ENTEROBACTER SAKAZAKII: PRESENTATION OF THE STUDY LED BY THE AFSSA

(EVALUATION OF THE ISO-IDF DRAFT STANDARD METHOD FOR THE DETECTION OF *ENTEROBACTER SAKAZAKII* IN MILK POWDER PRODUCTS AND POWDER INFANT FORMULA)

Summary of the talk presented by Mrs GNANOU-BESSE (AFSSA) at CECALAIT's AGM 2005

***Enterobacter sakazakii* is a Gram-negative bacillus, member of the *Enterobacteriaceae* family.**
It has been identified as a pathogenic agent in sporadic but serious neonatal infections, causing meningitis, septicaemia or enterocolitis. The disease frequency is very low (several tens of cases since the Sixties), but the mortality rate is 20 to 50%. There are few infections among adults suffering from serious underlying disease. In the majority of cases, the consumption of infant powdered milk formula has been blamed. A rather recent example was the outbreak of neonatal infections in France in December 2004, due to the contamination of powder infant formula.
As with all the coliforms, this pathogen is widely found in the food and domestic environments, and it is especially sought in dried milk. A Canadian study shows that this bacterium is present in 6.7% of dried milks, but the rates of contamination are generally lower than 1 cfu per 100 g. The infectious dose is around 1000 bacteria, therefore a problem of bottle preparation and storage can be blamed in the cases of neonatal infections.
In 2004, a draft standard for the detection of *Enterobacter sakazakii* in milk powder and powder infant formula was proposed by the IDF (see figure). The AFSSA LERQAP, as the European Community reference laboratory for milk and milk products (European directive 92/46), has decided to test this method. The objectives of this study were to evaluate its performances and its applicability to powder infant formula, and to compare several available selective media.
The following criteria of performance were studied: (i) the inclusiveness and exclusivity; (ii) the detection limit ; (iii) the method performances for the analysis of naturally contaminated products.

Inclusiveness and exclusivity were studied using 28 pure strains of *E. sakazakii* and 16 non-*E sakazakii Enterobacteriaceae* strains. The whole protocol of analysis was followed by the isolation on 4 agars: 3 chromogenous media: ESIA™ (AES Laboratoires), DFI™ (Oxoid, Dardilly, France), and ESIA™ fabricated in our laboratory according to the formula described in the draft standard; and a medium for Enterobacteria: VRBG.
The results obtained are similar for all the chromogenous media and no difference was observed between ESIA™ ready-to-use and ESIA™ produced in the laboratory.
Concerning the aspect of the colonies obtained on these media, the specificity and the sensitivity are good, but the specificity for the medium VRBG is only 50%. Indeed, on this medium, all the colonies of *Enterobacteriaceae* appear as typical.
Therefore, the step of selection of the yellow-pigmented colonies after spreading on the non-selective medium TSA decreased the performances of the method. The sensitivity of the 4 media tested hardly decreases after this step. Indeed, some strains of *E. sakazakii* (about 20%) did not produce typical yellow-pigmented colonies on TSA.
On chromogenous media, mainly for DFI™, the definition of typical colony colour should be widened as it does not allow strains of *E. sakazakii* to be detected.
To evaluate the detection limit, samples of infant powder formula with four different levels of contamination (from 5 cfu/100 g to 50 cfu/25 g, and a blank) were tested. The analyses were repeated six times per level, except the blank which was repeated twice. Then, the detection limit was assessed for 3 *E. sakazakii* strains.
The results show positive detections at all the levels of contamination, except for the blanks, with similar results for the 4 selective media tested.
All the detection limits obtained are lower than 10 cfu/100 g, the lowest was 4 cfu/100 g.

The analyses of naturally contaminated samples were realised with 100 g of 3 powder infant products implicated in neonatal infections. The draft standard protocol was followed and 4 selective media were tested with incubation at 37 and 44°C, which are temperatures recommended for DFI™ and ESIA™. In all the cases, *E. sakazakii* was detected. However, yellow or white pigmented colonies were obtained on TSA medium. For the medium DFI™, an incubation at 44°C is less effective, and the temperature recommended by the manufacturer is best.

To conclude, the detection method of *E. sakazakii*, as described in the draft standard (ISO/IDF), appears to be sensitive and selective, but could be improved by some modifications:
- the widening of the definition of typical colonies on chromogenous media,
- the taking into consideration of the problem of

the non-pigmented strains, which appears frequently in the study. One solution should be to re-isolate all the typical strains on chromogenous media, and perhaps to add an additional biochemical test before complete identification. (activity Tween 80 esterase)

Due to their similar performances, the 2 agars DFI™ and ESIA™ could be used in this method. The medium VRBG shows relatively correct performances because of the selectivity of the liquid enrichment step. Consequently, it could be used as a complement to a chromogenous medium.

In this study, we demonstrated that the results obtained on medium ESIA™ produced in the laboratory or on ready-to-use medium ESIA™ are equivalent.
A complementary study, based on the analysis of a larger number of naturally contaminated samples, is in process at the AFSSA LERQAP.

Concerning the evolution of the standardisation of this method, a new draft was diffused, in August 2005, for comments. The main modification concerns an incubation of the broth at 44°C instead of 45°C in the previous version.

Figure: ISO/IDF draft standard method flow scheme