ciation. The DNA used in this study was total cellular DNA and therefore may have contained plasmid DNA, if present. However, plasmid DNA being only a small fraction of total DNA should not appreciably affect the data presented here. $C_0t_{½}$ values were calculated from the C_0t corresponding to the 50% of observed maximum hybridization.

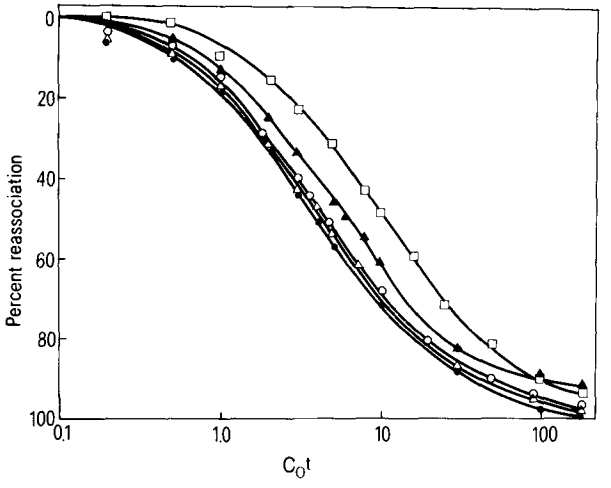
The $C_0t_{½}$ of DNA reassociation of the fast-growing species of *Rhizobium; R. meliloti* (SU216), *R. phaseoli* (CC365), *R. trifolii* (T19), *R. leguminosarum* (SU391), were found to be 4.1, 4.4, 3.9 and 4.9, respectively. These values are considered to be very close to each other. On the other hand the DNA of *R. japonicum* (SB16), a slowgrower, has a $C_0t_{½}$ of 9.8 which is about double that of the fastgrowing species studied, indicating that it has a genome size almost twice that of the fast-growing ones. The DNA of *E. coli* B was found to have a $C_0t_{½}$ of 2.8. The table presents the calculated genome size of the strains of fast- and slow-growing rhizobia. *E. coli* B has a molecular weight of 2.2×10^9 daltons for the genome size[19]. The value has been used as a primary molecular-weight standard for measurement of the rhizobial DNA genome by proportionality relationship of $C_0t_{½}$ of reassociation. The values for the genome size of *R. japonicum* (CC409), *R. lupini* (RL3001) and *R. sp.* Cowpea (U_8) were obtained from our previous study[11] and have been used for comparison (table). The genome sizes of the fast-growing and the slow-growing species fall in the range of 3.1 to 3.9×10^9 and 5.4 to 7.7×10^9 dalton, respectively. The fast-growers and the slow-growers, therefore, have genome sizes in 2 distinct ranges of values and the 2 groups differ by a factor of approximately 1.5 to 2. If this is confirmed in general for the 2 groups of rhizobia, this difference would support the proposal[7] to establish 2 distinct genera.

2 classes, distinct as regards their genome size, have already been found in mycoplasma[20]. On the basis of that, and of the frequency distribution of prokaryotic genome sizes, an evolutionary scheme by genome duplication has been suggested[10]. If increase in genome size is a primary step of evolution, we feel that slow-growing strains of rhizobia having a larger genome size are to be considered more advanced in the evolutionary scale than their fast-growing counterparts.

Genome size estimates for strains of *Rhizobium*

Strain	Calculated genome size in daltons $\times 10^{-9}$
R. meliloti (SU216)	3.22
R. phaseoli (CC365)	3.46
R. trifolii (T19)	3.06
R. leguminosarum (SU391)	3.84
R. japonicum (SB16)	7.69
R. japonicum (CC409)	7.62*
R. lupini (RL3001)	5.42*
R. sp. Cowpea (U8)	6.91*

*Values are quoted from our previous study[11].



The kinetics of reassociation of DNA of *Rhizobium* species. The samples were sheared by partial depurination and alkaline cleavage, made up to 0.12 M in phosphate buffer, pH 6.8, denatured by heat and incubated at 60 °C. Kinetic points were then taken. Each point was analyzed by fractionation on hydroxyapatite. *E. coli* B DNA was determined to have a $C_0t_{½}$ of 2.8 (not shown here). *R. trifolii* (T19), ●—●; *R. meliloti* (SU216), △—△; *R. phaseoli* (CC365), ○—○; *R. leguminosarum* (SU391), ▲—▲; *R. japonicum* (SB16), □—□.

1 Jordan, D.C., and Allen, O.N., Family Rhizobiaceae, in: Bergey's manual of determinative bacteriology, 8th edn, p. 261. Eds R.E. Buchanon and N.E. Gibbons. Williams and Wilkins, Baltimore 1974.
2 Lange, R.T., J. gen. Microbiol. *26* (1961) 351.
3 De Ley, G., and Rassel, A., J. gen. Microbiol. *41* (1965) 85.
4 Gibbins, A.M., and Gregory, K.F., J. Bact. *111* (1972) 129.
5 Vincent, J.M., and Humphrey, B., J. gen. Microbiol. *63* (1970) 379.
6 Graham, P.H., J. gen. Microbiol. *35* (1964) 511.
7 Crow, V.L., Jarvis, B.D.W., and Greenwood, R.M., Int. J. syst. Bact. *31* (1981) 152.
8 Jordan, D.C., Int. J. syst Bact. *32* (1982) 378.
9 Imaeda, T., Kirchheimer, W.F., and Barksdale, L., J. Bact. *150* (1982) 414.
10 Wallace, D.C., and Morowitz, H.J., Chromosome *40* (1973) 121.
11 Chakrabarti, S.K., Mishra, A.K., and Chakrabartty, P.K., Curr. Sci. *52* (1983) 779.
12 Marmur, J., J. molec. Biol. *3* (1961) 208.
13 Savitsky, J.P., and Stand, F., Nature, Lond. *207* (1965) 758.
14 Grouse, L., Chilton, M.D., and McCarthy, B.J., Biochemistry *11* (1972) 798.
15 Chakrabartty, P.K., J. Hered. *66* (1975) 213.
16 Chakrabartty, P.K., J. gen. appl. Microbiol. *23* (1977) 77.
17 Chakrabartty, P.K., Chattopadhyay, S.K., and Schneider, W.C., Cancer Res. *42* (1982) 421.
18 Britten, R.J., and Kohne, D.E., Science *161* (1968) 529.
19 Eigner, F., in: Methods in Enzymology, vol. 12, p. 386. Eds L. Grossman and K. Moldave. Academic Press, New York 1968.
20 Bak, A.L., Black, F.T., Christiansen, C., and Fraundt, E.A., Nature, Lond. *224* (1969) 1209.

0014-4754/84/111290-02$1.50 + 0.20/0

Bacterial growth in artificial capillary spaces

M.T. Bomar[1]

Federal Research Center for Nutrition, Engesserstrasse 20, D-7500 Karlsruhe 1 (Federal Republic of Germany), 16 November 1983

Summary. In experiments on the influence of microscopic capillaries on the growth of *Escherichia coli, Brevibacterium linens, Micrococcus flavus* and *Bacillus cereus*, a distinct delay in growth was observed. The difference in counts was greatest after 8 h. Later a slight equilibration of counts was noted. With *B. cereus*, only slight or no spore formation was observed under microcapillary conditions.

Key words. Bacterial growth; capillary space; spore formation; microcapillary conditions; ecological niche.

The growth rate and biochemical activity of microorganisms are regarded ecologically as responses to a number of physiological factors which are also controlled by the essential influence of external conditions. Within the human body, the dimensions of the habitats populated by microorganisms differ from site to site. Distinct biochemical and physiological properties result from the microscopic communities to which they are closely linked[2]. The host-regulated environments such as mouth or intestine produce varying levels of hydrostatic tensile stress on the microbial cells. It was found that an abnormally low pressure exists in several liquids in micron-size isolated capillarities[3]. O'Brien et al.[4] reported that the growth rates of bacteria were enhanced when the growth media were subjected to hydrostatic tensile stress.

The present study is concerned with changes in the growth rate of various microorganisms in living spaces of different volume. Different capillary properties were simulated by using glass beads of defined size in the nutrient medium.

Material and methods. Microorganisms. *Escherichia coli, Brevibacterium linens, Micrococcus flavus, Bacillus cereus.*

Preparation of the inoculum. All experiments were carried out with tryptone soya broth (Oxoid, CM 129) to which 0.3% yeast extract (Oxoid, L 21) was added. The inoculated broth was shaken at 32 °C in Erlenmeyer flasks. Subsequently 1-ml portions each of the bacterial suspensions were transferred to sterile tubes and frozen at −50 °C.

Determination of bacterial counts. The number of c.f.u. was determined by decimal dilution and the transferring of 0.1 ml of the suspension to the surface of a nutrient agar plate (tryptone soya broth +0.3% yeast extract +1.5% agar). The samples were incubated under aerobic conditions for 2 days at 30 °C.

Determination of spores. The suspension was heated for 10 min at 80 °C in a waterbath; the number of spores was determined in the same way as described above for c.f.u.

Hollow-space systems. The growth rates were studied in the following systems: nutrient broth without solid bodies, and nu-

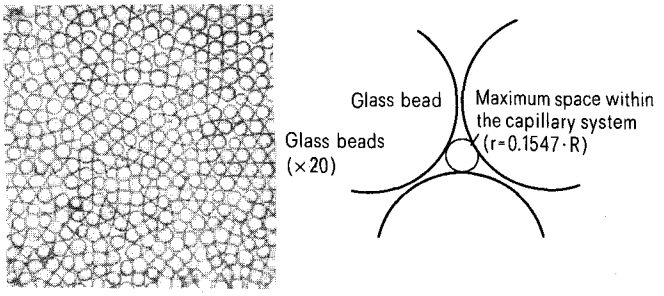


Figure 1. Geometry of capillary systems based on glass beads (two-dimensional representation).

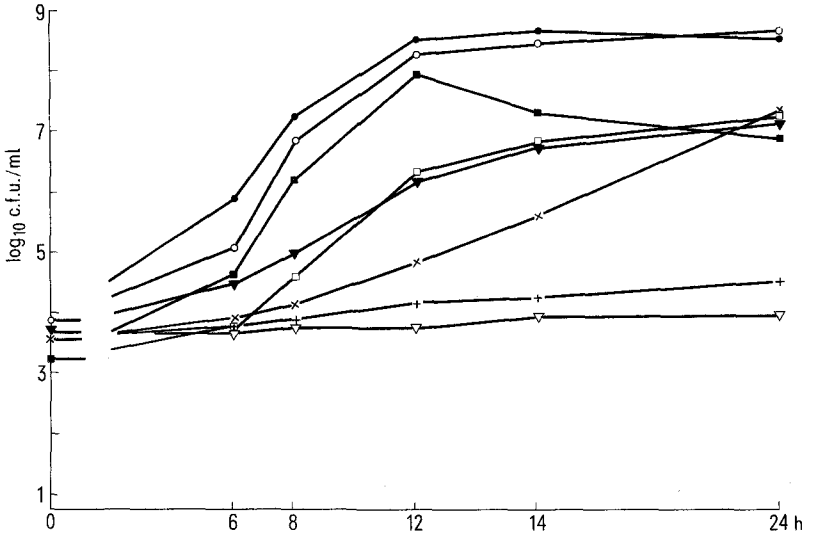


Figure 2. Growth of *E. coli, B. cereus, M. flavus* and *B. linens* in homogenous and heterogenous media (glass beads ∅ 100–110 µm) under microaerobic conditions. ●—●, *E. coli,* homogenous medium; ○—○, *E. coli,* heterogenous medium; ■—■, *B. cereus,* homogenous medium; □—□, *B. cereus,* heterogenous medium; ▼—▼, *M. flavus,* homogenous medium; ▽—▽, *M. flavus,* heterogenous medium; × —×, *B. linens,* homogenous medium; + —+, *B.* linens, heterogenous medium.

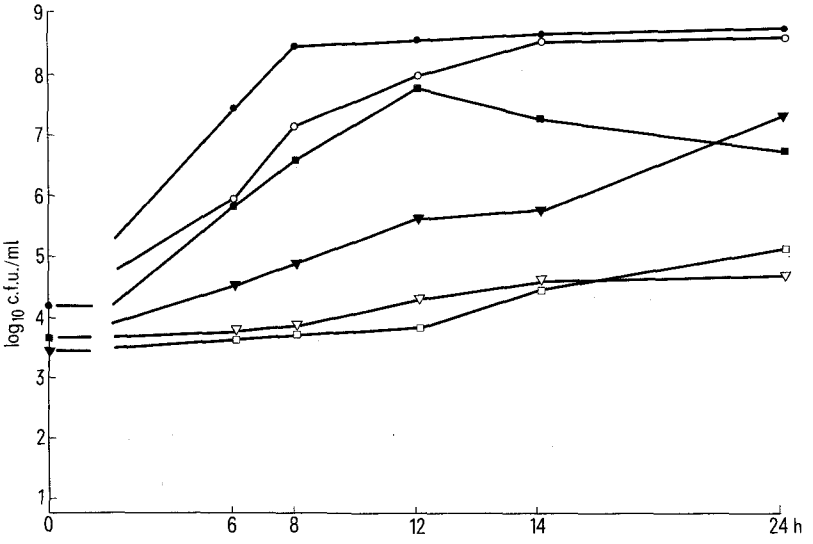


Figure 3. Growth of *E. coli, B. cereus* and *M. flavus* in homogenous and heterogenous media (glass beads ∅ 100–110 µm) under anaerobic conditions. ●—●, *E. coli,* homogenous medium; ○—○, *E. coli,* heterogenous medium; ■—■, *B. cereus,* homogenous medium; □—□, *B. cereus,* heterogenous medium; ▲—▲, *M. flavus,* homogenous medium; △—△, *M. flavus,* heterogenous medium.

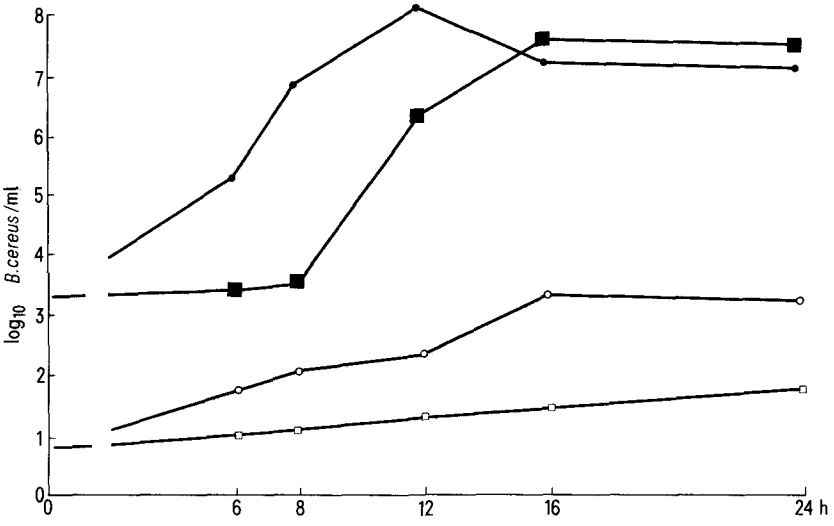


Figure 4. Growth and spore formation of *B. cereus* in homogenous and heterogenous media (glass beads of 250–300 µm) under microaerobic conditions. ●—●, Total c.f.u., homogenous medium; ○—○, total c.f.u., heterogenous medium; ■—■, c.f.u. of spores, homogenous medium; □—□, c.f.u. of spores, heterogenous medium.

trient broth containing glass beads. The diameter of the glass beads was 100–110 µm, 250–300 µm and 1000 µm (B. Braun, Melsungen, Cat. No. 854140/0, 854160/1 and 854180/9). The cleaned glass beads were sterilized for 2 h at 180 °C. The total volume of the liquid phase was 10 ml in each case. Immediately after inoculation of the nutrient broth the preheated beads (30 °C) were added to the test tubes and shaken, in order to eliminate any air bubble residues in the system. In addition, a layer of dry beads of about 10 mm height was added to the test tubes above the liquid surface to make sure that the conditions at the liquid surface corresponded to those of the capillary system. Immediately after the test tubes had been filled, they were incubated at 30 °C under aerobic conditions or under an atmosphere of nitrogen.

Geometry of the capillary spaces. If the beads are arranged regularly, the size of the hollow spaces formed may be characterized by an auxiliary parameter, namely the diameter of a sphere fitting into the hollow space (fig. 1).

Determination of the bacterial growth rates. At 6, 8, 12, 16 and 24 h after inoculation, 3 parallel samples (test tubes) were withdrawn. The entire content (nutrient broth plus beads) was quantitatively transferred to a sterile plastic pouch; 90 ml of sterile nutrient broth were added and the c.f.u. assay was carried out. In order to estimate the adherence of bacteria to the bead surfaces, the nutrient broth plus beads was treated in a Waring blender (Model 32 BL 79, Waring, Conn.) for 60 sec. Immediately after this procedure the c.f.u. was determined.

Results and discussion. In the experiments using *E. coli* the logarithmic phase of growth was delayed both under aerobic and anaerobic conditions in the heterogenous medium containing glass beads of 100 µm in diameter, with differences in counts being greatest after 8 h. Later, a slight equilibration of counts was observed. In order to find out whether the inhibitory effect is different in bacteria of different cell sizes, further experiments were made also with *M. flavus*, *B. cereus* and *B. linens*. These strains also showed a clearly reduced growth rate in heterogeneous media. Their growth is shown in figure 2 (aerobic conditions) and figure 3 (anaerobic conditions). *B. linens* is not shown in figure 3 because no growth was found in either system under anaerobic conditions.

The possibility that the ratio of microbial counts to nutrients available was the reason for the different growth rates of microorganisms was tested by experiments using different initial counts. For these experiments *B. cereus* was selected and used in 3 different counts ($2.92 \cdot 10^2$; $2.5 \cdot 10^1$; $4.5 \cdot 10^0$/ml). It was found that the growth phases of all samples were taking a similar course, i.e. the growth was delayed to a certain extent in the heterogeneous medium. So the influence of nutrient deficiency as the cause of the growth delay can be excluded. The question of sporulation was also studied in *B. cereus*. Conditions for the initiation of this morphological sequence have already been discussed[5] but the influence of very small living volumes was neglected. In the presence of beads of 100 µm in diameter no spore formation was observed within 24 h. A weak spore formation was observed in heterogenous medium with beads of 250 µm in diameter (fig. 4). Comparing studies with *B. cereus* using beads of 100–110 µm and 1000 µm in diameter showed that the delay in growth seems to be the greater the smaller the capillarity spaces (fig. 5).

None of the microorganisms studied form polymers from glucose, so no adhesion to the glass surface occurred. The data so far indicate a distinct decrease in the growth rate of bacteria as a function of the limited size of niches in which they live, and not as a function of a limited nutrient or oxygen concentration.

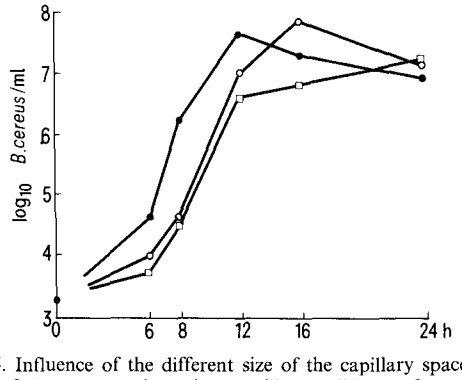


Figure 5. Influence of the different size of the capillary spaces on the growth of *B. cereus* under microaerobic conditions. ●—●, Total c.f.u., homogenous medium; ○—○, total c.f.u., glass beads ∅ 1000 µm; □—□, total c.f.u., glass beads ∅ 100–110 µm.

1 Acknowledgment. The author would like to thank Mr. Theo Wihan for his excellent technical assistance.

2 Alexander, M., in: Microbial Ecology, p. 447. John Wiley and Sons, Inc., New York 1971.
3 Shereshefsky, J. L., and Carter, C. P., J. Am. chem. Soc. 7 (1950) 3682.
4 O'Brien, J. W., Walker, W. C., Fau, P. L., and Johnston, W. M., Appl. envir. Microbiol. *36* (1978) 549.
5 Fitz-James, P., and Joung, E., in: The Bacterial Spore, p. 39. Eds G. N. Gould and A. Hurst. Academic Press, New York 1969.

0014-4754/84/111291-03$1.50 + 0.20/0