TLC plate in addition to the spot of original substrate. The solvent from the reaction mixture was then evaporated to dryness. The dry mass was again dissolved in a small quantity of benzene to remove the dye. The benzene was then evaporated and the white solid mass was subjected to column chromatography to separate the product in first fraction by using benzene as eluent. The ratio of silicagel compound was 20:1 (w/w). The yield of the product after 12, 24, 36 and 48 hours was about 10, 18, 25 and 30% respectively (m.p. 84°C). Calculated C, 49.7; H, 4.1 and N, 8.2 for $C_7H_7NO_4$ found C. 49.4; H, 4.3 and N, 8.0%.

(II) *Rose bengal sensitized photo-oxygenation of sym-diphenylthiourea*

Under exactly identical conditions as in experiment (i), solution of rose bengal sensitizer (2 ml, 1×10^{-4} M) was added in place of methylene blue. The same product was formed as shown by TLC. The yield was determined after 36 hr (about 23%).

(III) *Eosin-Y sensitized photo-oxygenation sym-diphenylthiourea*

Under similar experimental conditions as in experiments (i) and (ii), the oxidation of diphenylthiourea was carried out using solution of eosin-Y (2 ml, 1×10^{-4} M) instead of methylene blue or rose bengal. The same product was formed as shown by TLC. Yield was 20% after 36 hr.

Photo-oxygenation was also carried out in the presence of nickel chloride and cobalt chloride as singlet oxygen scavengers. The yield of the product was considerably decreased.

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SUCCESSFUL COLONIZATION OF *LACCOTREPHES GRISEUS* (HEMIPTERA: NEPIDAE)

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INFORMATION available on aquatic hemipterans are related to their biology, feeding habits and predatory behaviour[1-5]. Most of the nepids are continuous breeders with marked seasonal fluctuations. Thorough scanning of the literature revealed a paucity of information on the colonization of these animals. *Laccotrephes griseus* (Hemiptera: Nepidae) is a predaceous aquatic bug commonly called as nepa or water scorpion. It inhabits the littoral zones of ponds and temporary water system. Attempt was made to colonize the nepa in the laboratory for the following reasons:

(i) it is a potential feeder on mosquito larvae[6]
(ii) larval and adult stages of nepa co-exist with the mosquito larvae and hence it can be used as a tool for biological control
(iii) all the stages of this insect are easily adapted to varying conditions of laboratory rearing and
(iv) this bug reproduces throughout the year.

To get uniform and continuous production of the predaceous bug, attempt has been made to identify a set of standardized conditions for colonizing *L. griseus* in the laboratory. Final instar nymphs of *L. griseus* were collected from the ponds and maintained in the laboratory in a plastic aquarium. They were fed *ad libitum* with larvae of the mosquito *Culex quinquefasciatus*. Freshly emerged adult bugs were identified and sexed. 10 pairs (1♂ : 1♀) were introduced into an aquarium floored with fine sand and elevated sand margins (figure 1). Pebbles, the macrophyte *Chara fragilis* were also provided in the aquarium. Individuals were fed daily with *C. quinquefasciatus* larvae. Mating and reproductive behaviour of *L. griseus* were observed continuously. No detectable courtship behaviour was observed. Mating occurred after 15 days of adult emergence; though copulation was completed within a short period, (about 3–5 minutes), the pairs remained together even 2 days after

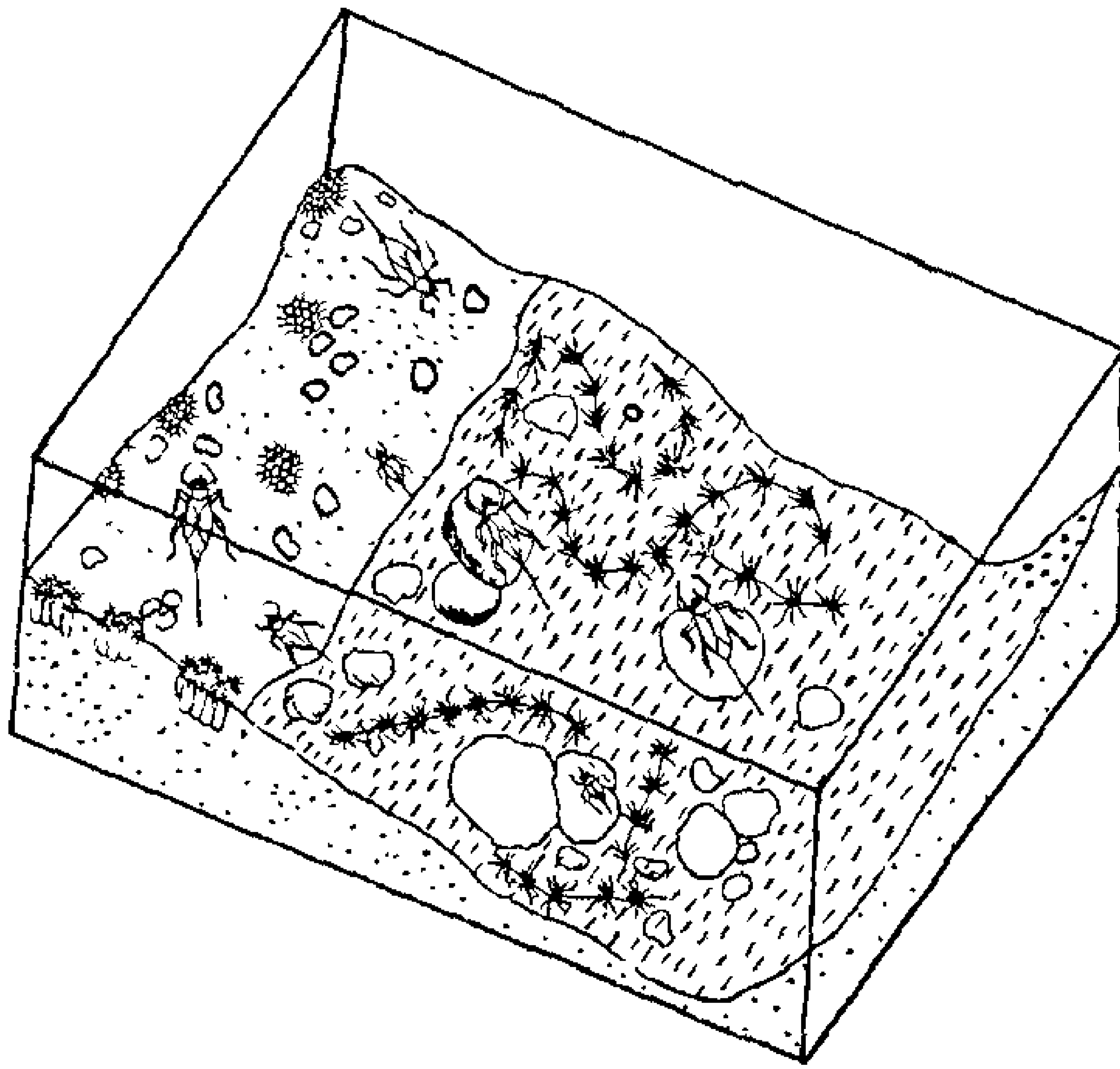

Figure 1. Simulated model aquarium for the colonization of *L. griseus*

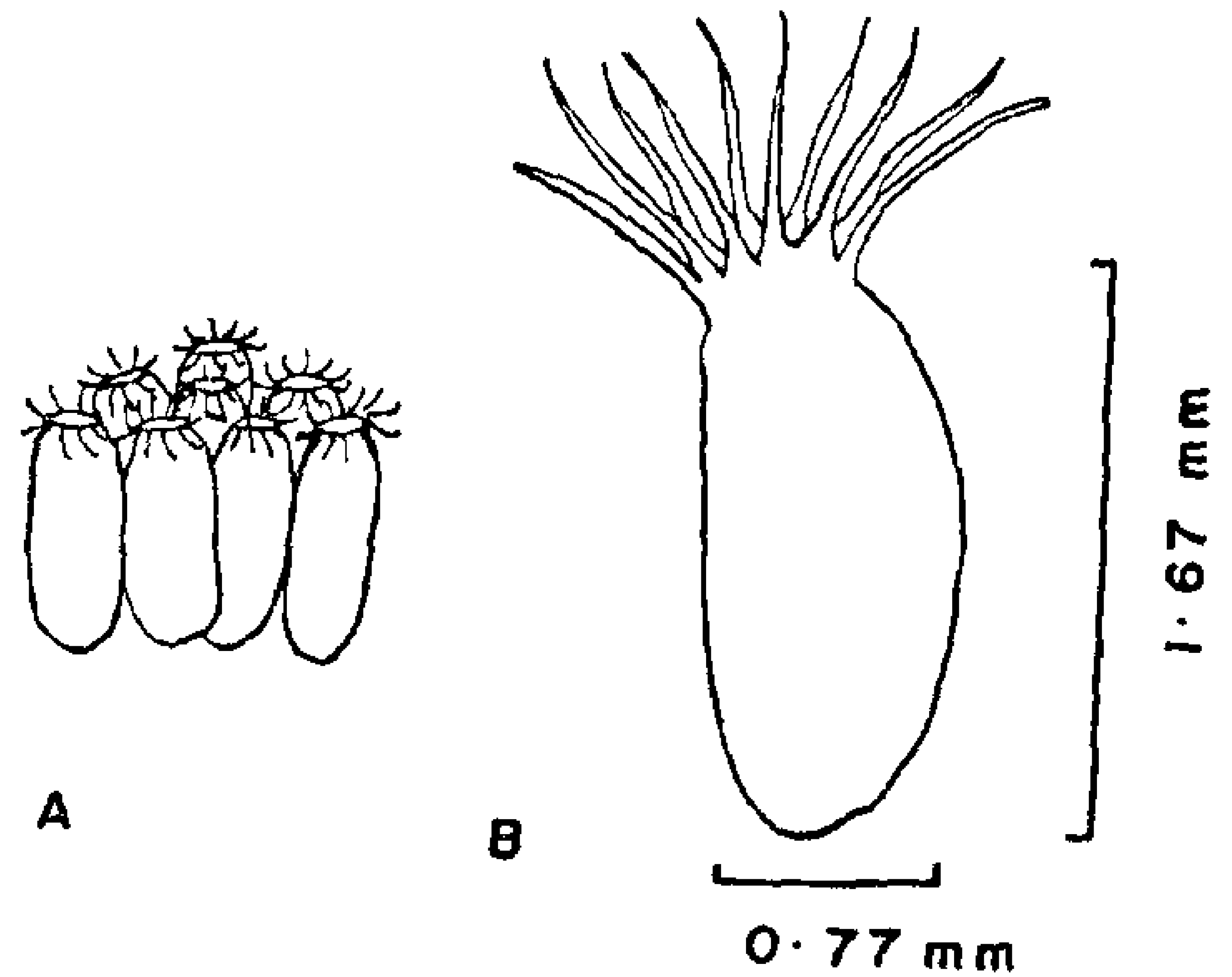

Figure 2. A. Eggmass of *L. griseus* with few eggs; B. Single egg of *L. griseus*

copulation. Since *L. griseus* oviposits on the sand margins[3] observations were continued to examine sand margins soon after mating was completed. Careful observations revealed the presence of bunch of eggs of *L. griseus* in the sand margins of the aquarium. First egg mass was observed 21 days after adult emergence or 7 days after mating. A maximum of about 23.75 ± 3.5 eggs ($n = 25$) and minimum 8.25 ± 2.06 eggs ($n = 25$) were observed in an egg mass. On an average a female laid about 15.75 ± 1.5 ($n = 20$) eggs during the first egg laying. The females repeated egg laying 3 or 4 times during its life time. After the first laying it required about 7.0 ± 2.16 days for every subsequent laying. However number of eggs/laying decreased in the third/fourth laying compared to the previous one. On an average a female laid about 44.25 ± 3.70 eggs during its life time.

The egg mass was embedded in the soil; the apical region of the egg mass with the respiratory filaments are projecting over the sand surface. Egg was oval in shape (length 1.67 ± 0.16 mm; width 0.77 ± 0.05 mm) with 10 apical respiratory filaments (length 0.81 ± 0.04 mm; figure 2). Freshly laid eggs hatched in 9.8 ± 1.4 days in the aquarium. Egg masses were carefully removed from the sand of the aquarium immediately after laying and the eggs were separated carefully. Individual eggs were placed on the sponge pads soaked in water. Care was taken to avoid drying of the sponge pads. Eggs embedded on the sponge pads developed normally similar to the eggs in the aquarium. The freshly laid eggs were pale yellow in colour; it turns to pink on the 6th day and subsequently black prior to hatching. Freshly laid egg weighed 0.69 ± 0.14 mg; it increased to 0.79 ± 0.21, 1.23 ± 0.5 mg on the 6th and 9th days respectively. Freshly emerged nymph weighed about 1.20 ± 0.13 mg.

Soon after hatching the nymphs tend to remain in the margin. They were fed on small size mosquito larvae. *L. griseus* passed through five nymphal stages in about 35.67 ± 5.50 days prior to adult at the water temperature of 30 ± 1°C. Instars I, II, III, IV & V required 5.58 ± 0.80, 6.33 ± 0.40, 5.30 ± 0.44, 7.33 ± 0.41 and 11.07 ± 1.10 day, respectively. There was no difference in the growth of male and female upto early V instar. However, freshly emerged adult males weighed 70.0 ± 5.0 mg and females 104.0 ± 6.0 mg. The males and females emerged in the laboratory copulated after 15 days and females laid eggs after a week.

From the eggs incubated in the sponge pads in the laboratory about 92% eggs hatched into I instar nymph. Of the hatched nymphs 23% successfully emerged into adults. These animals were reared in the laboratory and the colony is being maintained successfully. Cannings[7] successfully reared the hemipteran *Cenocorixa expleta* in the laboratory. He has reported that of the 20 eggs laid originally 12 adults emerged successfully. In otherwords 60% emerged as adults from the eggs laid. This value appears to be fairly high. Rearing the nymphs of dragonflies *Brachythemis contaminta* and *Orthetrum sabina* from egg to emergence, Mathavan[8] reported that about 1% of the eggs

only emerged as adults. Working on the biology of nepids Rao[3] showed the variation in the egg number in different months of the year. However, he has not observed repeated egglaying in any of the hemipterans. We have clearly recorded repeated egglaying by *L. griseus* and perhaps it may be the first report in this aspect.

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PROTOPLAST FORMATION AND REGENERATION IN *RHIZOBIUM* AND *AZOSPIRILLUM*

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PROTOPLAST of gram-positive bacteria have successfully been used for cell fusion between strains of same species[1-3] as well as of distinct species for transferring genetic factors[4-7]. The cell walls of gram-negative bacteria being complex, protoplast formation and their regeneration have been reported only in a few genera like *Escherichia*, *Providentia* and *Pseudomonas*[8-10]. *Rhizobium* and *Azospirillum* are the two major groups of gram-negative soil bacteria which have acquired significance, since the former fixes nitrogen in symbiosis with legumes and the latter in association with cereals and grass roots. Genetics of both the bacterial groups is poorly understood with regard to symbiotic association, host specificity and nitrogen fixation potential and because of that strain selection from native soil population is the only accepted method of getting better isolates. Since protoplast fusion provides a method to transfer genetic factors among distinct species and strains where normal methods of gene transfer is not possible, it was proposed first to standardize methods for protoplast formation and their regeneration in *Rhizobium* and *Azospirillum* so that it could be used subsequently for genetic recombinations. In this communication we report conditions for preparation of protoplasts and regeneration in *Rhizobium* strains Ca42 and Ca141 nodulating chick pea (*Cicer arietinum L.*), S24 nodulating green gram (*Vigna radiata Var. aureus*) and an associative nitrogen fixing bacterium of sorghum *A. brasilense* strain 12S. It was observed that protoplasting and regeneration frequencies varied with the strains as well as with the protoplasting method. However, by slight modification in plating technique 12 to 30% of the protoplasts formed showed regeneration.

Rhizobium strains Ca42, Ca141 and S24[11] were maintained on yeast extract mannitol agar slopes[12] whereas *A. brasilense* strain 12S was maintained on Dobereiner's malate medium[13]. For preparation of protoplasts, the basal medium used for the growth of *Rhizobium* was BMR medium containing: $g\,l^{-1}$ yeast extract, 0.5; mannitol, 10; K_2HPO_4, 0.5; $MgSO_4.7H_2O$, 0.2; NaCl, 0.1; sodium glutamate 0.32 and $mg\,l^{-1}$: $FeSO_4.2H_2O$, 27; $CaCl_2.2H_2O$, 78; EDTA disodium salt, 32; H_3BO_3, 3; $MnSO_4.H_2O$, 4; $NaMoO_4.2H_2O$ 5; $ZnSO_4.7H_2O$, 0.21; KI, 0.78 and $CoCl_2.6H_2O$ 0.025. For *A. brasilense* mannitol in the BMR medium was replaced with $3.2\,g\,l^{-1}$ of sodium malate (BMA medium). Hypertonic sucrose BMR or BMA broth contained 0.5 M sucrose in the basal medium. The broth was always boiled and filtered before autoclaving. In solid medium 1.5% agar was added. For preparation of protoplasts, *Rhizobium* was grown for 96 hr and *A. brasilense* for 48 hr in BMR and BMA broth, respectively. All incubations were done at $28 \pm 1°C$ and centrifugation at 4000 g for 30 min at 4°C. All solutions and media used, were sterilized by autoclaving.

Both, tris-sucrose-EDTA-lysozyme[8] as well as sucrose-cation-lysozyme[10] methods were tried for protoplast preparation. In the former method, cells harvested by centrifugation from BMR or BMA broth were resuspended in 100 mM tris-HCl buffer (pH 8.0) containing 0.5 M sucrose, washed twice and finally taken in the same buffer. Cell density in the buffer was then adjusted to have viable cell counts between 10^8 to 10^9 cells ml^{-1}. To 9 ml of the cell suspension, 1 ml of 100 mM EDTA (disodium salt) was added drop by drop