

The Application of Chromogenic Culture Media for Rapid Detection of Food and Water Borne Pathogen

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Abstract: One of the latest techniques that used in recent decade to rapid detection of pathogenic agent in water and food is chromogenic media. These media are very specific and their component act as substrate for specific enzyme and depending on enzyme exhibit special color. This study is an introduction to chromogenic media as a powerful tool to rapid identification of bacterial and fungal agent in water and food sample. In this survey we review studies on using chromogenic media in rapid identification pathogen microorganisms in water and food in recent decade. Studies in different countries confirmed that chromogenic media are specific, rapid and sensitive and by using of them the most important water and food borne pathogenic microorganism such as E.coli, Staphylococcus aureus, L. monocytogenes, Salmonella and Candida can identified easily. in compare with other diagnosis methods chromogenic media have more advantage and can be an appropriate alternative for conventional and routine procedure. Chromogenic media eliminate the need of subculture and further biochemical test for identification pathogenic agent and at the shortest period of time pathogenic agent can be identified.

Key word: Chromogenic culture media % Rapid diagnosis % Pathogenic microorganism food and water

INTRODUCTION

Isolation and identification of pathogenic microorganism is one of the most important aspects of food hygiene. Today identification and enumerations of microbes in food have done in two main techniques. Most of These conventional methods are time consuming and in spite of low cost often have difficulties for microbiologist. These depletion specially when rapid result report have importance from medical or economical view considered more significant [2]. These technique based on inoculating bacteria in various media and producing visible colony in solid selective media. In some bacteria like Salmonella it is necessary to take steps of pre-enrichment, enrichment and selective plating. Fortunately discovering rapid detecting methods have solved most of theses problem. These methods have high accuracy, rapid and with the help of them in shortest period of time the microorganism can be identified [1, 2]. About modern techniques that applied

in recent years for this purpose we can hint to genetically, physical and immunological method (Table 1). Hill believes that for each one of these technique equipped lab and experienced technician are necessary and have expensive performs cost beside each of them have their advantage and disadvantage however using technique like PCR and ELISA Gradually find their position in microorganism identification method [3]. As the matter of fact, today necessity of using rapid diagnosis method of food pathogen are more needed than ever. Lately using chromogenic media is one of the rapid diagnostic methods that introduced as appropriate alternative for conventional method in developed countries and applying these sensitive, accurate and specific methods in diagnosis process is a turning point in analytical microbiology and considered as powerful tools [18]. Although chromogenic culture media first time introduced in 1979 by Rambach but it officially offered and produced since 1991 [23].

Table1: Latest method of identification and isolation food pathogen microorganism

Method Name	Technique
1 Physical methods(diagnosis of microbe cell or their activities)	Impedance measurement, Flowcytometr direct microscopic examination
2 Chemical and biochemical methods	ATP assay (Bioluminescence) and Determination of microbial metabolism
3 Immunological methods(based on antibody)	Immuno fluorescence, ELISA Latex agglutination
4 Genetical method	Polymerase chain reaction, DNA Hybridation

In recent decades many research has been done in relation to rapid diagnosis pathogenic agent in food and water by chromogenic media and many paper has been published [4, 13, 5, 30, 25, 20].

In this study, we review latest decade studies on using chromogenic culture media in rapid detection of pathogenic microorganism in food and water in different countries.

Identification Water and Food Borne Pathogen by Chromogenic Media: By using chromogenic culture media following pathogenic microorganism can be identify:

Coliforms and *E. Coli*: Coliforms and specially *E.coli* considered as microbial contamination marker in food and water and they presence in drink water and food indicate that this material are contaminated with other enteric pathogens, so its isolation and enumeration have great important in determination of foods hygiene[28]. For detecting Coliforms acid and gas producing from lactose is the base of most diagnosis method, but occasionally some fecal Coliforms specially *E.coli* in some cases because of lack of ferment hydrogenase enzyme not produce the gas and this cause to new enzyme definition, among the rest can mentioned to existence of β -D-galactosidase in Coliforms and β -D glucuronidase in 94 to 96 percent of *E.coli*. Other species of *E.coli* doesn't produce this enzyme[21].

For evaluating the enzyme activity chromogenic material have been used. For instance with chromogenic (5-bromo 4-chloro 3-indol β -D-gluconoride) activity of β -D-glucuronidase can be evaluated. Also chromogenic substances (5-brmo 6-chlro 3-indol- β -D-galactosidase) have been used for demonstrating existence of β -D-galactosidase. Based on new definition variety commercial culture media for detecting Coliform especially *E.coli* by using chromogenic media has been invented that make it possible to identification them simultaneously in food and water. For instance can be mentioned to culture media like LMX broth media, ready cult media, chromo cult media (Merck Germany), Coli id media (Biomerio France) (Manafi *et al.* 2004, manafi andromans 1988). *Escherichia*

coli variety O157H7 is significant food born pathogenic agent and can cause diarrhea, hemorrhagic colitis and uremic hemolytic (HUS).this bacteria caused many epidemic by contaminated food[6]

Conventional culture media using for *E.coli* is Mccankey sorbitol agar that have a low exclusivity and high false positive rang. Also in case of long time incubation color changing occurred in colonies that can not easily identified[1]. Recently new selective culture media has been made that rises possibility of isolation of this variety of *E.coli*. Some of the most important of this media cultures are rain bow agar (Biolog America), BCM O157H7 (Biosynthesis Swiss). Chromagar O157 (Chromagar France). late medium because have high sensitivity and exclusivity made it possible to identification this pathogen bacteria easily. Sensitivity of this method is as high as 98% and purple colonies are identifiable. Other species of *Escherichia coli* are colorless or blue in this media[2,32,14].

Salmonella: Because routine and conventional culture media apply for *Salmonella* diagnosis employing non specific marker sulfide hydrogen producing (SH₂) have poor exclusivity that lead to numerous false positive result. (like *Citrobacter* and *Proteus*) [2]. In *Salmonella* id media (sm-id) (Biomerio France) *Salmonella* colonies identification by red color, Coliform in purple or blue and *proteus* species are colorless [14,12,11]. Biochemical characterizes used in the media are acid formation from gluconat incorporation with neutral red indicator and a chromogenic substrate material that make it possible to different *Salmonella* from other *Entrobacteriaceae*. Selective element in media consists of bile salt, brilliant green Rambach agar (Merck Germany) that synthesis from propilen glycol, peptone, yeast extract, sodium dexoxy colat and neutral red. *Salmonella* chromogenic culture media like Rambach agar and *Salmonella* ID thanks to the high selectivity power, make the lab technician needless to performing test on irrelevant colonies and hereby save sufficient time to concentrating on real *Salmonella* colonies. This feature specially has importance on salmonellosis outbreak. Above culture media are selective and permit to easily identify most of the *Salmonella* species based on distinct red colonies.

Table 2: identifiable microorganism by chromogenic media according to genus, color sensitivity and differential ability

No.	Genus	Colony color	Sensitivity (%)	Differential ability (%)
1.	<i>S.aureus</i>	Purple and red	95.5	99.4
2.	<i>S.epidermidis</i>	Blue	95.5	99.4
3.	<i>E.coli</i> O157	Purple	98.0	99.0
4.	<i>E.coli</i> spp	Blue or colorless	98.0	99.0
5.	<i>Salmonella</i>	Red	100.0	100.0
6.	<i>V. cholerae</i>	Blue	99.0	98.5
7.	<i>V. vulnificus</i>	Green	99.0	98.5
8.	<i>V. parahaemolyticus</i>	Purple	99.0	98.5
9.	<i>L. monocytogenes</i>	Blue with colorless halo	100.0	100.0
10.	<i>L. innocua</i>	Blue without halo	100.0	100.0
11.	<i>C. albicans</i>	Green	99.0	99.0
12.	<i>C. tropica</i>	Metallic blue	99.0	99.0
13.	<i>C. krusei</i>	Pinkish Velvet	99.0	99.0

The main defect of rambakh agar is that *Salmonella* type, *Salmonella paratyphi* and some scarce species have not detected. Beside that, β -D-galactosidase producing *Salmonella* species (e.g. *Salmonella* Arizona) exhibits purple bluish colonies on both media.

Staphylococcus Aureus: *Staphylococcus aureus* infections are one of the worlds most prevalence food infection and also are among the most significant pathogenic agent in clinical and hospital infection. The source of bacteria are skin, mucous and persons hand and many epidemic have been reported [25, 27]. significant conventional culture media applied for identification this bacteria are potato dextrose agar (PDA) and Manitol salt agar(MSA) which they act based on high salt concentration and manitol fermentation and have a lot of false positive and negative results. Also another current culture media (blood agar) because necessity of time consuming and expensive test like coagulase that should be perform at least on 5 to 10 presumptive colonies from each sample is not routines [7,13]. While *S.aureus* chromogenic medias are unique with high sensitivity more than 95% (equal to coagulase), make it possible to accurate and easy identification of the pathogens. *S.aureus*'s colonies have 99.4% differential ability and demonstrated with purple color and other staphylococcus species colonies are blue or colorless [6,7,12,14,22,31,30].

In recent years more hospitals are involving with methicillin resistance *S.aureus* (MRSA) and current culture media demonstrated unreliable result in detection of these species while chromogenic media produced for resistance strain with high differential and sensitivity as 100% just in one 24 hours incubation can isolate the pathogen and antibiotic sensitive strain not able to growth in this media [21, 22, 24]. Diedern and others in 2006 applied new chromogenic media to detecting MSRA and achieve the satisfactory results [15,17].

Listeria Monocytogenes: is a dangerous food poisoning agent and exhibit high resistance to environmental factor like low temperature, dryness and heat and isolated from many food even dairy pasteurized product [2]. Efficiency of Conventional culture media like Oxford and Palcam for culturing and isolating the organism considered uncertainly. Beside the lab technician in conservative method should perform a lot of consuming culture test for confirming the presumptive colonies. *Listeria* chromogenic media cultures (Chromoagar) with the help of advance technology make it possible to direct isolation *Listeria* in one step. This test method sensitivity is 100% and in *L. monocytogenes* have special blue color and surrounded with white color halo due the phospholipids activities, so easily identified. *L.innocuu* is blue (without halo) and other *Listeria* species are colorless [2,4,6,13,19].

Vibrio Cholerae: *Vibrio cholerae*, *Vibrio Parahaemolyticus* and *Vibrio vulnificus* are pathogens that cause food borne disease, generally contagion by polluted water and vegetable and lead to severe food poisoning [6,13,15]. Conventionally TCBS culture media have been used for isolation and identification which have poor sensitivity beside long turn around time, while specific *Vibrio* chromogenic media (Chromoagarr vibrio) easily isolated this 3 vibrio species from other species and the sensitivity of the method evaluated 98.8%. In this media the color produced by *Vibrio cholera*, *Vibrio parahaemolyticus* and *V. vulnificus* colonies are blue, green and purple respectively and other species are colorless [14].

Yeast (Variety Species of Candida): Among the various yeast *Candida* have significant rule in human infection and food spoilage and many report have been received relate to *Candida albicans* infections [13]. yeast isolating and diagnosis take a several day in lab and have not high

sensitivity, but chromagar Candida beside the ability to detecting yeasts, have ability to simultaneously isolating variety species of Candida according to their colonies color. Also this media can detect antifungal resistance species. Isolation ability in this method is 99% and Candida albicans species identified by green color, C. tropicalis metallic blue and C. krusei pink [15] in Table 2, identifiable microorganism by chromogenic culture media had been demonstrated.

RESULTS AND DISCUSSION

Conventional methods for detecting pathogen agent generally based on bacteria culture in different media and visible colonies production in a selective solid media and conducting biochemical test in liquid media. These methods are time consuming and for bacteria isolation in food sometimes need to perform following step of pre-enrichment, enrichment and culture in selective solid media and occasionally it is necessary to employed further test to confirm like biochemistry test e.g. catalase, oxidize, coagulase and IMVIC test [2]. In recent years biotechnology advance lead to change in food test technique and today, we benefit from methods that are more specific, faster and often more sensitive in compare with conventional method [22, 23]. One of the latest method introduced for rapid detection of pathogen microorganism in food is using chromogenic media. Utilizing these media can eliminate necessity of further subculture and biochemical test in identification process of bacteria. These technique based on production substrate material for specific microorganism enzyme, according to the produced color the microorganism can be identified easily [13].

As the matter of fact many substance produce colored or fluorescent substance after reaction with microbe enzyme or other component this feature has been used for identification. Most of the substrate utilize for chromogenic media are phenol and indol derivatives and often Comarin derivatives have been used for fluorescent substrate. Derivatives of β -D-gluconoride or β -D-galactoronid widely use in food pathogen rapid diagnostic kit. These component metabolized by respective decomposer microbial enzyme chromogenic or florescent product, produced from their metabolism [14].

Chromogenic media have many advantages like rapid detection, high sensitivity, highly specific, needles to further biochemical test in microorganism identification.

Research conducted all over the world has been shown that these technique have higher specifically,

exclusivity and performance in compare with other technique. Rahbar and other (2008) for MRSA identification have used four method and culture media that was consist Manitol salt agar, E-test Mic, Oxacillin screen agar, Plus and chromogenic culture media the result showed chromogenic media in compare with other method have had higher sensitivity and exclusivity as sensitivity and exclusivity of this method defined 100% [2, 14, 22, 25]. The result of this study are similar to other survey [12, 15, 21, 22, 26, 30].

It is clear that these techniques like other methods have defect. Specific media culture, expensive cost, dissimilar usages are the most important of this method disadvantages. For instance ready cult media can be used only for drink water and can not be used for food specimen. In this method identification of some genus of bacteria is not feasible for example clostridium perfringens, because often water contaminated with variety species of this bacteria after culture in chromogenic media there will be a lot of red colors so its not possible to identification bacteria species because all of the red colonies are not clostridium perfringens. Beside ammonium hydroxide presence in the media is reaky and sometimes the ammoniums itself eliminate the colonies. This problems lead to new chromogenic culture media production that are able differ clostridium species (C.P. chromagar). Late media have ability to produce variety of color and further research have been conducted related to this media [18].

One of the important aspect these media come useful is water contamination rapid identification and utilize this methods have huge impact on prevention of water borne diseases. 2.1 million people have died hence using contaminate water WHO announced. These issues especially in crises and military have great importance, because in such situation water microbial test result should reported at possible shortest period of time chromogenic media are very helpful. In conventional E.coli and Coliforms (that are water contamination indicator) identification methods are very time consuming furthermore there are many bacteria genus that are able to produce acid from lactose (ie. Klebsiella, Citrobacter, Entrobacter and etc) that their presence in water not mandatory result of contamination [4,5]. So we need rapid and specific method that be able easily identified Coliforms specially *E.coli*.

Our research showed according to these bacteria importance as indicator of food and bacteria contamination many research has been done on them. Herein Anfor and others tested 2500 water sample. In all

of cases test results was satisfactory and even variety species of *E.coli* have been identified [20,22] for this purpose LMX and ready cult culture media employed that make it possible to rapid and simultaneous detection of *E.coli* and Coliforms in unite culture media by consisting of following compound (5 bromo 4 chloro 3 indol β -D-galactopyranose) and fluorogenic component 4 methyl β -D-gluconoride. Also adding 1-isopropil β -D-thiogalactopyranose (IPTG) substance that easily produces lactose yield to produce and increase activity of β galactosidase. in this method 99% of *Escherichia coli* can be detected and identified exclusively.

In research conducted by Manafi and others on 1246 sample contaminated to varies species, 1240 species (99%) have been identified by chromogenic media and a few non Coliform bacteria such as *Serratia*, *Hafnia*, *Vibrio* and *Aeromonas* species have and false positive result. he believes that employing chromogenic media in compare with the other standard methods detects more Coliforms and *E.coli* within 24 hours[8]. Baunadonna and others studies (2007) shown this media when compared with other method and confirmatory tests, chromogenic media have more sensitivity when identifying *E.coli* in water sample[14, 6]. Pitkanen and others confirmed ability of detecting Coliforms in *E.coli* contaminated water by chromogenic media[13, 18, 19, 21]. Stampi and others in italy applied this method to identify *E.coli* O157H7 in cattle meat product. Also Gonzalez and others have used specific chromogenic media for detecting ready to eat food contamination[2, 5, 14].

Utilizing chromogenic and fluorogenic media to detecting beta galactosidase activity for differentiation verity spiciest of Enterococci get the great attention. Heretofore substrate substance like 4-methyl β -D-glycosidase[4, 6] and indoxil β glycosidase[7] described for β galactosidase enzyme activity detection. Ready cult Enterococci (Merck Germany) with using β galactosidase reaction as Enterococci persistence idicatore. In this reaction 5-brmo 4chlro 3-indol β -D-glucoopyranose realized and rapidly oxidized and convert to bromochloro-indigo.that give blue color in chromo cult and ready cult Enterococci broth media [4-7, 13]. Result of pure culture on 104 water sample tested by manafi and others showed 97% of positive result identified as Enterococci and 3% of result faced with false positive spices (some species of *Leuconostoc*, *Lactococcus lactis* and *aeromonas*). This methods have had satisfactory result with water sample (cfu 100ml) after 24 hours incubation. These selective media are rapid, sensitive and easy to use and

interpretation [14, 15].The result of this reviewed studies according to the last decade research all over the world [14, 15, 18, 22, 23, 30] chromogenic culture media are useful tool in rapid identification of food and water pathogen, and with utilizing them significant food and water pathogenic microorganism i.e. *Salmonella*, *E.coli*, *S.aureus* monocytogenes and *Candida* can be easily detected. In comparison with other method chromogenic media are more rapid, accurate and reliable and can utilize as alternative for conventional methods. Hence employing these media eliminate the need of subculture and further biochemical test for identification of pathogenic agent and at the shortest period of time possible, pathogenic agent can be identified. This feature especially in in disasters and military condition like maneuver and military camps have special important for preventing food and water borne outbreak

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