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Evaluation of the ß-Glucuronidase Test with the multiple tube technique for specific determination of *Escherichia coli*

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The use of *Escherichia coli* as an indicator of the sanitary quality of water and seafood products has been classically recommended by different official organizations (APHA, 1985; Greenberg & Hunt, 1985; WHO, 1977), since this microorganism provides a measure of fecal contamination. According to the APHA (1985) two methods are currently available for enumerating *E. coli* from those samples: the Most Probable Number (MPN) procedure and the membrane filtration (MF) technique. Both present advantages and disadvantages, but the former requires the need of presumptive and confirmatory tests, which are costly, time-consuming and arduous.

Recent reports of fluorogenic assay procedures have shown the promise for a rapid and specific detection of *E. coli* in seawater (Mates, 1987; Gauthier *et al.*, 1988), seafood (Rippey *et al.*, 1987) and foods (Robinson, 1984). This new method relies on the almost exclusively presence of the enzyme ß-glucuronidase in *E. coli*. This enzyme cleaves a fluorogenic substrate, 4-methylumbeliferyl-ß-D-glucuronide (MUG) to yield an end product, 4-methylumbeliferone, which is fluorescent under UV light.

In the present study, a comparative study of MPN technique using MacConkey purple supplemented with MUG as presumptive broth and nutrient agar with MUG as confirmatory test from seawater, sediment and shellfish samples was carried out.

All the strains isolated with positive fluorescence in nutrient agar-MUG from the gas-positive and fluorescence-positive tubes were identified as *E. coli*. On the other hand, the isolates positive in gas and fluorescence from the tubes and MUG-negatives on nutrient agar were identified as: *Klebsiella pneumoniae* (66.7%), *E. coli* (16.7%) and *Enterobacter agglomerans* (16.7%) from seawater samples; *K. pneumoniae* (100%) from sediment samples; and *K. pneumoniae* (33.3%), *E. coli* (33.3%), *E. cloacae* (14.8%), *E. sakazakii* (4.8%), *Citrobacter freundii* (4.8%), *Serratia liquefaciens* (4.8%), and *Buttiaux agrestis* (4.8%) from shellfish samples. The high frequency of *E. coli* MUG-negative detected in these samples from tubes presumptively positive may be explained by the negative effect exerted by the selective agents contained in the MacConkey-purple broth, which could affect the permease of the ß-glucuronidase activity, or may be a competence effect exerted by the background flora present in the shellfish, or a self-activity of ß-glucuronidase by the shellfish tissues (Koburger & Miller, 1985; Holt *et al.*, 1989).

A high proportion of the strains MUG-negative on nutrient agar isolated from the gas-positive tubes were identified as *K. pneumoniae*. This result agrees with those reported by Rippey *et al.* (1987) and Damoglou *et al.* (1988), being considered the mainly false-positive species in the coliform analysis.

In Table 1 the characteristics of MPN tubes in which *E. coli* was isolated are shown. *E. coli* was verified in about 90% of fluorescent tubes. Only one *E. coli* strain as isolated from fluorescence-negative and gas-positive tubes, and MUG-positive on nutrient agar. For this reason, this methodology is considered to increase the accuracy of the MPN technique to isolate *E. coli* from samples of different sources.

TABLE 1.- Percentages of *Escherichia coli* isolated from MPN tubes (MacConkey-purple broth).

SAMPLE	MUG Tubes +	MUG Tubes -	Gas Tubes +	Gas Tubes -	mFC Colony Typical	mFC Colony NonTypical	AN-MUG +	AN-MUG -
Seawater	91	17	64.7	ND	76.5	20	100	14.3
Sediment	100	17	78.6	0	93.8	0	100	22.6
Shellfish	84	0	70.8	0	71.4	21	100	0
Total	90	12	70.9	0	78.7	14.6	100	15,5

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Specific determination of *Salmonella* strains using a phage-typing scheme

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Phage-typing is a practical method for bacterial differentiation based upon the sensitivity and high specificity of the strains to bacteriophages.

There are numerous potentially pathogenic serotypes of *Salmonella* and some of them can possess an epidemiological relevance. The methods actually applied to study the epidemiological markers of these strains are based on serological and antimicrobial resistance characteristics. However, these markers offer a low discrimination rate and they are unable to determine the epidemic outbreaks. For this reason, a single, wide-spectrum phage-typing scheme may provide additional advantages with respect to the classical typing methods (Gershman & Markowsky, 1983).

A phage-typing scheme, comprising 25 bacteriophages isolated from sewage on 12 *Salmonella* serotypes, were used as a possible epidemiological marker and/or determinative tool of *Salmonella* isolates, regardless their source or serotype.

A total of 224 *Salmonella* strains, belonging to the 20 serotypes most frequently isolated from waters in Malaga (Spain) (Moriñigo *et al.*, 1988), were analysed using the phage-typing techniques described by Adams (1959) and Anderson (1962).

On the basis of the phage set lytic activity, 117 phage-types have been recognized. Table 1 shows the phage-patterns obtained from the different serovars of *Salmonella* tested. Only one strain could not be typable by this method, however self-agglutinable, non-motile and monophasic strains of *Salmonella* can be determined by the phage-typing method.

The detection frequency of the different phage-types of the same serotype is relatively low (less than 5%), which coincides with the results obtained by other authors (Gershman, 1976; Bouzoubaa *et al.*, 1985).

In short, the results obtained indicate that: (i) no significant relationship was observed between the susceptibility of the strains to specific bacteriophages and their somatic antigenic characteristics. (ii) All the strains belonging to C1 serogroup present a narrow and specific sensitivity pattern to bacteriophages, which implies the possible diagnostic use of this phage set. (iii) A close relationship between the phage-types on *S. typhimurium* and *S. enteritidis* was observed, which indicates a possible common source or epidemiological route.

TABLE 1.- Phagetypes from the different *Salmonella* serotypes tested.

Serotype	Serogroup	Number of Strains	Number of Phagetypes
S. blockley	C2	27	15
S. bovis-morbificans	C2	2	2
S. braenderup	C1	2	2
S. enteritidis	D1	21	6
S. infantis	C1	7	6
S. london	E1	20	10
S. menden	C1	1	1
S. montevideo	C1	1	1
S. muenchen	C2	4	2
S. ohio	C2	24	15
S. oranienburg	C1	1	1
S. paratyphi C	C1	1	1
S. potsdam	C1	4	3
S. richmond	C1	3	3
S. senftemberg	E4	6	4
S. taksony	E4	1	1
S. thompson	C1	15	9
S. typhimurium	B	45	24
S. virchow	C1	7	7
S. weltevreden	E1	3	3
Self-agglutinable	-	24	17
Non-motile	-	3	3
Monofasic	-	1	1

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