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## Journal of Fermentation Technology. Vol. 33, No. 1. 1955

### Studies on the Rôle of B-Vitamins in Sake-Brewing (I)

#### Effects of B-Vitamins on the Growth of *Saccharomyces sake*

Saburo FUKUI (Himeji Coll. Techn.),

Yoshio TANI (Dept. Indust. Chem., Facult. Engin., Kyoto Univ.),

and Tadanobu KISHIBE (TANI Sake-brewing Co., Ltd.)

In this paper, the authors have reported on the effects of B-vitamins upon the growth of *Sacch. sake* (strain No. 7) which is most widely used in sake-brewing in Japan.

The results obtained are as follows:

1) Under our experimental conditions, no growth of the yeast was observed in a vitamin-deficient medium, the nitrogen source of which consisted of organic compound such as a mixture of amino acids or casein hydrolyzate.

On the other hand, when ammonium sulphate was used as the sole nitrogen source, the yeast failed to grow at the initial stage, but began to grow after an incubation period of some days.

2) The media containing eight B-vitamins (thiamine, riboflavin, pyridoxine, niacin, PABA, biotin, inositol and pantothenic acid (PA)) permitted an excellent growth. However, in the media devoid of PA, the yeast exhibited the same behavior as in the vitamin-deficient media, and a considerable reduction in the growth was also found when inositol was omitted. The omission of the other vitamins showed no effect.

3) When the deficient medium was supplied with PA alone, the growth-promoting action occurred, but the effect was smaller than that of the complete medium.

Thiamine, biotin and inositol had respectively a cooperative effect on the growth with PA, and the combined use of these four vitamins exerted the effect identical with that of the eight vitamins.

The response of *Sacch. sake* (strain No. 7) to increasing concentration of PA and inositol (In) has also been studied.

When the amount of PA was below 200  $\text{m}\gamma$  per 10 cc of the final medium and the size of the inoculum was regulated to provide 4,000 or 120,000 cells per cc of that medium according to the experimental conditions, the yeast showed a quantitative growth response.

In the case of In, the limiting amount was 50 $\gamma$  per 10 cc of the final medium.

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### Studies on the Soy Manufacture by Enzymatic Method (III)

#### Estimation of Glutamic Acid by Buffered Paper Chromatography

Kunisuke ICHIKAWA (Dept. Indust. Chem., Facult. Engin., Kyoto Univ.)

Mc FARREN, LEVY and CHUNG have reported on the buffered paper chromatography. According to their studies, several amino acids were completely separated from one another by employing one-dimensional buffered paper chromatography. For example, glutamic acid, aspartic acid, glycine and serine were completely separated at pH 10.

(2)

In the previous paper, this author reported on the estimation of amino acids by two-dimensional paper chromatography. If one-dimensional method, however, were used for quantitative analysis, the efficiency of estimation would be very much increased. In this paper, the study for this purpose has been reported.

1) The area of spot developed on filter paper was found to be a simple function of the concentration of glutamic acid.

2) The R<sub>f</sub> values of the amino acids were very changeable by Cl ion.

3) Applying this method to the enzymatic digestion of soy bean cake, the behavior of glutamic acid was observed. The results showed that only 25~30% of glutamic acid in the raw material was isolated by enzymatic digestion for 4~6 days.

## Studies on the Nutrient Substances of Hiochi-kin (Putrefactive Bacteria of Japanese Sake) and their Antagonists (VI)

### Antagonisms between Several Amino Acids (1)

#### Inhibitory Effects of Isoleucine and Valine on Leucine Utilization

Shiro TERAMOTO, Wataru HASHIDA, and Eihachiro YASUDA

(Dept. Ferment. Techn., Facult. Engin., Osaka Univ.)

In a study of some effects of the excess amounts of amino acids on the growth of Hiochi-kin (strain T 101, T 105, T 107), DL-threonine, DL-tryptophan, DL-leucine, DL-isoleucine, DL-valine, and DL-aspartic acid were observed to be inhibitory, while DL-alanine, L-arginine-HCl, L-cysteine-HCl, glycine, L-histidine, DL-methionine, DL-norleucine, DL-norvaline, DL-phenylalanine, and L-tyrosine were found to be non-effective.

The mutual antagonism between leucine, isoleucine, and valine on the growth of Hiochi-kin strain T 107 was investigated by cultivating it in the synthetic medium described in the previous report (This Journal; 31, 502 (1953)). The utilization of L-leucine was inhibited moderately by L-isoleucine, and slightly by DL-valine. The antagonistic actions were observed especially in the low level of L-leucine. The utilization of L-isoleucine was not inhibited by L-leucine and DL-valine.

## Studies on the Classification of Streptomycetaceae and Actinomycetaceae (VI)

### Microscopic Morphology of *Streptomyces spec.* No. 254

Hirosuke NAGANISHI, and Ryosaku NOMI

(Dept. Ferment. Techn., Facult. Engin., Hiroshima Univ.)

We researched microscopic morphological characters of *Streptomyces spec.* No. 254.

This strain was isolated from soil in our laboratory. The morphological characters are considerably different from the three strains reported in preceding papers. On the CZAPEK-Dox agar and CB series media agar, the aerial mycelia are well developed, powdery or a little cottony, and brownish pink. The aerial mycelia are branched monopodially or in cluster and have many long straight or slightly bent hyphae.

The terminal filaments are long straight or long wavy and the ends of them often give rise to spirals, especially on CB-III agar. The forms of spirals are flat

(3)

and irregular, and the curvature is dextrorse or sinistrorse, compact, 1~5 turns,  $3.2\sim 9.6\mu$  (occasionally to  $12\mu$ ) in diameter.

The aerial hyphae have a diameter of  $0.8\sim 1.2\mu$ , and no septa. The oidiospores are oval or short rod-shaped,  $0.8\sim 1.2\mu \times 1.0\sim 2.4\mu$ .

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## Studies on the Prevention of White Clouding of Canned Bamboo-shoots (V)

### Detection of Organic Acids and Bases by Paper Chromatography

Mitsuharu FUJII, and Minoru UKITA (Shikoku Branch of the Osaka Industrial Research Institute)

The free organic acids and bases detected in the dried bamboo-shoots were as follows :

1) Six spots corresponding to oxalic, citric, tartaric, malic, lactic and fumaric acids were detected (Table 2).

For the detection of organic acids, a mixture of phenol, formic acid and water was more suitable than any other solvents.

2) Four spots corresponding to guanine, adenine, xanthine and hypoxanthine were detected, but pyrimidine bases were not (Table 4, Fig. 1).

The revelation method of REGUERA et al. gave excellent results with purine bases, etc.

3) The spots corresponding to choline and betaine and three spots presumable to be produced by alkaloid-like substances were detected (Table 5). Chromatograms with DRAGENDORFF's reagent were clear and permanent.

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## Studies on the Organic Acids in Sake (I)

### On the Paper Partition Chromatography of Keto Acids

Norio ANDO, Kazuhide KURIYAMA, and Satoshi AKAMIZAKA (Ōkura Shuzo Co., Ltd.)

1) Paper partition chromatography was applied for the determination of the keto acid 2,4-dinitrophenylhydrazones obtained from the samples that were the vacuum condensed solutions of sake, moto, and putrified sake.

2) When the 2,4-dinitrophenylhydrazones obtained from sake and moto were dissolved in a solution containing sodium hydroxide, the paper partition chromatograms showed several unknown spots, some of which gave a little fluorescence with ultraviolet light.

3) Paper partition chromatogram of pyruvic acid 2,4-dinitrophenylhydrazone always showed two distinct spots, and the spot of lower R<sub>f</sub> tended to move towards that of higher R<sub>f</sub> from time to time.

4) The amount of keto acids in sake has a tendency to increase when it putrifies. But, neither the amount nor the kind of keto acids is always to be said as the proof of putrification.

(4)

## Studies on the Carotene Production by Micro-organisms (XI)

### On the Large Scale Cultivation Methods of *Neurospora sitophila*

Ryuichiro ISHII (Osaka Municipal Hygienic Laboratory) and  
Toshihiko MIYAMOTO (Nippon Shiryō-Kogyō Co., Ltd.)

Both the koji-buta method and the cloth shelf method were employed for the cultivation of *Neurospora sitophila* on a large scale.

The latter method was somewhat inferior to the former method in the production of carotene, but was easier to deal with the medium on a large scale.

When the mold grew on wheat bran or a mixture of wheat bran and rice bran, the carotene contents of culture and conidia were 11.9 and 49.6 mg % respectively, while those obtained by the cloth shelf method showed the values 9.2 and 46.6.

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## Studies on *Aspergillus oryzae* Strains for Sake-Brewing (VII)

### On the Proteolytic Activities of Rice-koji (1)

#### On the Determination of Proteolytic Activity with FOLIN-CIOCALTEU's Reagent

Kimio KAGEYAMA and Noriyuki KUNISADA (Yamamura Sake-brewing Laboratory)

We determined the proteolytic activity with FOLIN-CIOCALTEU's reagent as follows:

Casein was digested by proteolytic enzyme under a specific condition, and tyrosine and tryptophan contained in that part of digested casein, which was soluble in 0.2 mol. trichloroacetic acid, were measured by colorimetry using FOLIN-CIOCALTEU's reagent. The reagent was very stable under various conditions, and the standard curve showing the correlation of optical density (E) with the concentration of pure tyrosine and tryptophan was very reliable, and in this case the concentration of trichloroacetic acid exerts no influence on the degree of colorization. We use for the development of colour a mixture of 1 cc of the original reagent (diluted five times), 1 cc of sample solution (containing less than 100γ of tyrosine) and 5 cc of 0.4 mol Na<sub>2</sub>CO<sub>3</sub>, keeping on a water bath at 37°C for 20 min.