



Fig. 1. Differentiation of somatic embryoids in hypocotyl explants of *Albizzia lebeck* L. (a) Emergence of young embryoids from the cracks of the hypocotyl segment after 2 weeks of culture on BM; (b) development of shoot from an embryoid in 3-4-week-old culture. Arrow points at an activated shoot meristem on a mature embryoid; (c) production of roots from the base of a shoot implanted on BM; (d-f) globular, heart-shaped and dicotyledonous embryoids, dissected from a 2-3-week-old hypocotyl explant; (g) L.S. of hypocotyl explant passing through a globular embryoid of radial symmetry

(BM [1]), containing 5×10^{-5} M FeSO_4 and 5×10^{-5} M $\text{Na}_2\text{-EDTA}$ as iron source, were used for all studies. Various explants, namely root, hypocotyl, cotyledon and leaflets, were inoculated on BM without any growth hormone. Cultures were maintained at 25 ± 2 °C and at 750 lux intensity. After two weeks of culture on BM, signs of cracking of the hypocotyl explants and subsequent emergence of young embryoids was observed (Fig. 1a). By the third week of culture, various developmental stages (i.e., globular to dicotyledonous embryoids) were discernible in the crevices formed on the hypocotyl segments (Fig. 1d-f). The average number of embryoids developed per hypocotyl explant varied from 20 to 25. But, by periodical excision and removal of the mature embryoids, the number of embryoids differentiating from an explant could be increased to about 50-60. On the other hand, when these embryoids were left as such on the hypocotyl, development of shoots without any sign of rhizogenesis was observed (Fig. 1b). However, on subsequent excision and implantation of these shoots on

the same medium, differentiation of roots took place giving rise to complete plantlets (Fig. 1c). Histological examination of the hypocotyl explants showing embryogenesis indicated the origin of embryoids from the inner cambial/cortical region (Fig. 1g). In contrast, the other explants cultured, namely, root, cotyledon and leaflet, did not show any sign of differentiation on BM. Nevertheless, supplementation of BM with α -naphthaleneacetic acid (2 ppm) and

Pelagic Bacteria: Extreme Abundances in African Saline Lakes

P. Kilham

Department of Ecology and Evolutionary Biology; University of Michigan, Ann Arbor, Michigan 48109

In freshwater lakes and in the ocean natural concentrations of pelagic bacteria determined by acridine orange direct-counts (AODC) are between 10^5 - 10^6 bacteria/ml [1-3]. I was therefore surprised to find con-

centrations of 10^7 - 10^8 bacteria/ml in three alkaline, saline lakes in Kenya: Lake Bogoria (formerly Hannington, $0^\circ 15' \text{N}$, $36^\circ 06' \text{E}$), Lake Elmenteita ($0^\circ 27' \text{S}$, $36^\circ 15' \text{E}$), and Lake Sonachi (formerly

6-benzylaminopurine (0.5 ppm) resulted in abundant shoot bud production in all cases which could in turn be subsequently rooted on BM to form complete plantlets. Surprisingly, the hypocotyl segments, when cultured on hormone-supplemented medium, exhibited caulogenesis instead of embryogenesis as observed on BM. The results indicate the autonomous state of hypocotyl segments for induction of embryogenesis at the time of culture. This is contrary to the often documented requirement of exogenous hormone for induction of somatic embryogenesis [2]. The presence of an undifferentiated state of tissue is also not critical, like in *Sorghum* [3]. In addition, unlike the important role of genotype in induction of embryogenesis in *Medicago sativa* (where the response varies even in different plants of the same cultivar [4]) and in *Trifolium pratense* [5], no such genotypic control is observed in *Albizzia lebeck*.

We are grateful to Prof. R.S. Mehrotra of Kurukshetra University for supplying seeds of *Albizzia lebeck*. This work was supported by financial assistance in the form of a Research Fellowship to PKG from the University Grants Commission, and a grant from the Indian Council of Agricultural Research to SCM.

Received April 22, 1981

1. Gamborg, O.L., Eveleigh, D.E.: Can. J. Biochem. 46, 417 (1968)
2. Kohlenbach, H.W., in: Frontiers of Plant Tissue Culture 1978, p. 59 (Thorpe, T.A., ed.). Calgary: Int. Asso. Plant Tissue Culture 1978
3. Wernicke, W., Brettell, R.: Nature 287, 138 (1980)
4. Kao, K.N., Michayluk, M.R.: Z. Pflanzenphysiol. 96, 135 (1980)
5. Keyes, G.J., Collins, G.B., Taylor, N.L.: Theor. Appl. Genet. 58, 265 (1980)

centrations of 10^7 - 10^8 bacteria/ml in three alkaline, saline lakes in Kenya: Lake Bogoria (formerly Hannington, $0^\circ 15' \text{N}$, $36^\circ 06' \text{E}$), Lake Elmenteita ($0^\circ 27' \text{S}$, $36^\circ 15' \text{E}$), and Lake Sonachi (formerly

Table 1. Bacterial abundances and selected environmental parameters

Lake	Date (1980)	k_{25} at 25 °C [mS/cm]	pH	Bacteria/ml (\pm S.D.)
<i>Elmenteita</i>	3 Jan			
0.0–0.4 m		18.8	10.1	$3.6 \pm 0.3 \cdot 10^8$
1.1–1.5 m		–	–	$3.5 \pm 0.4 \cdot 10^8$
<i>Sonachi</i>	1 Jan			
1 m		5.25	9.6	$3.6 \pm 0.5 \cdot 10^7$
4 m		5.40	9.6	$2.4 \pm 0.3 \cdot 10^7$
5 m		10.9	9.6	$3.5 \pm 0.5 \cdot 10^7$
7 m		14.5	9.5	$4.8 \pm 0.9 \cdot 10^7$
<i>Bogoria</i>	10 Jan			
1 m		71.0	10.4	$3.5 \pm 0.4 \cdot 10^7$
<i>Naivasha</i>	14 Jan			
1 m		0.21 ^a	8.1 ^a	$3.7 \pm 0.4 \cdot 10^6$

^a Data from Table 3 in [9]

Naivasha-West Crater, 0°46' S, 36°15' E). Bacterial abundances in Lake Naivasha (freshwater, 0°46' S, 36°21' E) were similar to those found in freshwater lakes in other parts of the world.

Lake water samples (20 ml) were preserved immediately after collection with formaldehyde (1 ml, saturated). Subsamples were diluted 10–100 times, when appropriate, with filter-sterilized de-ionized water and mixed using a Virtis mixer (5 min). I then followed the standard AODC procedure [1] with 0.2 μ m, 25 mm Nuclepore filters. Bacterial abundances and selected environmental parameters are given in Table 1.

These alkaline, saline lakes are extreme ecological environments characterized by high rates of primary production [4], and faunas and floras comprised of only a few species that occur in great numbers [5]. Even though bacterial concentrations of the magnitude observed in these lakes do not occur in unpolluted natural waters, similar high concentrations have been found in polluted waters directly contaminated by raw sewage [2]. Because these African lakes receive little or no domestic sewage one can assume that their bacterial populations are supported by organic compounds of autochthonous origin. Regression equations relating BOD₅ (5-day biochemical oxygen demand; an accepted index of oxidizable organic matter) and direct counts of bacteria [2] indicate that BOD₅ values corresponding to the bacterial counts given in Table 1 range between ca. 70–700 mg/l O₂. To my knowledge there are no data available for either BOD₅ or DOC (dissolved organic carbon) for African, saline lakes. However, if comparable high BOD₅ values were found to exist in

these waters these data would support the argument that bacterial concentrations in both natural and polluted aquatic ecosystems are primarily a function of ambient concentrations of biochemically oxidizable organic matter.

Bacterial numbers are also in part a function of the population dynamics of the pelagic organisms that feed primarily on bacteria (ciliated and flagellated protozoans), but their biology is still poorly known [6]. I observed numerous paramecium-like organisms in an unpreserved water sample from Lake Sonachi and a chilomonad (ca. 10³/ml) occurs in Lake Bogoria. Jenkin [5] reported finding ciliates in Lake Elmenteita. Zooplankton that potentially prey on protozoans [7] are found in all of these lakes. *Paradiaptomus africanus* Daday (a copepod) and various species of rotifers are often abundant in Elmenteita and Sonachi, and a pelagic chironomid larva occurs in Bogoria.

Because the bacterial abundances observed are only slightly less than those commonly

reported in sediments (10⁹/ml) [8] one could argue that the concentrations found were the result of sedimentary contamination. This is improbable, however, because little sedimentary debris was seen in the samples counted and only Lake Elmenteita is shallow enough (<3 m) to permit significant sedimentary resuspension.

Not only are these alkaline, saline lakes remarkable for their high concentrations of bacteria, their simple-food chains make them an ideal natural environment in which to study the population dynamics of pelagic bacteria and protozoa.

I thank J.M. Melack, J.E. Hobbie, S.S. Kilham and R. Menzell for help and advice. The National Science Foundation (U.S.A.) supported this research.

Received March 31, 1981

- Hobbie, J.E., Daley, R.J., Jasper, S.: Appl. envir. Microbiol. 33, 1225 (1977)
- Straškrabová, V.: Limnologica 6, 29 (1968)
- Watson, S.W., et al.: Appl. envir. Microbiol. 33, 940 (1977); Hobbie, J.E., Wright, R.T.: Ergebn. Limnol. 13, 85 (1979); Griffiths, R.P., et al.: Can. J. Microbiol. 24, 1217 (1978); Daley, R.J., Hobbie, J.E.: Limnol. Oceanogr. 20, 875 (1975); Ferguson, R.L., Palumbo, V.A.: ibid. 24, 697 (1979)
- Melack, J.M., Kilham, P.: ibid. 19, 743 (1974); Melack, J.M.: Ph. D. thesis, Duke Univ. (1976)
- Hecky, R.E., Kilham, P.: Limnol. Oceanogr. 18, 53 (1973); LaBarbera, M.C., Kilham, P.: ibid. 19, 459 (1974); Jenkin, P.M.: Ann. Mag. nat. Hist. Ser. 10 18, 133 (1936)
- Fenchel, T.: Hydrobiologia 46, 445 (1975); Fenchel, T.: Microb. Ecol. 6, 13 (1980)
- Porter, K.G., Pace, M.L., Battey, J.F.: Nature 277, 563 (1979)
- Rublee, P., Dornseif, B.E.: Estuaries 1, 188 (1978)
- Kilham, P.: Ph. D. thesis, Duke Univ. (1972)

Tumor Stem Cell Cloning in Agar-Containing Capillaries

H.R. Maurer and F. Ali-Osman

Pharmazeutisches Institut der Freien Universität, D-1000 Berlin

Recently techniques have been developed to grow, in vitro, tumor cell colonies representing clones of tumor stem cells [1, 2]. The so-called Human Tumor Stem Cell Assay has proved valuable in predicting

clinical drug sensitivity or resistance to cytostatics as well as in screening for new drugs [3]. The assay utilizes a 2-layer soft agar in Petri dishes. Here, we report that the assay can be simplified and refined by