New Process for Reducing the Nucleic Acid Content of Yeast

It has been shown that if single-cell protein is to be used as a primary protein source for human populations, the nucleic acid content should be reduced to a level which would allow a maximum intake in the range of 2 g of nucleic acid per day[1]. For a processing method to be acceptable, it must not only decrease the nucleic acid level to the required degree, but it must also be inexpensive and must not contaminate the product with undesirable chemicals.

Candida utilis was grown in continuous culture[2] with glucose as the carbon source at *p*H 4·0 and at a dilution rate of 0·19 h^{-1} ($\mu_{max} = 0{\cdot}4\ h^{-1}$). The initial nucleic acid content of the cells in these growth conditions was approximately 7 per cent. After collection, the cells were treated in a three-step heating process to produce cells with 1·0–1·5 per cent nucleic acid and approximately 50 per cent protein. The successive steps involved were: (1) heat shock of a few seconds duration at 54°–70° C; (2) incubation at 45°–50° C; and (3) incubation at 55°–60° C. All three steps seem to be necessary for optimum results although a compromise temperature between 50 and 60° C is possible for the last two steps.

The heat shock was performed by passing a suspension of *C. utilis* in spent medium through a 56 cm length of 1/32 inch inner diameter (1/16 inch outer diameter) stainless steel tubing immersed in a vigorously stirred water bath at the desired temperature. For the remaining steps a nominal residence time of 5·5–6·0 s and a temperature of 68° C were chosen. After heat shocking, the cells were collected at 0° C or were treated immediately at 45°–50° C. The method of collection did not affect the final results.

The data in Fig. 1 indicate an optimum temperature range of 62°–68° C for a heating time of 17 s and an optimal temperature of 68° C for treatments of shorter duration. Other experiments have shown that longer heating times at lower temperatures or shorter heating times at higher temperatures do not lead to an optimal process under any conditions of subsequent incubation. When the heat shock is omitted the remaining two steps result in only a 10 per cent reduction in nucleic acid compared with an 80–85 per cent reduction when heat shock was used. After heat shock alone the nucleic acid content of the cells is not significantly altered.

Recalculation of data presented by Bodley[3] for the rate of *E. coli* ribosome denaturation indicates a half life of 0·6 s at 68° C. Haight and Ordal[4] have shown a ribonuclease associated with staphylococcal ribosomes which is activated by heat and attacks only the RNA closely bound within the same ribosome. The heat shock in our process may serve either or both of these functions. The final two steps probably allow a complex series of enzymatic hydrolysis reactions to occur culminating in the leakage of these products from the cells into the surrounding medium.

One of the principal functions of the third step is to allow leakage of the accumulated hydrolysis products from the cells, but there is also a significant amount of additional hydrolysis of polynucleotides. If the second step is conducted at 45° C there is essentially no leakage of UV absorbing material into the medium in the absence of step 3. The optimal time and temperature of incubation of this third step were found to be 1 h at 55° C when the second step was conducted at 45° C. A slightly higher temperature of 60° C results in poorer nucleic acid removal, possibly because of the denaturation of the endogenous ribonucleases preventing further degradation of polynucleotides or alternatively of the formation of RNA aggregates resistant to enzyme action. The optimum range for the overall process is between *p*H 5·0 and 6·5.

In view of the close similarity between the optimum conditions of heat shock for breakdown of *C. utilis* RNA and the conditions required for irreversible thermal denaturation of *E. coli* ribosomes[3], we expect that our method can be applied to other types of microbial cells. We have begun to scale up the process of *C. utilis* in order to prepare enough product to conduct feeding studies. We are also trying to determine the exact nature of the reactions involved in each of the steps.

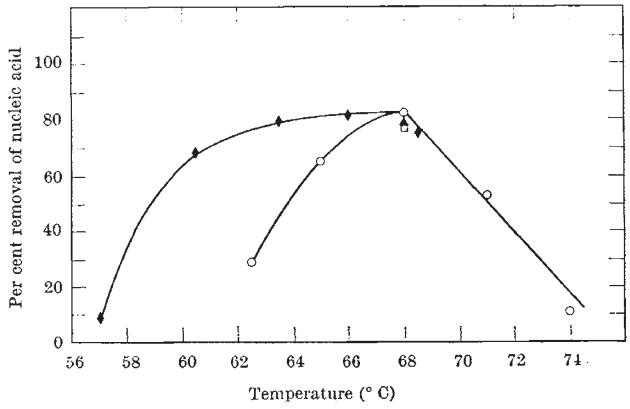

Fig. 1. Effect of heat shock temperature and time on nucleic acid removal. Step 1: variable; step 2: 45° C, 2 h; step 3: 55° C, 1 h. Residence time: ▲, 3 s; ○, 6 s; □, 12 s; ◆, 17 s.

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S. B. Maul
A. J. Sinskey
S. R. Tannenbaum

Department of Nutrition and
Food Science,
Massachusetts Institute of Technology,
Cambridge, Massachusetts 02139.

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Absence of the Pigments of Photosystem II of Photosynthesis in Heterocysts of a Blue–Green Alga

All filamentous blue-green algae capable of fixing elementary nitrogen have heterocysts. Stewart *et al.*[1] have strong evidence that these differentiated cells are the sites of nitrogen fixation. They did not, however, show that photosystem II, responsible for the evolution of molecular oxygen (O_2), is not functional in heterocysts. Because high oxygen tension inhibits nitrogen fixation, heterocysts should not possess the pigments of photosystem II. Fay[2] produced evidence *in vitro* suggesting that heterocysts do not contain c-phycocyanin (c-PC), a principal constituent of photosystem II. Stewart *et al.*[1] found much less c-PC in heterocysts than normal cells and believed that photosystem II could be absent in heterocysts. Others[3,4], however, have demonstrated the evolution of O_2 from blue-green algae in the absence of c-PC. Thus the points to be resolved are whether heterocysts *in vivo* contain c-PC and the other pigments comprising photosystem II, and whether, in the absence of one or more of these, photosystem II is functional in heterocysts. A comparison of the *in vivo* pigment composition of normal cells and heterocysts has indicated that heterocysts lack a functional photosystem II.