

Short Communication

Different Nitrogen Fractions in Normal and Low-Nitrogen Cells of Microorganisms

ARTTURI I. VIRTANEN and JORMA K. MIETTINEN

Laboratory of the Foundation for Chemical Research, Biochemical Institute, Helsinki, Finland

In many papers, Virtanen and collaborators¹ have dealt with the dependence of the enzymatic activity of cells on their nitrogen content. The disappearance or the great decrease in activity of certain enzymes, accompanying the lowering of N-content, has been ascribed to the insufficient protein supply necessary for the building up of all the possible cell enzymes.

Lately, we have examined to what extent the content of different N-fractions has changed in low-nitrogen cells compared with normal nitrogen cells. The first object of research was *Pseudomonas fluorescens*. Low-nitrogen cells were produced according to Virtanen and Kokkola². The nucleic acid nitrogen and the "protein nitrogen" insoluble in trichloroacetic acid were primarily examined. A sample containing the same amount of nitrogen was taken from both normal and low-nitrogen cell masses, NaOH-solution was added to make the suspension 0.1 N to NaOH, and the suspension was shaken vigorously. After 10 min. standing, 50 % trichloroacetic acid was added to make the solution 6.9 % with regard to trichloroacetic acid, and the

mixture kept in boiling water bath for 10 minutes. It was found in the control experiments that the amount of NA extracted in this way was at least 92 % of the amount extractable with 0.5 N NaOH in 1 hr in boiling water bath. However, in the latter stronger alkali extraction yellowish brownish compounds were formed which make the measurements uncertain and surely partly cause the higher absorption. The milder extraction was therefore adopted. Ahlström and v. Euler *et al.*³ have used the same method for the splitting of nucleoproteides and for the extraction of nucleic acids.

The estimation of nucleic acids from the trichloroacetic acid extracts, clarified by centrifugation, was made by determining the absorption spectrum between 240 and 300 m μ . The curves obtained (Fig. 1) were in fairly good agreement with the absorption curve found by Ahlström, v. Euler *et al.*³ for purified desoxyribonucleic acid 99. Evidently the extract did not contain substances interfering with the absorption within the stated region, and the method is thus suitable for the determination of total nucleic acids. In calculating the nucleic acid content, the value $a_{262} = 29.2$ was used. A value of 14 % was taken for the N-content of nucleic acids. The amounts of different nitrogen fractions in normal and low-nitrogen bacterial masses are presented in Tables 1 and 2.

The results show that the percentage of the fraction soluble in trichloroacetic acid is higher in low-nitrogen cells than in normal nitrogen cells. Nucleic acid nitro-

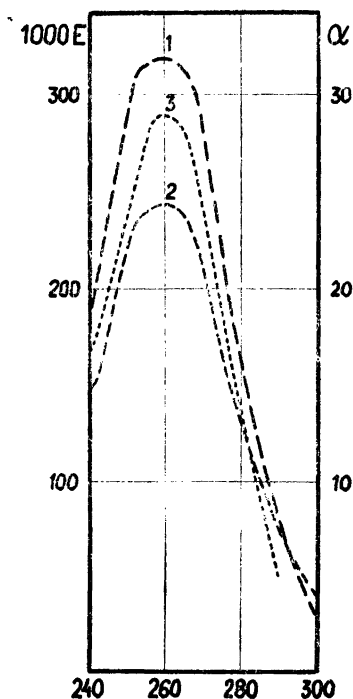


Fig. 1. Absorption spectra of extracts from normal N and low-N *Ps. fluorescens* and of a nucleic acid preparation by Ahlström, v. Euler et al.³

Extinction values

$$1000 \times E \quad (\text{Curves 1 and 2})$$

$$\alpha \quad (\text{Curve 3})$$

Curve 1: Extract from normal N bacteria
 » 2: » » low-N » »
 » 3: DNA-preparation 99 (Ahlström, v. Euler et al.)

gen has decreased considerably more than total nitrogen, whereas no drop occurred in other soluble nitrogen, and accordingly, this nitrogen fraction, in per cent of total N, is in the low-nitrogen cells much higher than in the normal nitrogen cells. This nitrogen fraction comprises the amino acids and small peptides. The lack of nitrogen nutrition has the greatest effect on the decrease of the protein and nucleic acid fractions.

After hydrolysis with hydrochloric acid, amino acids were qualitatively determined

Table 1. Different nitrogen fraction in normal and low-nitrogen bacterial masses.

	Normal N	Low-N	Decrease
g per 100 g dry matter	bact. mass	bact. mass	%
1. Total N	12.7	7.8	38.6
2. "Protein N"	9.18	5.52	39.9
3. N soluble in trichloroacetic acid	3.50	2.30	34.3
4. NA-N	2.27	1.05	54.7
5. Other soluble N	1.23	1.25	0

"Protein-N" = calc. difference between 1 and 3.

Other soluble N = calc. difference between 3 and 4.

Table 2. Different nitrogen fractions in % of total nitrogen.

% of total N	Normal N	Low-N	Difference
	bact. mass	bact. mass	%
"Protein N"	72.3	70.5	- 2.5
Soluble N	27.7	29.5	+ 6.5
NA-N	17.9	13.4	- 24.0
Other soluble N	9.7	16.0	+ 65.0

both in normal nitrogen and low-nitrogen bacterial masses by means of paper chromatography. No difference could be detected in the amino acid composition of the masses. The intensity of the colour spots, estimated by the eye, was also similar in both cases. Thus the sharp decrease in the protein content of cells does not cause changes in the amino acid composition of proteins that could be detected by means of paper chromatography.

The corresponding determinations as with *Ps. fluorescens* were also made with *Torula utilis*. Low-nitrogen yeast was prepared by driving, in a Kluver-flask, a strong current of air through sugar nutrient solution (without combined nitrogen) in which normal-nitrogen yeast was suspended⁴. The analytical data appear from Table 3.

The nucleic acid content of low-nitrogen *Torula* has decreased approximately as much as in low-nitrogen *Ps. fluorescens*. Instead, the insoluble "protein-N" has