Elucidation of the stationary-phase specific amino acid catabolic pathway in budding yeast and its application to cell longevity

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Research objective

The budding yeast *Saccharomyces cerevisiae*, which has been used in the field of brewing for thousands of years, has been recognized as a model organism to study the basic mechanisms of biological phenomena. The process by which budding yeast cells in liquid culture lose their viability after the stationary phase is called chronological senescence, and has been analyzed as a model for the aging of non-proliferating cells. Extensive studies have shown that the senescence process involves internal factors, such as the production of reactive oxygen species and the storage of carbohydrates during the stationary phase, as well as external factors, such as the amount of carbon sources and amino acids and changes in the pH of the culture medium.

In most aging studies of budding yeast, synthetic defined (SD) medium, a minimal synthetic medium containing ammonium sulfate as a single nitrogen source, has been used. When budding yeast is cultured in this medium, the pH of the medium, which is approximately 4.5, drops rapidly to approximately 2.2 the next day. When synthetic complete (SC) medium[1)] with an additional 14 amino acids was used, the decrease in pH was suppressed and the pH was maintained at approximately 2.8. We have shown that the amino acid-mediated suppression of medium acidification prolongs the lifespan of yeast[2)]. The purpose of this study was to analyze the mechanism of delayed aging induced by the addition of amino acids, focusing on metabolic changes in yeast cells. This study is expected to lead to the elucidation of new adaptation pathways to physiological stresses and to novel strategies for extending the lifespan of industrial yeasts.

Methods

Yeast: *S. cerevisiae* strain YOM36 (MATa wild-type)[2)]

Medium: SD medium (2% glucose, 0.17% Bacto-yeast nitrogen base without amino acids and ammonium sulfate, and 0.5% ammonium sulfate) and AYD medium (Patent 6429627, Kohjin Life Science Co., Ltd.).

Measurements of the medium composition: The culture medium was centrifuged at 3,000 rpm for 2 min, and the supernatant was used for various measurements: pH was measured with a pH electrode (Mettler Toledo), ammonia concentration with an ammonium electrode (Toko

Chemical Laboratories Co., Ltd.), and serine concentration with a DL-Serine assay kit (Sigma Aldrich).

Results

(1) Determination of amino acids that inhibit acidification of the culture medium

We prepared a medium with only one of the amino acids present in the SC medium at the same molar concentration and measured the pH of the medium during the stationary phase (9 days after culturing). The results showed that L-glutamic acid and L-serine had inhibitory effects on medium acidification, and L-serine had a remarkable inhibitory effect.

We found that the pH of the medium increased during the stationary phase when cultured in the AYD medium containing high concentrations of amino acids, and the increase in the pH was not observed when L-serine was removed from the medium. This observation confirmed that L-serine had the greatest effect on inhibiting acidification of the medium environment. This effect was not observed when D-serine was used, suggesting that serine uptake and metabolism in yeast cells is involved in this phenomenon.

(2) Time course of serine consumption in the medium

In the medium supplemented with L-serine, the pH increase occurred after more than one week in the stationary phase. The amount of serine in the medium decreased at a constant rate from the beginning of the culture. In addition, cultivation in an L-serine-supplemented medium suppressed the consumption of ammonium ions in the medium.

(3) Involvement of the metabolic pathway induced by serine supplementation

Among the genes induced by L-serine addition, *CHA1* encodes serine threonine dehydratase[3]. This enzyme has the ability to degrade serine into ammonium and pyruvate. In fact, the Cha1 protein, which is rarely expressed under normal culture conditions, was induced from the logarithmic growth phase when L-serine was added to the culture.

In the *CHA1*-deficient strain, the suppression of ammonium ion consumption by serine addition was no longer observed, and the pH of the culture medium was greatly reduced, as was observed when the wild-type strain was cultured in serine-free medium. This result indicates that the ammonium produced by the degradation of serine by the Cha1 protein is utilized as a nitrogen source during the logarithmic growth phase and thereafter.

(4) Function of serine as a carbon source

Although *S. cerevisiae* can utilize a single amino acid as the sole nitrogen source, it cannot grow in a medium containing amino acids as a carbon source[4]. This means that this microorganism typically grows in environments with limited nitrogen sources.

Cha1, which plays an important function in the growth of yeast populations after the stationary phase, has the ability to degrade threonine in addition to serine, but the addition of threonine did not reproduce the effect of serine. This suggests that pyruvate, rather than

ammonium ions, is responsible for the observed effect. The fact that the maximum yield of cells increases in serine-supplemented cultures suggests that serine is also used as an energy source under certain conditions. Moreover, the fact that the pH of the medium increased early in the culture using the medium fraction recovered from the late stationary phase suggests that a change in the composition of the medium promotes an increase in pH.

Conclusion

In the process of analyzing the medium conditions that delay chronological senescence, we observed that the survival rate increased when a certain amount of a specific amino acid was present in the medium, and that amino acid utilization continued during the stationary phase when growth had stopped. Although it is known that budding yeast cannot grow using free amino acids as a sole carbon source, our results indicate that metabolic reactions using specific amino acids as a source occur in stationary phase cells with limited carbon sources, and that these reactions contribute to the maintenance of viability.

Elucidation of the function of amino acid metabolism in *S. cerevisiae* under extreme conditions will lead to the elucidation of the ability of this yeast to utilize amino acids, which has not been observed under normal conditions. Based on the results of this study, we propose that the use of yeast with constitutively activated amino acid catabolism to avoid the problem of medium acidification in fermentation using a carbon source, and the suppression of senescence and cell death caused by acidification of the culture medium. In the future, we hope to genetically modify the metabolism of microorganisms to increase their longevity and contribute to the improvement of material production capacity in the fermentation industry using *S. cerevisiae* and other microorganisms.

References

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