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Short Communications

Different Nitrogen Fractions in Normal and Low-Nitrogen Cells of Microorganisms. II. Changes in the Free Amino Acids of Low-Nitrogen *Torulopsis utilis* Yeast during Nitrogen Enrichment by Various Nitrogen Sources

Preliminary communication

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The enrichment experiments reported earlier [1] have been continued with other nitrogen sources. Low-nitrogen *Torulopsis utilis* yeast (N appr. 4 % of dry weight) was prepared from normal *Torulopsis utilis* yeast (N appr. 8 % of dry weight) by driving, in a Kluyver-flask, a strong current of air through a sugar nutrient solution (without combined nitrogen) in which the normal yeast was suspended [2].

The enrichment experiments were made as follows: The washed low-nitrogen yeast was suspended in water in a Kluyver-flask and a strong current of air was driven through the suspension. A 0-sample was taken, whereupon one of the following nitrogen compounds: ammonium sulphate, sodium nitrate, glutamic acid, aspartic acid, glycine, alanine, or asparagine, was added in concentrated water solution to make the initial molarity of the added nitrogen 0.03–0.2. Samples were taken 5, 15, 30 min and 1, 2, and 4 h after the addition of the nitrogen compound.

Each sample was rapidly chilled and washed, and an aliquot was extracted with 70 % ethanol. The ninhydrin-positive compounds of the ethanol extracts were determined semiquantitatively by paper-chromatography using the spot dilution technique [3]. The chromatograms were made by the "continuous development technique" [4] using 1-dimensional runs with *tert.* amyl alcohol for leucine, *iso*-leucine, phenylalanine, methionine, valine, and tyrosine (see ref. 4), and 2-dimensional runs (see Fig. 1) for the other amino acids.

From other aliquots, measured simultaneously, dry weight, total-N, N soluble in hot (10 min extraction at 100° C) and cold (1 h at 0° C) 8.3 % trichloroacetic acid, total nucleic acids (by a slight modification of the method of Di Carlo *et al.* [5]), and desoxyribonucleic acid (Dische-method) were determined and microscopic preparations made. Protein-N and ribonucleic acid were calculated as differences.

The 70 % ethanol extract of low-nitrogen Torulopsis utilis contains:
richly: glutamic acid and alanine,
considerably: arginine, lysine, unknown no. 26 (probably a peptide), aspartic acid, glutathione, glycine, serine, threonine, unknown no. 30 (probably a difficultly hydrolysable peptide), valine, tyrosine, unknown no. 43 (probably a peptide) and unknown no. 41 (unhydrolysable basic compound),
slightly: leucine, isoleucine, phenylalanine, glutamine, asparagine, proline, and methionine.

Histidine, cystine, hydroxyproline, ethanolamine phosphoric acid esther, sarcosine, taurine, γ-aminobutyric acid, α-amino-*n*-butyric acid, α-amino-*iso*butyric

acid and β-alanine have not been present in identifiable amounts.

In hydrolysis (20 % HCl 24h 108° C) glutamine, glutathione, asparagine, and the unknown compounds nos. 26 and 43 disappear completely and compound no. 30 partly and considerable amounts of amino acids, mainly glutamic acid, glycine, alanine, and unknown 41 are set free. In addition an unknown 42 appears.

The presence of *peptides* containing mainly glutamic acid, glycine and alanine, but in smaller amounts other amino acids, has been further confirmed by cutting strips (corresponding to compounds 26 and 30) out from a set of chromatograms, eluting them with water, and examining the concentrated eluate paperchromatographically before and after hydrolysis.

The 70 % ethanol extract of normal Torulopsis utilis was studied for comparison. In general, it contains the same free amino acids as the low-nitrogen yeast but in considerably higher concentrations, arginine, glutamine and alanine being (relatively) most enriched.

The most remarkable changes in the concentrations of the free amino acids in the *enrichment experiments* were the following ones:

I. *Ammonium sulphate:* Ammonium nitrogen was rapidly assimilated and a very powerful formation of glutamine (but not asparagine) rapidly (in less than 5 min) started. Glutamic acid and alanine, too, were rapidly enriched. The unknown no. 30 disappeared completely (in one experiment) or partly in 5 min. Later (after about 1—2 hours) a slow and continuous enrichment of other amino acids, especially of the basic ones, took place.

II. *Sodium nitrate* was only very slowly assimilated and no change in the free amino acids took place within the first 30 min. In 1,2 and especially 4-hours' samples an enrichment of glutamine, alanine and glutamic acid and probably glutathione was to be noticed.

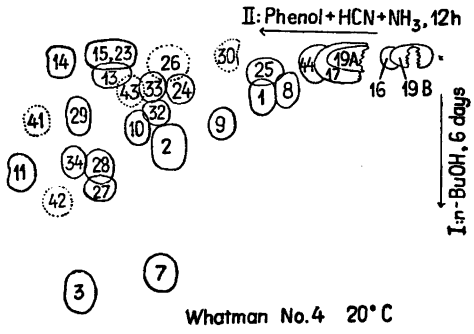

Fig. 1. 1 = Gly, 2 = ala, 3 = val, 7 = tyr, 8 = ser, 9 = thr, 10 = hypro, 11 = pro, 13 = his, 14 = arg, 15 = lys, 16 = asp, 17 = glu, 19 = glutathione, 23 = orn, 24 = glutamine, 25 = asparagine, 27 = α-amino-n-butyric acid, 28 = α-amino-iso-butyric acid, 29 = γ-aminobutyric acid, 32 = β-alanine, 33 = sitr, 34 = sarc, 35 = tau, 44 = phosphorylaminoethanol.

III. *Aspartic acid* was rapidly assimilated but not much enriched in the yeast cells, since it was rapidly metabolised further. Instead, glutamic acid, glutamine, and alanine were strongly enriched. In the 4 hours' sample asparagine was enriched.

IV. *Glutamic acid* was rapidly assimilated and metabolised. At first (5 to 30 min) mainly glutamine and alanine, but later (1 to 2 hours) other amino acids were enriched. In the 4 hours' sample asparagine was enriched.

V. *Glycine* was rapidly assimilated — in 15 min a large amount of glycine was enriched in the cells — but the only change in the other amino acids was at first the disappearance of the compound no. 30. Not until after 2 hours was a formation of glutamine, alanine and glutamic acid noticeable.

VI. *Alanine* was rapidly assimilated but only after about 30 min did a more considerable enrichment of glutamine start.

VII. *Asparagine* was the most potential of the nitrogen sources studied. It was rapidly assimilated and (in addition to

asparagine) a rapid enrichment of glutamine, glutamic acid, aspartic acid, and probably glutathione immediately started. A distinct but less intense enrichment of the other amino acids started rapidly, too.

Ribonucleic acid was rapidly enriched with all the other nitrogen sources, expect sodium nitrate, during the first and second hour, but no greater change took place in the *desoxyribonucleic acid.*

Roine [2] has earlier studied in this laboratory with other methods changes in the free amino acids during the enrichment of low-nitrogen *Torulopsis* with ammonium nitrogen being able to show enrichment of amides — glutamine and asparagine — and glutamic acid and alanine. His results have now been confirmed paperchromatographically.

By the sensitive paperchromatographic method many other free amino acids have now been shown to be present and other changes have been observed.

Virtanen *et al.* [6] have studied enrichment with NO_3-nitrogen finding that amino acid and amide content of the cells rises hereby much less than with ammonium nitrogen, which is in accordance with the results in this paper.

The rapid enrichment of *alanine* in these experiments must be emphasized as well as the great activity of *asparagine* as a nitrogen source, and the presence of *peptides*, which in their amino acid composition resemble more the free amino acid fraction of the yeast than the protein of the yeast.

Full details of this work are being published elsewhere.

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Notiz über die Reaktion zwischen Trialkyl-amino-silanen und Schwefelwasserstoff

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Eaborn [1] hat gezeigt, dass Trimethyl-jod-silan und Silbersulfid mit einander unter Bildung von Hexamethyl-thiodisiloxan reagieren. In entsprechender Weise entstand Hexaäthyl-thiodisiloxan aus Triäthyl-jod-silan. Im folgenden werden wir eine Methode angeben, nach der Hexaalkylthiodisiloxane ohne Verwendung der schwierig zugänglichen Trialkyl-jod-silane dargestellt werden können.

Gasförmiges Schwefelwasserstoff wurde während einigen Stunden in Triäthyl-amino-silan eingeleitet. Bei der Destillation des Reaktionsgemisches wurde *teils* Triäthyl-thiosilanol vom Sdp. 158°, *teils* Hexaäthyl-thiodisiloxan vom Sdp. 128° (7 mm) erhalten. 163 g Triäthylamino-silan ergaben in dieser Weise 34,5 g Triäthyl-thiosilanol und 95,2 g Hexaäthyl-thiodisiloxan. Weiter wurden aus 89,5 g Tri-*n*-propyl-amino-silan und Schwefelwasserstoff 18,4 g Tri-*n*-propyl-thiosilanol vom Sdp. 83—84° (7 mm) und 54,3 g Hexa-*n*-propyl-thiodisiloxan vom Sdp. 168° (7 mm) erhalten.

Versuche mit Trimethyl-amino-silan konnten nicht ausgeführt werden, weil dieses Silan noch nicht dargestellt worden ist. Man hat immer statt dessen Hexamethyl-disilazin erhalten. Dieses reagierte sehr schlecht mit Schwefelwasserstoff. Dagegen ergab Trimethyl-N-phenyl-amino-silan mit Schwefelwasserstoff Trimethyl-thiosilanol vom Sdp. 75—76°. In einem Versuch betrug aber die Ausbeute nur 14 %.

Über die obigen Versuche und einige andere Umsetzungen zwischen Verbindungen $R_3Si \cdot N\begin{smallmatrix}R'\\R''\end{smallmatrix}$ (wo R Alkylgruppen und