THE IMPORTANCE OF THE BRAND OF THE BEEF EXTRACT IN RELATION TO THE GROWTH OF *FLEXIBACTER PSYCHROPHILUS* IN ANACKER & ORDALS MEDIUM

By Ellen Lorenzen

During the last 8-9 years a disease known as rainbow trout fry syndrome (RTFS) has resulted in serious losses in rainbow trout hatcheries throughout the world (Baudin-Laurencin et al., 1989, Holt et al., 1989, Lorenzen et al., 1991, Santos et al., 1992). The disease has been found to be caused by a flexibacterium named *Flexibacter psychrophilus*. Therefore, the need of an efficient medium for isolation and cultivation of this bacterium has increased.

According to Anacker & Ordal (1955, 1959) a low nutrient medium consisting of 0.05% tryptone (Difco), 0.05% yeast extract, 0.02% sodium acetate, 0.02% beef extract and agar adjusted to pH 7.2 is suitable for cultivation of *Flexibacter columnaris*. This medium has been used by several authors also for cultivation of *Flexibacter psychrophilus* (Bullock, 1972; Schaechte, 1983; Baudin-Laurencin et al., 1989; Bernardet & Grimont, 1989; Holt et al., 1989; Lehmann et al., 1991; Bruno, 1992; Santos, 1992). Some of the authors have reported improvement of the medium e.g. by increasing the amount of tryptone to 0.5% (Bernardet & Grimont 1989), adding 10% foetal calf serum (Obach & Baudin-Laurencin 1991) or 5% new-born calf serum (I. Dalsgaard, personal communication). In the present laboratory we obtain a good growth by combining these experiences and using 5% new-born calf serum and/or 0.5% tryptone (Lorenzen & Karas, 1992). The concentration of agar used seems to depend on the brand of agar and/or the user: Anacker & Ordal (1959) thus suggest 0.9% (Difco), I. Dalsgaard (personal communication) 1.0% (Gibco) and we use 0.7% (Oxoid). In all cases, however, the agar is very soft and moist, which together with freshness of the preparation seems to be important for the growth of the bacteria (Freireich 1984).

In some laboratories, however, problems with very poor growth or no growth at all of *Flexibacter psychrophilus* during sub-cultivation have been encountered even when using the recipe above. In at least two cases these problems apparently were related to the brand of the beef extract used (R. Rangdale, FDL, UK, personal communication; author's own experiences). It turned out that neither the beef extract produced by Oxoid (Cat. No. L 29), nor the one produced by Gibco (Cat. No. 152-00004) was efficient in the concentration recommended (0.02%), whereas the product from Difco (Cat. No. 0126-01) in the same concentration gave an excellent growth. In the case of Oxoid and Gibco products, the beef extract is a powder whereas the Difco product is a semifluid substance. Since beef extract is not a well defined product, the reason for the observed differences in relation to the growth of *Flexibacter psychrophilus* is unclear. One possible explanation might be that the semifluid extract contains important components in a higher concentration.

In addition to the above it deserves mentioning that the readiness of *Flexibacter psychrophilus* to multiply seems to depend very much on the strain as well as on its previous history (freezing, lyophilization, semi-fluid agar, usual agar, broth, fish etc.). Primary isolation of *Flexibacter psychrophilus* apparently can be done on several media, which do not allow successful sub-culture.
Author's address
National Veterinary Laboratory, Haugevej 2, DK-8200 Århus N, Denmark

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References


