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**Culture medium for the identification of Salmonella  
and process for its use**

5 The present invention relates to an isolation medium permitting the identification of bacteria of the Salmonella species.

The identification of the bacterium Salmonella, which is pathogenic for man, is a major problem in medical bacteriology and in the monitoring of food hygiene.

10 Thus, in the case of epidemics transmitted by chicken farms, the birds infested in the intestinal tract are not ill but constitute a reservoir of Salmonella. The latter can be propagated, especially by eggs, in the diet following these epidemics. In fact, Salmonella is a bacterium whose notification is obligatory.

15 Nowadays, it is becoming essential to provide for the large scale detection of infected sites and more particularly of farms infected with the Salmonella bacterium in order to reduce these epidemics.

20 Indeed, Salmonella are generally to be recognised among other microorganisms such as the commensal species Escherichia coli and Proteus.

25 The detection of Salmonella is normally carried out on agar medium for the selective isolation of enterobacteria which permits the differentiation of pathogenic enterobacteria and the detection of suspicious Salmonella colonies. An ideal isolation medium should permit the growth of enterobacteria, the differentiation of the various species present so as to permit subsequent identification of a colony of each type, and the detection of suspicious  
30 Salmonella colonies.

The object of the present invention is to make it possible to carry out highly simplified routine tests for Salmonella, for example methods for monitoring the microbiological quality of water using soaking methods, or the monitoring  
35 of the microbiological quality of food factories using contact methods,

the diagnosis or alternatively the detection of pathogenic microorganisms using traditional microbiological Petri dish methods on solid medium or on semi-solid selective medium, by means of media according to the invention.

5 The object of the present invention is to permit the detection and differentiation of Salmonella by revealing an activity specific to these bacteria.

The Salmonella bacterium has among its range of enzymes esterases which cleave in particular caprylate derivatives such as nitrophenyl caprylate.

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It could therefore be envisaged to use in an isolation medium a chromogen attached to the caprylate and its derivatives, a substrate for esterases, which would be liberated into the medium under the action of these enzymes, allowing the identification of Salmonella colonies.

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However, tests on chromogens such as 5-bromo-4-chloro-3-indolyl caprylate in a conventional culture medium for Salmonella have not made it possible to identify colorations specific for the inoculated colonies.

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It has been shown, surprisingly, that the addition of a detergent to the culture medium at an appropriate concentration, and which is compatible with a high Salmonella growth, made it possible to obtain a coloration of the Salmonella colonies.

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The present invention therefore relates to a culture medium intended for the identification of Salmonella bacteria, comprising, in addition to a culture medium for Salmonella, as chromogenic agents, a combination of:

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- a chromogenic compound linked to a C<sub>7</sub>-C<sub>10</sub> fatty acid, and
- an appropriate detergent which promotes the liberation of the chromogenic compound.

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Preferably, the chromogenic compound is chosen from caprylic acid esters, advantageously chosen from derivatives of indolyl caprylate and their salts, in particular 5-bromo-4-chloro-3-indolyl caprylate.

Preferably, the 5-bromo-4-chloro-3-indolyl caprylate will be used at a concentration of between 40 and 200 mg/l.

5 The appropriate detergent will be advantageously chosen from fused polycyclic detergents, in particular salts of carboxylic acid derivatives-cholane polyols.

Preferably, the appropriate detergent will be chosen from the alkali metal salts of cholic acid or its derivatives, in particular sodium deoxycholate.

10 The sodium deoxycholate may be used at a concentration greater than 1 g/l, preferably of between 1.5 and 5 g/l, a concentration which is perfectly compatible with a high Salmonella growth.

15 Advantageously, the chromogenic compound/appropriate detergent weight ratio is between 1/10 and 1/100.

It should be noted that the present invention does not consist in a mere addition of detergent to an identification medium, and that it is necessary to consider the chromogenic compound/appropriate detergent pair.

20 Thus, for the indolyl caprylate derivatives, only the fused polycyclic detergents, derivatives of cholane, make it possible to obtain a coloration of the Salmonella colonies, whereas its replacement with a detergent such as the standard anionic detergents (especially the detergents marketed under the trade mark TERGITOL by the company UNION CARBIDE Corp.) does not  
25 make it possible to identify a coloration.

It is also particularly important to note that the chromogenic agent of the medium according to the invention does not result in coloration with many  
30 other enterobacteria even if they are allowed to incubate for several weeks.

A person skilled in the art will of course know how to supplement the medium according to the present invention so as to eliminate exceptional non-Salmonella bacteria, especially by identifying other Salmonella-specific  
35 properties, in particular the properties relating to cleavage or metabolism of beta-galactosides and beta-glucosides.

Advantageously, the medium according to the present invention will also contain beta-glucosides and/or beta-galactosides.

5 Thus, a coloured Salmonella colony will be easily identified on a microbiological device even in the presence of thousands of other colonies, or on a semi-solid medium in order to increase the specificity of detection.

10 A deep blue coloration characteristic of the presence of Salmonella is generally obtained. However, other colorations may be envisaged depending on the chromogenic compound used.

15 Moreover, the detection will be facilitated by the fact that it necessitates neither the use of a non-viable coloured indicator nor that of a specific illumination.

20 The present invention also relates to a process for detecting strains of Salmonella in any sample, for which the culture medium as described above is inoculated with the said sample and in that the coloration characteristic of the presence of Salmonella is at least identified.

Depending on the form and presentation of the medium and depending on the test carried out, the medium is preferably inoculated by contact, by soaking, by surface inoculation or by inoculation within the mass.

25 Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The preferred specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the disclosure in any way whatsoever.

30 The entire disclosures of all applications, patents, and publications cited above and below, and of corresponding application French 92-12 533, filed October 20, 1992, are hereby incorporated by reference.

35 The examples below are intended to illustrate other characteristics and advantages of the present invention.

**EXAMPLE:** Detection of Salmonella strains

5 Various concentrations of sodium deoxycholate are added to the medium S (in g/l: meat extract 1; yeast extract 2; peptone 5; sodium chloride 5; agar 15; 5-bromo-4-chloro-3-indolyl caprylate 0.2).

10 The dishes are incubated at 37°C and the blue coloration of the colonies of the bacterium Salmonella AR3001 and of the control bacterium E. coli AR2901 is examined after incubating for 24 hours at 37°C.

Salmonella is identified only with the chromogenic substrate and deoxycholate combination.

	Salmonella AR3001	E. coli AR2901
15 Deoxycholate 0.5 g/l	colourless	colourless
Deoxycholate 1.0 g/l	light blue	colourless
Deoxycholate 1.5 g/l	blue	colourless
Deoxycholate 2.0 g/l	darkish blue	colourless
20 Deoxycholate 2.5 g/l	dark blue	colourless
Deoxycholate 3.0 g/l	dark blue	colourless

25 Various enterobacteria strains are isolated on the medium T (in g/l): meat extract 1; yeast extract 2; peptone 5; sodium chloride 5; agar 15; neutral red 0.03; Cellobiose 10; sodium deoxycholate 2.5; 5-bromo-4-chloro-3-indolyl caprylate 0.2).

30 The dishes are incubated at 37°C and the coloration of the colonies is examined after incubating for 24 hours.

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The coloration of the colonies makes it possible to distinguish *Salmonella* from the other enterobacteria.

	<i>Salmonella</i>	AR3001	blue
5	<i>Salmonella</i>	AR3002	blue
	<i>Salmonella</i>	AR3003	blue
	<i>Salmonella</i>	AR3004	blue
	<i>Salmonella</i>	AR3004	blue
	<i>Citrobacter</i>	AR30033	pink
10	<i>Citrobacter</i>	AR3437	pink
	<i>Citrobacter</i>	D9316	pink
	<i>Citrobacter</i>	D9352	pink
	<i>Serratia</i>	AR3493	pink
	<i>Enterobacter</i>	AR3494	pink
15	<i>Klebsiella</i>	B2497	pink
	<i>Klebsiella</i>	B2593	pink

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**CLAIMS**

1. Culture medium intended for the identification of Salmonella bacteria, characterised in that it comprises, in addition to a culture medium for Salmonella, as chromogenic agents, a combination of:  
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  - a chromogenic compound linked to a C7-C10 fatty acid, and
  - an appropriate detergent which promotes the liberation of the  
10 chromogenic compound.
2. Culture medium according to Claim 1, characterised in that the chromogenic compound is chosen from caprylic acid esters.
- 15 3. Culture medium according to Claim 2, characterised in that the chromogenic compound is chosen from derivatives of indolyl caprylate and their salts.
- 20 4. Culture medium according to Claim 3, characterised in that the chromogenic compound is 5-bromo-4-chloro-3-indolyl caprylate.
- 25 5. Culture medium according to Claim 4, characterised in that the 5-bromo-4-chloro-3-indolyl caprylate is used at a concentration of between 40 and 200 mg/l.
- 30 6. Culture medium according to one of Claims 1 to 5, characterised in that the appropriate detergent is chosen from fused polycyclic detergents, more particularly from the salts of carboxylic acid derivatives-cholane polyols.
- 35 7. Culture medium according to Claim 6, characterised in that the appropriate detergent is chosen from the alkali metal salts of cholic acid or its derivatives.
8. Culture medium according to Claim 7, characterised in that the appropriate detergent is sodium deoxycholate.

9. Culture medium according to Claim 8, characterised in that the sodium deoxycholate is used at a concentration greater than 1 g/l, preferably of between 1.5 g/l and 5 g/l.
- 5 10. Culture medium according to one of Claims 1 to 9, characterised in that the chromogenic compound/appropriate detergent weight ratio is between 1/10 and 1/100.
- 10 11. Culture medium according to one of Claims 1 to 10, characterised in that it is presented in a liquid, semi-liquid or solid form.
- 15 12. Process for detecting Salmonella strains in any sample, characterised in that the culture medium according to any one of Claims 1 to 11 is inoculated with the said sample, and in that the coloration characteristic of the presence of Salmonella is identified.
- 20 13. Detection process according to Claim 12, characterised in that the culture medium is inoculated by contact, by soaking, by surface inoculation or deposition or by inoculation within the mass.

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## INTERNATIONAL SEARCH REPORT

International Application No  
PCT/EP 93/02659

A. CLASSIFICATION OF SUBJECT MATTER IPC 5 C12Q1/04 C12Q1/10 C12N1/20		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols) IPC 5 C12Q C12N		
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Electronic data base consulted during the international search (name of data base and, where practical, search terms used)		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EUROPEAN JOURNAL OF MEDICAL CHEMISTRY vol. 25, no. 8, 1990, PARIS F pages 679 - 700 A. AGBAN ET AL. 'Synthèse de substrats indigogéniques. Mise en évidence de l' activité estérastique des Salmonella.' see the whole document -----	1-13
<input type="checkbox"/> Further documents are listed in the continuation of box C. <input type="checkbox"/> Patent family members are listed in annex.		
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