(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 025 467**
A1

(12) EUROPEAN PATENT APPLICATION

(21) Application number: **79400638.7**

(22) Date of filing: **12.09.79**

(51) Int. Cl.³: **C 12 Q 1/04**, C 12 Q 1/12

(43) Date of publication of application: **25.03.81**
Bulletin 81/12

(84) Designated Contracting States: **CH DE FR GB NL SE**

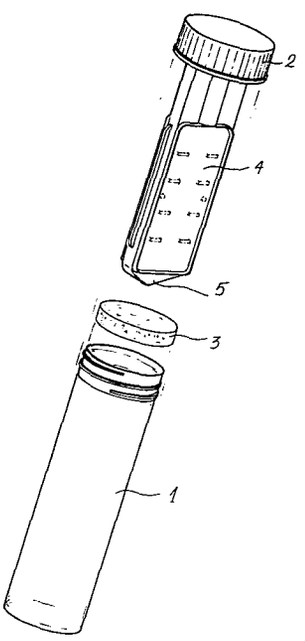
(71) Applicant: **Rambach, Alain, 75, rue Madame, F-75006 Paris (FR)**
Applicant: **Société TECHNOGRAM, 19, rue Théodore Deck, F-75015 Paris (FR)**

(72) Inventor: **Rambach, Alain, 75, rue Madame, F-75006 Paris (FR)**

(74) Representative: **Martinet, René et al, Cabinet Martinet 62, rue des Mathurins, F-75008 Paris (FR)**

(54) Chromogenic chemical substrates for identification of microbial colonies.

(57) Chromogenic substrate member for identifying Escherichia coli bacteria. It comprises a growing medium for said bacteria and container means (1) for the growing medium (4) and a liquid sample to be tested. The container means also contains a chromogenic reagent compound consisting in 8-hydroxyquinoline-β-D glucuronide and an activator compound consisting in X-glucuronide where X is selected from the group consisting in alkyl, aryl or indoxyl radical, the halogeno substitutes and the nitro substitutes thereof.



EP 0 025 467 A1

Chromogenic chemical substrates for identification of microbial colonies.

Field of the invention

The present invention concerns chromogenic chemical substrates permitting to recognize microbial colonies, particulary Escherichia coli colonies, by incorporation ot the chemical substrates into the growth medium. The identification is obtained from the specific coloration of the colonies.

Escherichia coli, is with, few exceptions, found in the fecal wastes of all warm-blooded animals. This characteristic, along with its simple nutritional requirements, makes this organism a well-chosen candidate as an indicator of fecal contamination. A simple method and means for chromogenically detecting Escherichia coli colonies is therefore of practical importance.

Description of the prior art

Chromogenic substrates are already used to identify enzymes and examples of such known substrates will be given in the following. However, these substrates are inadapted to certain microbial identifications on colonies.

In certain cases, the coloration specific for the particular enzyme does not appear spontaneously after the enzymatic reaction on the substrate, since the degradation product is colorless and it is necessary to further add a developer in order to cause the color to appear. As a result, not only another dispensation of liquid (the developer) has to be made, but it is impossible to continually observe the reaction to be studied.

Other substrates proposed in the prior art liberate colored pro-

ducts volatil or diffusible and this limits the time during which the solution or the biological suspension can be left in the presence of the substrate before important problems of diffusion or evaporation are encountered.

Finally, other substrates previously proposed become yellow after the enzymatic reaction, which is a color difficult to detect and visualize, as many biological media already have this color per se.

Jonas S. FRIEDENWALD and Bernard BECKER "The Histochemical Localization of Glucuronidase", J. Cell. and Comp. Physiol., 31, 303-310, 1948, have developed a sensitive photocolorimetric method for the quantitative assay of glucuronidase activity. The tissue to be tested is incubated with 8-hydroxyquinoline glucuronide in the presence of ferric ions in a solution saturated with ferric hydroxyquinoline. The enzymatically liberated 8-hydroxyquinoline is precipitated as ferric hydroxyquinoline at the presumption site of the glucuronidase activity. This black precipitate is then converted into Prussian Blue.

Further it is known Escherichia coli has the capability of synthetizing the glucuronidase enzymatic activity. One could thus expect that, if 8-hydroxyquinoline-glucuronide is incorporated in a microbiological medium in which Escherichia coli is caudes to grow, the corresponding colonies would be detected in the form of black blobs.

Unfortunately, these black blobs are not formed neither observed by the bacteriologist when he treats Escherichia coli by 8-hydroxyquinoline glucuronide reagent.

Summary of the invention

I have found that, by supplementing the 8-hydroxyquinoline glucuronide reagent with an activator, the Escherichia coli colonies give rise to black blobs.

This activator is X-glucuronide where X is a radical selected from the group comprising alkyl, aryl, indoxyl and the halogeno or nitro substitutes of these radicals.

Preferably, X is methyl, benzyl, phenyl, halogenomethyl, nitro-

methyl, halogenobenzyl, nitrobenzyl, halogenophenyl and nitropheny

The colored product of degradation is insoluble. This is most useful when, for example, several microorganisms develop on the same solid medium and give rise to colonies well separated from each other, because the colored product does not diffuse from one colony to the other. It is thus possible to see directly by the color of the colony whether the corresponding microorganism is Escherichia coli or not.

Another advantage of the chromogenic substrates according to the invention is that they can withstand 120° C so that the microbiological medium which contains them can be sterilized by simple autoclaving without disparition of the chromogenicity.

Brief description of the drawing

Fig. 1 is a perspective view of a commercially available kit for detecting bacterial contamination by Escherichia coli.

Description of the preferred embodiments

A test kit commonly used for detecting microbiological contamination in liquids and which can be used for putting the invention into practice is shown in fig. I. It consists of a sterile container I with a pad 5 connected to the cap 2 of the container. The pad 5 has a cav ty filled with a nutrient 4. Absorbent cotton 3 is located at the botto of the container for absorbing excess liquid. The pad is immersed i the test liquid and returned to its container to be incubated for the appropriate time and temperature. The nutrient medium 4 has inco porated thereto the reagent and activator previously defined.

First example

The reagent is

8-hydroxyquinoline-β -D glucuronide

in the presence of ferric salts

and the activator is

phenyl- β -D glucuronide

The degradation product is ferric hydroxyquinoline which is black and insoluble.

Second example

The reagent is

8-hydroxyquinoline -β-D glucuronide

and the activator is

bromophenyl -β-D glucuronide

Third example

The microbiological control of liquids is often performed with the aid of a sampler containing a solid nutrient medium on which the microorganisms present in the liquid can grow and form colonies visible to the naked eye. It is important to know whether fecal bacteria are present. The former procedure is to detect whether bacteria, capable of fermenting lactose and growing at exactly 44.5°C, are present.

Instead of this procedure which requires a very precise temperature of growth, thus a delicate instrument, one can use according to the invention a much simpler procedure. It consists in taking as reagent a solution of

8-hydroxyquinoline-β-D glucuronide at I/I000 containing ammoniacal ferric citrate

and as activator

nitrophenyl-β-glucuronide at a concentration of 500 µg. per ml.

Since precise temperature, 44.5°C, is no longer necessary in this new procedure, the user of a pool can simply dip a sampler containing a medium ready to use into the pool water and let the sampler incubate either in the sun or in any lukewarm place to see whether black colonies appear, thus indicating the presence of fecal bacteria. In a more general fashion this approach permits systematic control of the presence or absence of bacteriological contamination of water, and environment, without requiring costly instruments sometimes difficult to transport to the sites.

What I claim is :

1 - Chromogenic substrate member identifying Escherichia coli bacteria comprising :

a growing medium for said bacteria ;

container means for said growing medium and a liquid sample to be tested ; said container means also containing :

a chromogenic reagent compound consisting in 8-hydroquinoline- β-D glucuronide ; and

an activator compound consisting in X-glucuronide where X is selected from the group consisting in alkyl, aryl or indoxyl radical, the halogeno substitutes and the nitro substitutes thereof

whereby Escherichia coli bacteria form dark pigmented blobs on said medium.

2 - Chromogenic substrate member according to claim 1, in which the activator compound is :

methyl-glucuronide

3 - Chromogenic substrate member according to claim 1, in which the activator compound is :

a halogeno-methyl-glucuronide

4 - Chromogenic substrate member according to claim 1 in which the activator compound is :

a nitro-methyl-glucuronide

5 - Chromogenic substrate member according to claim 1 in which the activator compound is :

benzyl-glucuronide

6 - Chromogenic substrate member according to claim 1 in which the activator compound is :

a halogeno-benzyl-glucuronide

7 - Chromogenic substrate member according to claim 1 in which the activator compound is :

a nitro-benzyl-glucuronide

8 - Chromogenic substrate member according to claim 1 in which the activator compound is :

phenyl-glucuronide

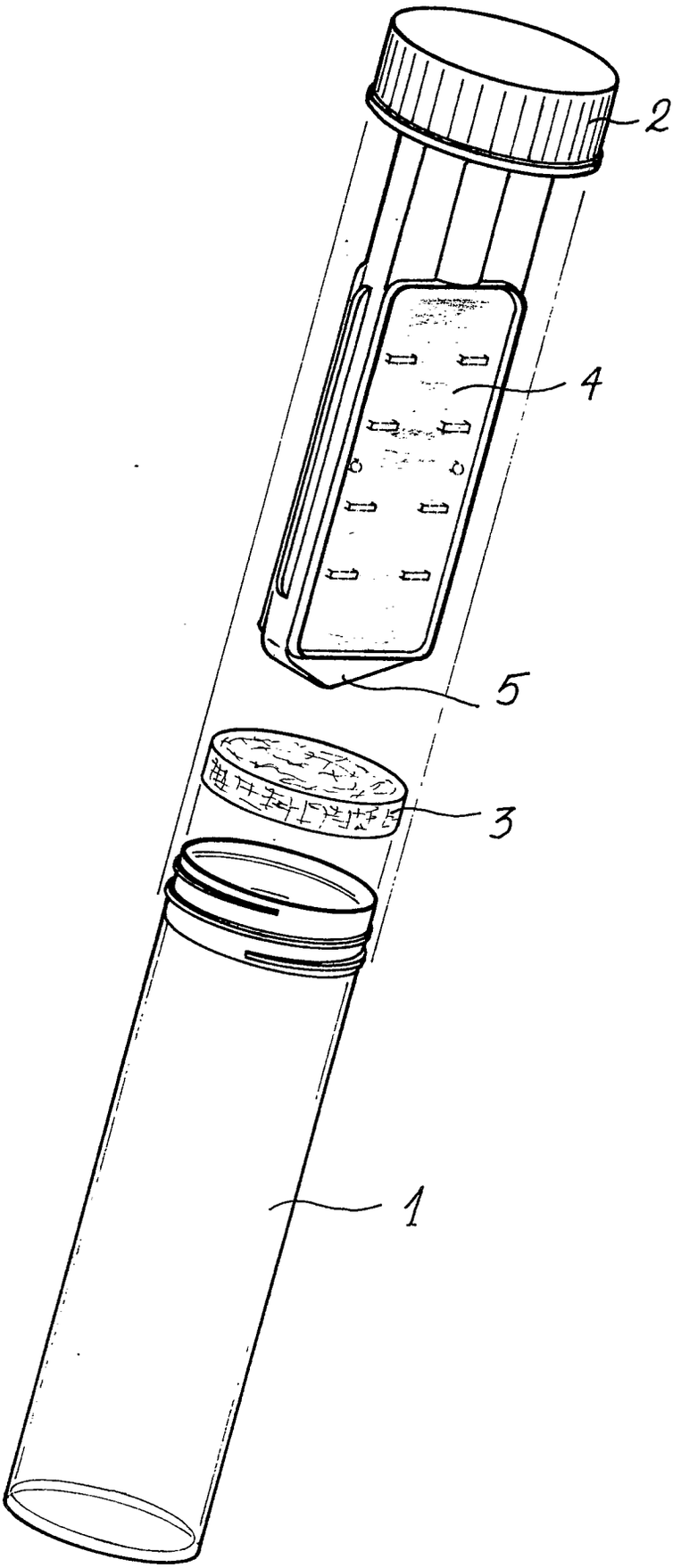
9 - Chromogenic substrate member according to claim 1 in which the activator compound is :

a halogeno-phenyl-glucuronide

10 - Chromogenic substrate member according to claim 1 in which the activator compound is :

a nitro-phenyl-glucuronide

FIG.1



European Patent Office

EUROPEAN SEARCH REPORT

0025467

Application number

EP 79 40 0638

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim
	CHEMICAL ABSTRACTS, vol. 86, no. 25, June 20,1977, page 268, abstract 185673p. Columbus, Ohio, USA MANDRAND-BERTHELOT, M.A. "Gratuitous induction of Escherichia Coli K12 β-glucuronidase and its double mechanism of repression". & Biochimie 1977, 59(2), 163-70. * The whole abstract * --	1,3
D	CHEMICAL ABSTRACTS, vol. 42, no. 21, November 10, 1948, abstract 8913c Columbus, Ohio, USA FRIEDENWALT et al. "The histochemical localization of glucuronidase" & J. Cellular Comp. Physiol, 31, 303-9 (1948) * The whole abstract * --	1
	CHEMICAL ABSTRACTS, vol. 85, no. 11, September 13, 1976, page 233, abstract 74277x. Columbus, Ohio, USA & JP - A - 76 63687 (YATORON K.K) (02-06-1976) * The whole abstract * --	1
A	CHEMICAL ABSTRACTS, vol. 78, no. 25, June 25, 1973, page 172 abstract 156487k. Columbus, Ohio, USA NOVEL, G. et al. "Mutants of Escherichia coli K12 unable to grow on methyl-β-D-glucuronide. Map	2

./.

CLASSIFICATION OF THE APPLICATION (Int. Cl. 3)

C 12 Q 1/04
C 12 Q 1/12

TECHNICAL FIELDS SEARCHED (Int.Cl. 3)

C 12 Q 1/02
1/04
1/12

CATEGORY OF CITED DOCUMENTS

X: particularly relevant
A: technological backgrounc
O: non-written disclosure
P: intermediate document
T: theory or principle underl the invention
E: conflicting application
D: document cited in the application
L: citation for other reasons

&: member of the same pate family, corresponding documen

[X] The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
The Hague	18-04-1980	DE LUCA

EPO Form 1503.1 06.78

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int. Cl. 3)
tegory	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
	location of uid A locus of the structural gene of β-D-glucuronidase". & Mol. Gen. Genet. 1973, 120(4), 319-35 * The whole abstract * --		
A	CHEMICAL ABSTRACTS, vol. 81, no. 21, November 25, 1974, page 454, abstract 136459m. Columbus, Ohio, USA & JP - A - 74 69663 (05-06-1974) * The whole abstract * ----	1	TECHNICAL FIELDS SEARCHED (Int. Cl. 3)