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A SIMPLE TECHNIQUE FOR OBTAINING CLEAN *PANAGRELLUS REDIVIVUS* FROM AXENIC CULTURES FOR STUDIES WITH NEMATOPHAGOUS FUNGI

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Summary. A new method, based on layered discontinuous density gradient of potassium tartrate is proposed to obtain clean *Panagrellus redivivus* from axenic cultures.

The nematode *Panagrellus redivivus* (L.) Goodey is a useful bait (Gray, 1983) for studying nematophagous fungi and has therefore been used for their isolation from soil (Dackman *et al.*, 1987). It can be grown in axenic culture (Barron, 1977), but debris in the medium can be difficult to eliminate and makes the further handling of the nematodes difficult. We propose a simple method for obtaining clean *P. redivivus* from culture media.

Panagrellus redivivus was axenically grown in Neutralized Soya Peptone (Oxoid) as in Jansson and Nordbring-Hertz (1979). Ten days after medium inoculation, a 1 ml sample of the medium (containing *c.* 10,000 nematodes) was axenically sampled (Fig. 1A). The medium containing the nematodes was placed on top of a recently layered discontinuous density gradient of potassium tartrate. The gradient consisted of six layers (2 ml each) of the following concentrations: 1, 0.75, 0.625, 0.5, 0.375 and 0.25 g. ml^{-1} within a 25 ml sterile centrifuge tube (modified from Clarke *et al.*, 1967). The tubes were then centrifuged at 650 g for 5 min. After centrifugation the nematodes were located in the top of the first step of the gradient where they could be easily collected. Conversely, particles from the medium were found within the 2nd or 3rd steps of the gradient. The liquid which contained the nematodes still had pigment from the culture medium. The pigment was removed by three 5 min centrifugations at 1956 g in sterile 1.5 ml Eppendorf centrifuge tubes containing sterile distilled water, each time discarding the supernatant. The nematodes, clean and alive (Fig. 1B), could then be used as bait for isolation of nematophagous fungi.

The method can be modified including sonication at 0 °C (exceeding 90 sec.) before centrifugation in the discontinuous density gradient. This bursts the nematodes and isolated cuticles can be obtained (e. g. second-stage juveniles of *Globodera* spp. (Lopez-Llorca, 1988)).

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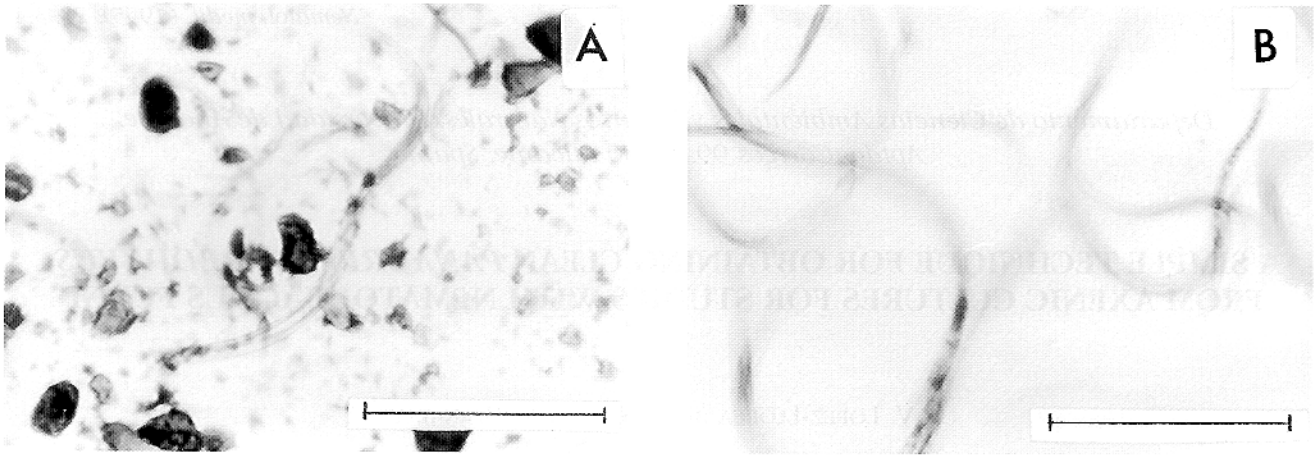

Fig. 1: A, sample of axenic culture of *Panagrellus redivivus* in Neutralized Soybean Peptone, containing medium particles together with nematodes; B, *P. redivivus* grown in axenic culture after centrifugation in discontinuous density gradient of potassium tartrate and futher centrifugation in sterile distilled water showing no debris from the medium (bar = 1 mm).

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