Communications to the Editor

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Occurrence of Nucleotides in the Culture Fluid of a Microörganism

During the past decade a great amount of information has been accumulated on the occurrence of purines, pyrimidines, and their precursors in the culture fluid of microorganisms. The information has provided proof that microörganisms are apt to accumulate or excrete purine or pyrimidine compounds under unusual conditions such as genetic block,[1~4] lack of nutrients,[5] presence of antimetabolites,[6~8] suspended microorganisms in appropriate buffer solution,[9] or irradiation of X-ray or ultraviolet ray.[10]

On the other hand, there seems to be few reports which dealt with purine and pyrimidine compounds occurring in the culture fluid of normally growing microörganisms, although their occurrence has been suggested by several experiments.[11,12] The authors have found that a newly isolated *Brevibacterium* strain grown in normal condition excretes a considerably large amount of nucleotides in the culture fluid.

At first, more than 300 bacterial strains isolated from drainage and 70 type-culture bacterial strains were tested for their properties to excrete purine compounds using non-exacting purine-auxotrophic mutant of *Escherichia coli*. A strain thus obtained, which is designated as *Brevibacterium liquefaciens* novo sp., was cultivated on a glucose-casamino acid medium with shaking for 48 hours at 28°. Five hundred cc. of the broth was centrifuged to sediment bacterial cells. The nucleotides in the supernatant was absorbed on charcoal and eluted with 50% ethanol containing 2% of ammonia. The

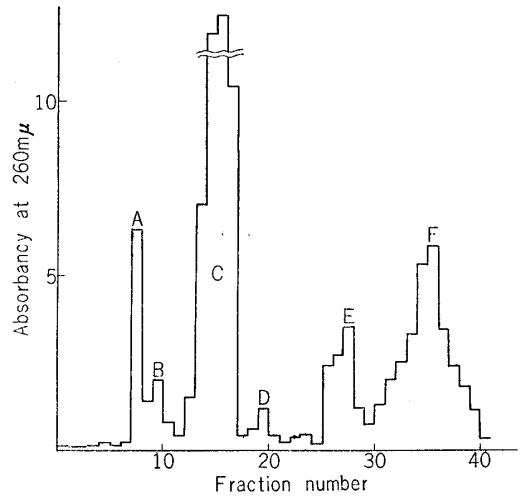

Fig. 1. Gradient Elution of Nucleotides in the Culture Fluid of *Brevibacterium liquefaciens* novo sp.

Five hundred cc. of broth of shaken culture of *Brevibacterium liquefaciens* novo sp. was treatd as described in the text and eluted from Dowex-1 X2 (chloride form, 200~400 mesh). Column, 2.8×35 cm.; fractions, 30 cc. each; mixing chamber, 1.12 L. filled with 0.005*N* HCl; reservoir with 0.01*N* HCl+0.1*N* $CaCl_2$.

Tentative assignment of each peak is as follows:

Peak A : Unknown compound
Peak B : 5′-CMP+unknown substance
Peak C : 5′-AMP
Peak D : adenosine compound + unknown substance
Peak E : CDP
Peak F : 5′-UMP+UDP-peptide

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2) *Idem* : Nature, **172**, 256(1953).
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effluent was evaporated *in vacuo* to dryness, the residue was redissolved in a small amount of water, and the clear supernatant was submitted to ion exchange chromatography on Dowex-1 X2 (chloride form). Fig. 1 shows a typical elution pattern of these nucleotides. Each nucleotide was isolated as calcium or ammonium salt and was identified by its absorption spectrum, organic P and labile P content,[13] paper chromatography with four different solvent systems, carbazole reaction,[14] and pentose determination by the orcinol reaction.[15] Some analytical date are given in Table I.

Table I. Analytical Data on Effluent Fractions

Substance	Spectrum type[a]	P-nucleoside ratio[b]	Labile P-nucleoside ratio	$R/_{adenosine}$[c] with EtOH-$AcONH_4$[f] (pH 7.5)	$R/_{adenosine}$[d] with EtOH-$AcONH_4$[f] (pH 3.8)	Absorption maximum in carbazole reaction[e] (mμ)
C	Adenosine	1.07	0	0.43	0.81	600, 710
E	Cytidine	2.08	0.90	0.325	0.37	600, 710
F_1	Uridine	1.0	0	0.59	0.93	600, 710
F_2	〃	1.86	0.96	0.80	0.65	600, 710

a) Determined in neutral, acid (0.1*N*), and alkaline (0.1*N*) solutions.
b) Calculated from the total phosphate and the extinction coefficient of substances as found in the literature.
c) $R/_{adenosine}$ value of each nucleotide varied because of lack of temperature control. In all cases authentic nucleotides were run as controls. $R/_{adenosine}$ values: 5′-AMP, 0.44; CDP, 0.33; 5′-UMP, 0.59; 3′-UMP, 0.66; 3′-CMP, 0.55.
d) $R/_{adenosine}$ values: 5′-AMP, 0.82; CDP, 0.38; 5′-UMP, 0.93; 3′-UMP, 0.98; 3′-CMP, 0.54.
e) Pyrimidine nucleotides were reduced with Na-amalgam by the method of Haavaldsen, *et al.* and then treated with carbazole reagents (L. Haavaldsen, S. Laland, J. Mckinley Mckee, E. Roth: Biochim. Biophys. Acta, **33**, 201(1959)).
f) A. C. Plandini, L. F. Leloir: Biochem. J., **51**, 426(1952).

Substances in peak F were poorly separated in pure form under the procedure employed. Rechromatography of peak F with the solvent system of HCl+NaCl gave at least two nucleotides (Fig. 2). One was found to be 5′-UMP*[1] and the other was UDP-peptide (UDPX). Examination of UDPX has shown that on hydrolysis with 6*N* HCl it gave three amino acids which were identified as alanine, glutamic acid, and lysine by paper chromatography with four different solvent systems. It also gave on paper chromato-

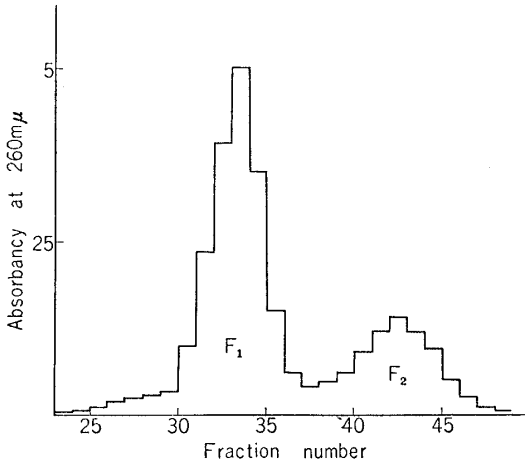

Fig. 2. Rechromatogram of Peak F on Dowex 1

About 23 μ*M* of nucleotide (calculated from absorbancy) was submitted to gradient elution on Dowex-1. Column, 1.5×30 cm.; fractions, 10 cc. each; mixing chamber 1.12 L. filled with 0.035*N* NaCl+0.01*N* HCl; reservoir with 0.01*N* HCl+0.1*N* NaCl.

*1 Abbreviations used: 5′-AMP, adenosine 5′-phosphate; CDP, cytidine 5′-pyrophosphate; 5′-UMP, uridine 5′-phosphate; UDP-peptide, uridine 5′-pyrophosphate-peptide.

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gram the spot positive to the Elson-Morgan reaction[16] on hydrolysis with 0.1*N* H_2SO_4.

The quantity of each nucleotide in 1 L. of culture fluid of *Brevibacterium liquefaciens* novo sp. amounted to 400 μ*M* of 5′-AMP (peak C), 90 μ*M* of CDP (peak E), 68 μ*M* of 5′-UMP (peak F_1) and 15 μ*M* of UDPX (peak F_2).

The presence of UDP-peptides in cell wall of penicillin-inhibited bacteria has called much attention in connection with the biosynthesis of cell-wall material of bacteria.[17,18]

Recently, Gilbert, *et al.*[19] reported the occurrence of UDP-peptide that contains aspartic acid, glutamic acid, arginine, and alanine in exponentially growing *Torulopsis utilis*.

The presence of UDPX in the broth of *Brevibacterium liquefaciens* novo sp. is very interesting because it seems to offer the first evidence that UDP-peptide occurs in the culture fluid of microörganism.

A more thorough investigation of the nucleotides occurring in the culture fluids of various microörganisms is now in progress and an attempt is also being made to elucidate the structure of UDPX.

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Über Taxinin*

Früher gaben wir über die Reduktionsergebnisse des Taxinins mittels Lithiumaluminiumhydrid und Palladium-Schwarz einen kurzen Bericht.

Da seitdem die eingehenden Untersuchungen einige der vermutlichen Beschaffenheiten der im Taxinin enthaltenen Sauerstoffe ins Klare gebracht haben, möchten wir nun darüber in der vorliegenden Mitteilung in wenigen Worten Angaben machen.

Die Reduktionsprodukte des Taxinins mittels Lithiumaluminiumhydrid wurden näher verfolgt, dabei konnte ausser Äthanol, 3-Phenylpropanol, der ölige Kohlenwasserstoff von $Sdp_{0.03}$ 215° und Taxinol, $C_{16}H_{26}O_4$, das bei 250° sintert und bei 263° scharf schmilzt, und eine als 1,1-Dimethyläthylenglykol vermutete Substanz, die vorher unbekannt war, isoliert werden.

* Mitteilung Ⅶ. von "Chemische Untersuchung der japanischen Eibenblätter." Ⅵ. Mitt. : Dieses Bulletin, **6**, 728(1958).