DETECTION OF β-HEMOLYTIC STREPTOCOCCI

➢ REGULATION

β-hemolytic streptococci are pathogens responsible for septic sore throat and scarlet fever in humans and for mastistis in cattle. However there exists no EC microbiological criteria concerning these microorganisms, the French regulation [1] specifies that **for raw milk intended for direct human consumption,**

there should be no β-hemolytic streptococci in 0.1ml.

It also specifies that the β-hemolytic streptococci belong to A, B, C, G or L Lancefield serological groups [2]

➢ NON STANDARDISED METHOD

An official text [3] lists the standardised methods applicable to all other microbiological criteria, but there is no recommanded method for the detection and enumeration of these streptococci.

The litterature [4] explains that « there exists no satisfactory selective medium for the quantitative estimation of β-hemolytic streptococci in foods ». However, it is claimed that the TKT medium, described in 1953 by Hauge and Ellingsen, quoted in [4], allows detection and selective growing of all β-hemolytic streptococci. [4] and [5]. This medium is a blood agar, containing thallium sulfate (T), crystal violet (K) and a titrated quantity of toxin from a *Staphylococcus* β-hemolytic culture (T). The toxin accentuates the hemolytic reaction of the streptococci looked for. A few years ago, this toxin was manufactured and allowed easy preparation of the medium. But production has stopped ; so the users have to prepare the toxin themselves, which is not easy. It seems that AFNOR therefore cancelled, a few years ago, a draft standard, based upon this medium.

At the present time, there is no alternative commercial medium. Identification strips imply prior isolation, whereas commercial tests kits for fecal streptococci are usually intended for those belonging to Lancefield group D, not concerned here.

➢ AFSSA METHOD

AFSSA recently developped a method. For isolation purposes, it recommends to use a classical blood agar medium, but with a slightly modified procedure. Indeed, this medium is not selective enough. Therefore, β-hemolytic streptococci may be completely undetectable because of overgrowth by other bacteria, particularly by those that grow rapidly and produce large colonies. That's why AFSSA recommends to streak 0.1 ml of the initial suspension on more than one Petri dish, in order to favour isolated colonies.

Colonies having β-hemolytic zones must then picked for confirmation and identification. First, they have to be examined microscopically. Typical cocci chains must then be prepared for :

- Gram staining : streptococci are Gram-positive,
- catalase test : streptococci are catalase-negative,
- for biochemical screening, such as the CAMP-test, esculin or hippurate hydrolysis, or sugar fermentation screening. This can also be done by using identification strips for streptococci, which allow to identify rapidly pathogenic species of streptococci.

In some special cases, further serological tests may be necessary.

➢ IN CONCLUSION

As regulation exists and users demand it, it seems really necessary to define a reference method. The AFSSA method is applicable, but not well known. It would be highly interesting to launch a standardisation procedure based upon this method.

Abbreviations : AFNOR : French Standardization Body
AFSSA : French food safety agency, former CNEVA

Bibliographic references are in « La Lettre de CECALAIT »