Reductive Cleavage of Demeton-S-Methyl by Corynebacterium glutamicum in Cometabolism on More Readily Metabolizable Substrates

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Corynebacterium glutamicum is able to biotransform demeton-S-methyl, an organophosphorus compound, during cometabolism with more readily metabolizable substrates. Among the cosubstrates used, fructose is the growth substrate that is most favorable for demeton-S-methyl biotransformation. The reaction mechanism of demeton-S-methyl biotransformation involves reductive cleavage of an S-C bond, which leads to accumulation of dimethyl thiophosphate in the culture medium.

Synthetic organophosphorus (OP) compounds are used extensively as agricultural and domestic pesticides and could be used as chemical warfare agents. Most of these xenobiotic compounds have common organic phosphorus-ester bonds. The extremely toxic military OP compounds, such as soman, VX, and malathion (in 100 mM Tris-HCl [pH 7.0]) were used as internal standards. One microliter of an extracted sample was injected (splitless) into a Hewlett-Packard model 5890A chromatograph equipped with an HP-5 M.S. (cross-linked 5% phenyl methyl silicone) column (30 m by 0.25 mm by 0.25 μm). Helium was the carrier gas (flow rate, 0.9 ml min−1) and the auxiliary gas (flow rate, 20 ml min−1). The hydrogen and air flow rates were 2.9 and 100 ml min−1, respectively. The oven temperature was programmed as follows: 30 s at 80°C, linear increase (at a rate of 30°C min−1) to 200°C, 30 s at 200°C, linear increase to 230°C in 1 min, 30 s at 230°C, linear increase (at a rate of 30°C min−1) to 310°C, and 2 min at 310°C. The injector and nitrogen-phosphorus detector (NPD) temperatures were 260 and 300°C, respectively. The demeton-S-methyl quantification threshold was 0.1 mg liter−1.

Figure 1 shows the demeton-S-methyl concentrations during batch growth on each growth substrate. No additional peak resulting from demeton-S-methyl cleavage was detected by gas chromatography in the cell suspensions after extraction. Biodegradation was initiated rapidly, indicating that no adaptation.

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FIG. 1. Demeton-S-methyl concentrations during C. glutamicum biodegradation under batch conditions when acetate (D), glucose (E), and fructose (F) were used as growth substrates.
accomplished by using the WALTZ16 composite decoupling.

The number of scans was set to 16,000, and proton decoupling
were applied at a 90° flip angle and a 2.6-s repetition rate. The
probe (5 mm). A spectrum width of 20,450 Hz was used. Pulses
performed at 25°C and 145.7 MHz by using a Brucker Avance
model DPX 360 spectrometer equipped with a quadrupole

period was necessary, but was not complete since some residual
demeton-S-methyl was found in the stationary phase. The global
demeton-S-methyl consumption rates were 0.21, 0.75, and
0.78 mg liter⁻¹ h⁻¹ on glucose, acetate, and fructose,
respectively. The abiotic rate of degradation of demeton-S-
methyl, as estimated by monitoring concentrations in control
cultures lacking C. glutamicum, was less than 4.6 µg liter⁻¹ h⁻¹
over a 200-h period, which indicated that C. glutamicum is able
to degrade demeton-S-methyl.

Demeton-S-methyl biodegradation occurs by a cometabolism process. Demeton-S-methyl (10 mg liter⁻¹) was added to
each bioreactor when cells were entering the stationary phase
due to substrate exhaustion, and the concentration of deme-
ton-S-methyl was measured for 150 h. The global demeton-S-
methyl consumption rates calculated for nonproliferating cells
grown on acetate, glucose, and fructose were 0.080, 0.065, and
0.108 mg liter⁻¹ h⁻¹, respectively; these values were consider-
ably lower than the values obtained during the growth phase.
Addition of a carbon growth substrate to stationary-phase cul-
tures resulted in an immediate increase in the demeton-
S-methyl consumption when either acetate or glucose was the growth
substrate, while the pesticide was consumed in medium containing acetate, indicating that demeton-
S-methyl degradation depends on the functioning of primary
metabolism. When fructose was the growth substrate, the rate
of demeton-S-methyl consumption was greater than the rate of consumption when either acetate or glucose was the growth
substrate (the global rate was 0.78 mg liter⁻¹ h⁻¹, and the maximum instantaneous rate was 1.4 mg liter⁻¹ h⁻¹).

Demeton-S-methyl biotransformation involves reductive
cleavage of an S-C bond. The identities of the degradation products of demeton-S-methyl were investigated by 31P nuclear magnetic resonance (NMR) spectroscopy. 31P NMR was performed at 25°C and 145.7 MHz by using a Brucker Avance model DPX 360 spectrometer equipped with a quadrupole probe (5 mm). A spectrum width of 20,450 Hz was used. Pulses
were applied at a 90° flip angle and a 2.6-s repetition rate. The
number of scans was set to 16,000, and proton decoupling
was accomplished by using the WALTZ16 composite decoupling
sequence. Chemical shifts were referenced to external phos-
phoric acid (δ = 0 ppm). Before analysis 50 µl of D₂O was
added to each sample (volume, 500 µl). Demeton-S-methyl has a characteristic singlet at 35.7 ppm (Fig. 2); this singlet
decreased during incubation with C. glutamicum grown on fruc-
tose, and an upfield singlet at 33.8 ppm appeared. This singlet
could not be attributed to the spectrum of dimethyl phosphate
but was attributed to the spectrum of dimethyl thiophosphate.
Abiotic degradation of demeton-S-methyl at pH 14 resulted in a
decrease in the 35.7-ppm singlet and the appearance of two
upfield singlets at 2.5 and 20.5 ppm (data not shown). The signal at 2.5 ppm corresponded to the spectrum of dimethyl
phosphate. These results demonstrated that abiotic degrada-
tion at pH 14 resulted from P-S bond hydrolysis but that C.
glutamicum did not cleave the P-S bond. Attempts to detect thiol
liberation in crude cell extracts were unsuccessful, which
confirmed that enzymatic hydrolysis of the P-S bond did not
occur. Demeton-S-methyl is biotransformed by breaking the
S-C bond, and the resulting dimethyl thiophosphate is not
degraded by C. glutamicum. The biotransformation reaction is
not a hydrolysis reaction but is reductive cleavage of the S-C
bond due to a dehydrogenase-oxidoreductase activity accord-
ing to the following reaction:

CH₃CH₂S-CH₂-S-P-(O-CH₃)₂ → CH₃CH₂S₂CH₂S₂CH₃ + HS-P(O-CH₃)₂

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