Successful freeze storage and lyophilisation for preservation of *Helicobacter pylori*

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Abstract

Long term storage techniques for the preservation of *Helicobacter pylori* were developed. The cells survived at −75°C in the presence of glycerol and at +4°C after freeze-drying. Both techniques are suitable for routine use.

Most bacteria survive freeze-drying without loss of viability even after 30 years.[1] One important exception is *Helicobacter pylori*. This fastidious organism which is associated with human active chronic gastritis[2] was reported to be extremely sensitive to freezing and freeze-drying.[3-7]

Methods

Strains of *H pylori* Hp151 obtained from the Institut für Medizinische Mikrobiologie, Freiburg (Germany) were cultivated in Brucella broth (Difco) containing 2% fetal calf serum (FCS; Serva) and 10 mg vancomycin (Sigma) per litre on a rotary shaker at 37°C for three days in an anaerobic jar under a gas mixture consisting of 5% oxygen, 10% carbon dioxide, and 85% nitrogen.

For preservation at −75°C, cells of a 200 ml culture were harvested by centrifugation (20 minutes, 4°C, 11000 × *g*) and suspended in 6 ml of a sterile solution of 10% skimmed milk powder (Glücksklee, Germany) containing 17·4% glycerol. This suspension was stored at −75°C in sterile cryophials (Greiner Labortechnik, Germany) in portions of 2 ml. After one, three, eight, and 11 months some of these cultures were thawed and incubated with 48 ml Brucella broth, as described above.

Results

All cultures yielded profuse growth. The identity of the micro-organism was confirmed by microscopy, characteristic oxidase activity (oxidase test according to the method of Steel[8]), catalase activity (adding a 10% H_2O_2 solution to the cell sediment of *H pylori*), and by urease activity using a modified urease test according to the method of Romano *et al*[9] with a 10 mM phosphate buffer (pH 6), 1 mM urea, and 0·01% (w/v) Cresol red. Moreover, golden pigmented colonies, which are characteristic for *H pylori*, developed on modified Belo Horizonte agar (BHM), according to Queiroz *et al*,[10] with Brucella agar base and Campylobacter Selective Supplement (Merck). The agar plates were incubated in an anaerobic jar with the Anaerocult-C system (Merck) at 37°C for three days.

For lyophilisation, 0·5 ml aliquots of 20% skimmed milk powder solution (Oxoid) were sterilised at 115°C for eight minutes and freeze-dried under vacuum for 24 hours. Portions of 50–100 µl volume of a dense *H pylori* suspension were added on to this carrier material, followed by freezing and drying under vacuum (GT2-Heraeus freeze-dryer) for 24 hours. The tubes were sealed and stored at 4°C for one, two, and 12 weeks. Cultures were successfully recultivated by incubation in Brucella broth; the identity and the purity of the organism was confirmed as described above. All cultures yielded profuse growth.

We thank Mrs A Sauer and Mrs R Schepp for their valuable technical assistance.

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Accepted for publication 31 January 1992.

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