Soil Adenosine Triphosphate: Extraction, Recovery and Half-Life[1]

by A. R. CONKLIN, JR. and A. N. MACGREGOR
Department of Agricultural Chemistry and Soils
University of Arizona, Tucson, Ariz. 85721

Several investigators have recently suggested that the ATP-content of natural environments be used as a measure of their microbial biomass (4,7,8). There are only two instances, however in which anyone has determined the ATP-levels in a natural system. Lee, et. al. extracted ATP from lake muds and soil, and Hamilton and Holm-Hansen extracted ATP from the ocean. Lee, et. al. compared the recovery of ATP from lake muds using five different extraction methods. The method giving the greatest recovery of ATP was based on sulfuric acid extraction followed by cation exchange clean-up. However, this method gave a variable recovery of ATP from sample to sample (7) and involves several manipulations.

Investigations in our laboratory into the ability of various desert soils to biologically immobilize nitrate, as indicated by changes in ATP-levels (Terminal Report, Western Regional Research Project W- 5, 1970) have shown that some ATP-extractants are apparently not as efficient as others. We were thus lead to look for a simple extraction procedure which would give a reasonable recovery of ATP under varying soil conditions. We were interested in keeping the procedure as simple as possible in order to facilitate the large number of extractions required to determine the vertical distribution of microbial biomass in desert soils(2). Since we are interested only in viable microflora, it was important to determine the half-life of non-cellular ATP in soil. Although some authors have suggested that non-cellular ATP may persist for significant periods in soils, its nature and extent is unknown (6).

Experimental

Three main types of desert soil were used in these experiments: Sonoita sandy loam, Grabe loam and Vekol clay. These soils had been stored dry in the labora-

1. This work was supported in part by funds allocated from the Desert Biome project NSF grant GB-15886 and from Regional Research Project W-111, University of Arizona Experiment Station.

Bulletin of Environmental Contamination & Toxicology,
Vol. 7, No. 5, 1972, © by Springer-Verlag New York Inc.

tory for six months prior to use. The soil designated as "active" was a 200 g. sample of Sonoita sandy loam which had been amended with 2 g. of ground-up alfalfa (leaves and stems) and 100 mg. of ammonium nitrate and kept moist. The soil designated as 88h was taken from the 6-foot depth of a Sonoita sandy loam soil profile.

In all experiments 5 g. of soil were used for ATP extraction. Various moisture levels were obtained by adding sterile water to the samples just before extraction. Soil moisture content was determined by adding an equivalent amount of water to a replicate soil sample and drying for 24 hrs. at 105°C.

Sterilization of soil samples was accomplished by autoclaving for 60 minutes at 15 lbs./in.^{2} on three successive days. Sterility was checked by plate count on soil extract (8) nutrient agar (Difco) and by ATP analysis.

All filtrations were carried out using Millipore microanalysis filter holders fitted with Millipore filter membranes (0.45 μ.).

Standard suspensions (2 x 10^8 cells/ml.) of Pseudomonas fluorescens (ATCC-18679) in sterile soil extract were prepared by harvesting cells after 24 hrs. incubation (AC-broth, Difco) at room temperature (22°C.). One milliliter of standard suspension and 5 g. of soil were mixed and extracted as described below. Also, three, 1 ml.-portions of freshly prepared standard suspension were extracted without mixing with soil. In order to obtain soil-organism mixtures at the 1.5% moisture level, sterile soil samples were oven dried at 100°C. overnight and 0.1 ml. of standard suspension (2 x 10^9 cell/ml. in soil extract) added. These mixtures were similarly extracted as below.

Extraction of soil was carried out using the following methods: the formic acid method described by Klofat, et. al. (5), the n-bromosuccinimide method described by MacLeod, et. al. (8) and the hot-Tris method described by Hamilton and Holm-Hansen (4).

Soil was also extracted with n-butanol-octanol following the procedure developed by D'Eustachio and Johnson (3) with the following modifications. Five grams of soil were mixed with 1.0 ml. of n-butanol. Three milliliters of arsenate buffer (0.02 g. sodium arsenate, 0.02 M. ethylene diaminetetraacetic acid, EDTA, pH 7.5) was added to the soil butanol mixture, the combination mixed for 30 seconds on a Vortex mixer and allowed to stand 3 minutes before filtering.

In all cases, ATP-extracts were quick-frozen in a dry ice-acetone bath and kept frozen at -10°C. until analysis.

ATP analysis

Firefly extract (Sigma Chemical Co.) was rehydrated according to instructions supplied by Sigma. The

resulting solution was allowed to stand at room temperature (22°C.) until the light emission from 100 µl. of firefly extract solution fell to 2,000 counts per 50 seconds. This background count resulted from endogenous ATP and was thus constant in all ATP-analyses.

Standardized firefly extract (100 µl.) was placed in a small test tube which was placed through a hole in the cap of a liquid scintillation vial filled with toluene-fluor (Packard Permafluor). The vial-test tube-firefly extract combination was placed in a Packard Tricarb 407 liquid scintillation counter sample changer, just before it advanced the sample. Just prior to entering the counting chamber, 50 µl. of sample were injected (Hamilton Cr 400- 50 syringe) into the firefly extract. Counting was continued for 50 seconds with discriminators set at 50-1000 and high gain set at 14,000 volts. Counts were related to µg. of ATP by means of a standard curve constructed according to the method of Stanley and Williams (10).

Results and Discussion

The ability of various ATP extraction procedures to extract ATP from intact soil samples of differing type, texture, moisture level and microbial content is shown in Table I. In all cases, the n-butanol-octanol method gave the greatest yield of ATP. EDTA was added to the arsenate buffer to remove interfering ions extracted from soil (1). After mixing the buffer with soil -n-butanol combinations, a 3-minute settling time was observed to facilitate filtration.

TABLE I

Amount of ATP (X 10-3 µg. ATP/g. soil) extracted from soil of varying texture and moisture contents

		----Extractant----------			
Soil Sample	% H_2O	Formic acid	Hot Tris	n-bromo-succinimide	n-butanol-octanol
"active"	15	0.59	0.84	5.0	53
88h	1	N.D.	0.13	0.29	0.53
Sonoita	1	N.D.[a]	N.D.	0.95	53
sandy	15	N.D.	N.D.	3.0	4.0
loam	33	N.D.	N.D.	1.5	3.0
Grabe	1	0.29	0.16	N.D.	0.89
loam	15	N.D.	0.22	0.57	7.9
	33	N.D.	N.D.	1.0	7.9
Vekol	1	N.D.	0.66	3.3	8.9
clay	15	N.D.	N.D.	0.5	5.0
	33	N.D.	0.84	2.1	16

(a) N.D. - not detectable, i.e. less than 1 x 10^{-4} µg. ATP per ml.

The extraction procedure giving the second greatest yield of ATP was the one involving n-bromosuccinimide. This procedure extracted ATP from all but one soil sample. The other two extraction procedures involving formic acid and hot-Tris, failed to show measurable ATP from several intact soil samples. Although the hot-Tris method appears to be somewhat better than the formic acid method, it is still less reliable than the n-butanol-octanol method.

Most ATP extraction methods (except for the n-bromosuccinimide and hot-Tris methods) have been developed to extract ATP from microorganisms obtained from pure culture. However, these methods are not equally effective in extracting ATP when applied to such a complex medium as soil. The failure of certain extraction procedure-soil type combinations to give measurable ATP in soil extracts may be due to several factors. These factors have been reviewed recently by several authors (6,8). One factor not covered in these reviews is that ATP has been reported to transphosphorylate its terminal phosphate group to apatite. This reaction has been reported to occur at a rate of 3μM. of ATP per 100 μg. apatite per 15 minutes (5). Although this particular reaction has not been shown to occur in soil it could contribute to a loss of ATP during long extraction times. It would appear from these considerations that any extraction procedure using long extraction times would be unreliable when applied to soil.

The recoverability of ATP from soil using the n-butanol-octanol method was measured by extracting suspensions of P. fluorescens with and without addition to sterile soil.

$$\% \text{ recovery of ATP} = \frac{\text{ATP from cells + sterile soil}}{\text{ATP from cells alone}} \times 100$$

Soil moisture level and texture appear to have little effect on the recoverability of ATP from the desert soils examined. Table II shows that the n-butanol-octanol method gives a good recoverability of ATP from soils of various types, texture, microbial and moisture contents.

In addition to the recoverability of ATP from soil, the half-life of non-cellular ATP must be known if one is interested in obtaining meaningful information about the viable microfloral content of soil. Standard ATP-solutions (50×10^{-2} μg. ATP/g. soil) were added to untreated soil samples and the amount of ATP remaining

TABLE II

Percent recoverability of ATP from soil

Moisture content %	Soil Sample		
	Sonoita	Grabe	Vekol
1.5	76	76	77
15	68	68	70
33	65	66	63

after various periods of incubation checked by washing the soil with buffer minus n-butanol. Table III shows that the half-life of non-microbial ATP in soil is less than one hour.

TABLE III

Percent of added ATP recovered from soil after various incubation periods

Elapsed time	Soil Sample		
	Sonoita	Grabe	Vekol
0 hr	100	100	100
1 hr	0.3	0.03	0.05
2 hrs	0.06	N.D.	N.D.
3 hrs	N.D.	N.D.	N.D.

Conclusions

Extraction of ATP from microorganisms in desert soil is best carried out using the n-butanol-octanol method. Using this method, one obtains an average ATP-recovery of 68% under widely varying soil moisture and textural conditions. We have also shown that the half-life of non-microbial ATP in soil is not more than one hour and thus the possibility of a build-up of non-microbial ATP in desert soil is unlikely. This method can be used for rapid survey of the microbial content in soil environments with a minimum of soil disruption. It is also a valuable tool for investigating the effect of various environmental factors on fluctuations in soil microflora content.

BIBLIOGRAPHY

1. Aledort, L. M., Weed, R. and Troup, S. B., Anal. Biochem. 17:268 (1966).
2. Conklin, A. R. and Macgregor, A. N., Forty-seventh Annual Meeting, Southwestern and Rocky Mountain Division American Association for the Advancement of Science, Abstract No. 55., April 22, 1971, Tempe, Arizona.
3. D'Eustachio, A. J. and Johnson, D. R., Fed. Proc. 27:761 (1968).
4. Hamilton, R. D. and Holm-Hansen, O., Limnol. Oceanogr. 12:319 (1967).
5. Klofat, W., Picciolo, G., Chappelle, E. W. and Freese, E., J. Biol. Chem. 244:3270 (1969).
6. Krane, S. M. and Glimcher, M. J., J. Biol. Chem. 237:2991 (1962).
7. Lee, C. C., Harris, R. F., Williams, J. D. H., Armstrong, D. E. and Syers, J. K., Soil Science Proceedings. 35:82 (1971).
8. MacLeod, N. H., Chappelle, E. W. and Crawford, A.M., Nature. 233:267 (1969).
9. Patterson, J. W., Brezonik, P. L. and Putman, H. D., Environmental Science and Technology. 4:569. (1970).
10. Stanley, P. E. and Williams, S. G., Anal. Biochem. 29:381 (1969).