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SHORT COMMUNICATION

THE UTILIZATION OF EXOGENOUSLY SUPPLIED NUCLEOTIDE BY *XANTHOMONAS ORYZAE*[1,2]

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Many reports showed that phosphorylated compounds were barred from entering bacterial cells (Lin *et al.*, 1962; Fraenkel *et al.*, 1974). For phosphorylated nucleoside the failure of the incorporation of deoxyadenosine-α-^{32}P-triphosphate into *Escherichia coli* was reported (Buttin and Kornberg, 1966). Similar result was also demonstrated in the incorporation of deoxythymidine-^{3}H-triphosphate into *Bacillus subtilis* (Rinehart and Cophenland, 1973). Although deoxyribonucleoside triphosphates are believed to be the direct precursors of DNA, owing to the impermeability of bacterial cells to nucleotides, these substances can not be used in the study of DNA biosynthesis with intact bacterial cells. In order to study the DNA biosynthesis in bacterial cells the structure of cell walls was usually modified by certain treatments to make the cells permeable to nucleotides (Kornberg *et al.*, 1956; Buttin and Kornberg, 1966; Mosses and Richardson, 1970; Mordoh and Jacob, 1970; Vosberg and Hoffman-berling, 1971; Kuo, 1972; Miller *et al.*, 1973). Since nucleotides can not be uptaken by bacterial cells, the exogenous suppliment of nucleotides can not be utilized by bacterial cells. During the study of the methylation of DNA of Xp12 phage in *Xanthomonas oryzae* cells, it was found that when labelled deoxycytidine triphosphate (dCTP) was mixed with *X. oryzae* cells, the radioactivity was rapidly detected in acid precipitable fraction. It appeared that dCTP was able to enter bacterial cells, however, the mode of entering and utilization was unknown. To clarify this point the following experiments were conducted.

X. oryzae 507 was grown exponentially in synthetic medium (Chow *et al.*, 1972) with continuous aeration at 28°C. The cells were harvested and washed

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once with new synthetic medium. Finally the cells were concentrated in synthetic medium at concentration of 3×10^9 cells per ml. From this cell suspension 1.2 ml was taken and inucbated with 0.1 μc of dCTP-2-^{14}C. At different time intervals, 0.2 ml of reaction mixture was withdrawn and mixed with 0.5 ml of cold 5% TCA. The solution was filtered through Millipore filter. The precipitates left on the filter paper were washed twice with 10 ml of 5% TCA. The precipitates were dried and placed in scintillation fluid. The radioactivity was counted by using a liquid scintillation spectrometer (Parkard Model 3375). The incorporation of adenine-2, 8-^{3}H-triphosphate (ATP) was also proceeded in the same way. The results are shown in Fig. 1, following the incubation time the incorporation of radioactivity from dCTP-2-^{14}C and ATP-2, 8-^{3}H into TCA insoluble materials is gradually increased.

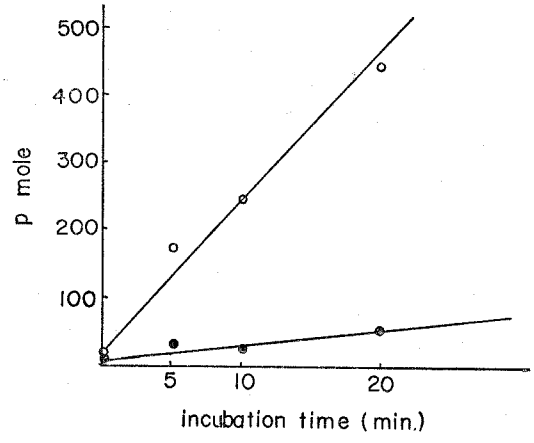

Fig. 1. Incorporation of labelled nucleotides into *X. oryzae* cells.
—○—○—: incorporation from dCTP-1-C^{14};
—●—●—: incorporation from ATP-2 8-H^3.

To clarify the mode of incorporation and utilization, the metabolic intermediates inside and outside the bacterial cells were analyzed. Under the condition used in above experiment the reaction was too fast to detect the metabolic intermediates, therefore, the reaction was slowed down by incubating the cells in tris buffer at 20°C. Two ml of cell suspension at 2×10^{10} cells per ml were incubated with 0.4 μc dCTP-2-^{14}C at 20°C. At zero time and after 10, 30, and 60 minutes, 0.5 ml of sample was withdrawn and filted by Millipore filter. The filtrate was harvested for analysis of metabolic intermediates of dCTP. The intermediates obtained from this fraction was considered as the intermediates of dCTP outside the bacterial cells. The cells left on filter paper were washed twice with 10 ml of distilled water, resuspended in 0.5 ml of 5% TCA and then boiled for 5 minutes to extract the metabolic

intermediates inside the cells. The intermediates obtained from this extract was considered as the intermediates existed inside the cells. Both filtrate and extract from cells were concentrated to dryness and resuspended in a small amount of distilled water, which was then spotted on Whatman No. 1 filter paper and run for paper chromatography in a solvent containing isobutyric acid-ammonium hydroxide-EDTA-distilled water (66:1:1:32, V/V/V/V) for separation of these metabolic intermediates. Authentic deoxycytidine (dCt), deoxycytidine monophoshate (dCMP) deoxycytidine diphosphate (dCDP), and dCTP were also used as references. After chromatography, paper was dried and cut to strip, the radioactivity was quantitatively measured with Nuclear-Chicago Actigraph III. The metabolic change of dCTP outside the bacterial cells is shown in Table 1. Following the incubation time dCTP was gradually decreased. The decrease of dCTP was accompanied by the appearance of dCDP dCMP and dCt. With further incubation all dCTP, dCDP and dCMP disappeared and their disappearance was accompained by the appearance of more dCt. The metabolic intermediates detected inside the cells are also shown in Table 2. The first intermediate detected inside the cell was dCt and following the incubation time the dCMP was detected and gradually increased. From these results it was concluded that dCTP did not directly incorporate into cells, it was degradated extracellularly into dCDP, dCMP and finally became dCt. When nucleotide was dephosphorylated into nucleoside, the nucleoside would be taken up by bacterial cells. After nucleoside was

Table 1. *Metabolic change of dCTP outside the bacterial cells*

Metabolic intermediates \ Incubation time (min.) / Amount (p moles)	0	10	30	60
dCTP	1,620	270	110	0
dCDP	0	168	0	0
dCMP	0	30	0	0
dCt	10	376	613	633

Table 2. *Metabolic intermediates detected inside the cells*

Metabolic intermediates \ Incubation time (min.) / Amount (p moles)	0	10	30	60
dCTP	0	0	0	0
dCDP	0	0	0	0
dCMP	0	37	57	56
dCt	17	65	79	81

incorporated into bacterial cells the nucleoside was coverted into dCMP and probably to dCDP, dCTP and finally into DNA. The conclusion was strongly supported by Huang *et al.* (1973). They isolated a non-specific nucleotidase from the surface of *X. oryzae* cells, the enzyme dephorylated all nucleotides. Similar phenomenon has been reported by Lichenstein (1960), he demonstrated that deoxycytidine monophosphate could be dephosphorylated by a nucleotidase on *Eschericia coli* cells.

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Xanthomonas oryzae 對於 nucleotides 的利用

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一般 phosphorylated compounds 如 nucleotides 等物質因不能滲入細菌細胞壁故不能被利用來做爲營養。本研究發現 *Xanthomonas oryzae* 能利用 nucleotides 來做其 DNA。利用的方式係此細菌能在體外把 nucleotides 分解爲 nuclosides。以 nucleosides 的方式滲入細胞壁。進入之 nucleosides 再轉變爲 nucleotides 而被利用到 DNA 上。