The Presence of Intracellular Proteolytic Enzyme Activities in 
Azotobacter vinelandii Strain O 
Cultured in Iron-deficient Medium

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Iron is essentially required for nitrogen fixation and 
other electron-transfer systems in microorganisms. 
On nitrogen fixation by Azotobacter the metabolic 
processes controlled or altered by iron deficiency in 
the cultured medium are less well defined. The ex 
tracellular products, for example, the formation of 
a yellow-green fluorescent peptide1) and of two fluores 
cent phenolic compounds2) in iron-deficient cultures of 
Azotobacter vinelandii have been reported. Fukasawa 
and Goto have previously reported the determination 
of the structure and biosynthesis of the yellow-green 
fluorescent peptide.3,4) In the course of the culture 
of Azotobacter vinelandii in iron-deficient medium, the 
growth of cell populations was extremely reduced by 
comparison to that of iron-containing medium. In 
order to obtain information on the relationship between 
biosynthesis of the yellow-green fluorescent peptide 
and low cell numbers of Azotobacter vinelandii strain 
O in iron free media, the proteolytic activity with and 
without iron in the culture medium was determined 
in water soluble protein fraction of the bacteria.

Cells were cultured in Bulk's nitrogen-free medium,5) 
1 liter, at 30°C for 60 hr in a glass jar with aeration. 
Cultivation was performed 10 times under the same 
condition as described above. The cells were separated 
from the medium by centrifugation (6,000 × g, 20 min). 
The sediments were pooled and stored at −20°C for 
the enzyme preparation. Iron content of the medium 
was determined by the method of Shinohara.6) The 
media contained 1.725 μg/ml (with Fe) and below 0.725 
μg/ml (without Fe) respectively.

The crude enzyme preparation: the sediments (10 g) 
were suspended in water (0.5 g/ml), then sonicated on 
10 Kc at 5°C for 5 min. The sonicated suspension was 
centrifuged (20,000 × g, 15 min at 5°C). The super 


<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Enzyme activitya)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Without Fe</td>
</tr>
<tr>
<td>Proteinase</td>
<td>23</td>
</tr>
<tr>
<td>Carboxypeptidase</td>
<td>33</td>
</tr>
<tr>
<td>Aminopeptidase</td>
<td>5.3</td>
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a) Units of the enzyme activities are defined in the text. 
— not detectable.

Amino acid and/or peptide in concentration of the 
culture medium was determined by the method of 
Yemm and Cocking,10) using concentrated supernatant
from the medium. For this the supernatant (100 ml) was evaporated to dryness in vacuo not exceeding 40°C and then dissolved in water to a suitable concentration so as to determine spectrophotometrically ninhydrin color development. From these determinations, the amino acid and/or peptide was found to be 0.22, 0.48 and 0.58 µmoles in the iron containing media and also 0.12, 0.24 and 0.34 µmoles in iron deficient media, respectively, in each 24, 48 and 72 hr, incubation periods. The number of cell population was estimated by the spectrophotometric measurement at 660 nm to be of the same order as in the previous experiment and was calculated to be 0.86 x 10⁸ cell/ml (−Fe) and 1.23 x 10⁹ cell/ml (+Fe) using the relationship between turbidity and cell numbers. Therefore, the cells in iron-deficient culture released more amino acid and/or peptide than in iron containing culture.

From these observations it was concluded that the iron-deficiency indirectly causes the production of high level of proteolytic enzymes. These enzymes produced the free amino acids and/or peptides in the culture medium and may maintain the life cycle of the cell. The yellow-green fluorescent peptide produced under the same condition was not hydrolysed by peptidases (pepsin, bacterial proteinase, and carboxypeptidase). Therefore, the yellow-green fluorescent peptide might be an accumulation product in the culture medium.

REFERENCES
6) K. Shinohara, J. Biochem., 29, 57 (1939).