%, —◐— 62.3%

of hydrating media used; at a given temperature it depends upon the moisture content of the dry yeasts. In general, the more dried the sample is and the lower the medium temperature is, the lower activity level is obtained.

In Fig. 4 are shown the dependence of methylene blue values and amounts of released substances upon the moisture content. These cells were rehydrated by using water at 10°. As seen from the figure, the cold medium effect is marked only for yeasts of water content less than 20%. Therefore, this effect of the cold medium can be prevented by prehumidifying the sample up to the range above 20%.

In Fig. 5 is shown the dependence of the cold medium effect upon the temperature of the medium used. As seen from the figure, the effect was observed for all media below 30°, showing a 50% decrease in the viable count at 15°. The temperature of 50% viability rises as the cell moisture decreases. The inactivation effect of a cold medium mentioned above seems essentially to depend upon the cell state as function of the cell temperature

Fig. 4. Cold medium effect of dry yeast as function of water content.

The effect observed in a rehydration at 10° is shown with dry yeasts of various water contents. Killed Fraction, stained fraction as observed by the methylene blue test; Carbohydrate, amount of released carbohydrates in arbitrary units as analysed by the anthrone reagent.

before and during the rehydration procedure. In fact, a similar protective effect as shown by prehumidification was found with samples preheated prior to the rehydration procedure. In Fig. 6, open circles represent the viable counts of the yeasts heated in a sealed tube for 30 min at respective temperatures and cooled before hydration. These data show that, if observed under a specified condition of rehydration, the cold inactivation varied in accordance with the cell state of yeasts prior to hydration.

If the heated cells were left at room temperature for a prolonged period before the hydration procedure, the protective effect of preheating was observed to disappear. The time course of the disappearance of the protective effect is given in Fig. 7, where the viable counts and the amounts of released substances are shown to approach the control levels of untreated cells after several hour standing at room temperature. The preheated cells were at approximately room temperature after several minutes of cooling, these curves seem to indicate that the related biological state of the cells

Fig. 5. Cold medium effect as function of rehydration temperature.

Viable count (—Δ—) denotes viable counts as expressed in (cells/ml) $\times 10^{-8}$. Other notations, i.e., 260 mμ (—⊙—), Killed Fraction (—□—) and Carbohydrate (—●—) are the same as in Fig. 4.

Fig. 6. Protective effect of preheating for the cold medium inactivation.

Open circles represent data on dry yeasts preheated for 30 min at respective temperatures. The solid one shows the value for untreated sample used as a control.

Fig. 7. Time course of disappearance of the protective effect.

The upper half of the figure shows a relative increase in the amount of released substances as revealed by optical density measurement at $260\text{ m}\mu$. The lower half shows a decrease in the viable count. The horizontal chain line indicates the level of $260\text{ m}\mu$ and Viable Count for the unrelaxed cells preheated at 55° for 30 min. All samples are relaxed at 20° and rehydrated at 9° .

was relaxed with a time constant of several hours order of magnitude at room temperature. The time delay in this cell state transition might be due to the hindered mobility of the constituent molecules of yeast cells under the dry conditions.

Domain map

The biological experiments mentioned above combined with biophysical results described elsewhere (1), indicate that the dry yeast cells always

Fig. 8. Domain map for the cell state of dry yeasts.

Open circles represent phase points where the yeast cell did not show a noticeable cold medium effect, while crosses indicate those where the effect was observed. Since curve AC was drawn in a somewhat arbitrary way, it does not mean the existence of a sharp boundary of phase transition.

assume equilibrium states defined by their temperature and their moisture content, provided that preparation procedures are carried out in a quasi-static way. If so, the state of the cells can be expressed as a point on a phase map where the ordinates denote the cell temperatures and the abscissas the cell moistures.

According to the cold medium effect, the phase map can be divided into two domains, α and β , as shown in Fig. 8. The cells in the α -domain will show the effect, while those in the β -domain will not be damaged when in contact with a cold medium. The phase transition between α and β can be realized reversibly. However, the time rate of transition is rather slow and this is especially the case when the transition takes place in the direction from β to α .

The heating survival data show that the β -domain has its limit in the north-east direction as shown with curve BC in Fig. 8. Beyond this border line, there is another domain (γ), where the cells cannot be alive. Therefore, the transition from β to γ is an irreversible one and, when it occurs,

it means the death of the cells. Another transition which occurs directly from α to γ is also of irreversible nature. As seen from Fig. 2, those two irreversible transitions seem to have the aparent activation energies of 80 kcal/mole and 60 kcal/mole, respectively. The nature of these phase transitions, both reversible and irreversible, is unknown at present. However, the transition between α and β seems to be accompanied by some kind of physical transition between different states in the cell membrane, giving rise to a remarkable change in its permeability as observed after being rehydrated.

SUMMARY

By using a series of dry yeasts prepared to have various water contents, the resistivity to heat inactivation and cold medium treatment was studied.

The heat resistance of the samples showed a marked dependence upon the water content, giving activation energies of 80 kcal with relatively wet cells and 60 kcal with relatively dried cells, respectively. The lowering of the biological activity shown after the rehydration with a cold medium was found to be prevented, at least partially, by preheating the sample at specified temperature before the rehydration procedure. This protective effect disappeared after several hour standing at room temperature, suggesting that the related physical state relaxed with a time constant of hours in order of magnitude.

A domain map is presented to show diagrammatically the relationship between biological properties of the dry yeast and its related physical states in terms of the cell temperature and the cell water content.

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