RESEARCH ARTICLE

Comparison of a new chromogenic medium with standard media for isolation and identification of *Bacillus cereus*

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Özet

Tewari A, Singh SP, Singh R, Kumar D. *Bacillus cereus*'un izolasyon ve identifikasyonunda standart besi yerleri ile yeni kromojenik besi yerinin karşılaştırılması. **Eurasian J Vet Sci, 2013, 29, 1, 39-42**

Amaç: Bu çalışmanın amacı yeni bir kromojenik besi yeri olan ve izolasyon, identifikasyon ve bakteri sayımı için önerilen HiCrome *Bacillus cereus* agar (HIMEDIA®)'ın standart selektif besi yerleri (PEMBA ve MYP) ile karşılaştırılmasıdır.

Gereç ve Yöntem: Etten izole edilen ve PZR ile doğrulanan 29 B. *cereus* izolatı 3 çeşit besi yerinin test edilmesinde kullanıldı. Bu çalışmada kullanılan tüm izolatlar araştırmada önceden tiplendirildi.

Bulgular: Tipik koloniye sahip izolat oranlarına bakıldığında, MYP'de 29 kolonin 27'sinin, HiCrome BCA ve PEMBA'da ise 25'şer adet kolonin olduğu belirlendi. Her 3 çeşit besi yerinde de zayıf reaksiyon görülmekle birlikte bu üremeler PEMBA, MYP ve HiCromeBCA'da sırası ile 4, 2 ve 4 izolat idi. Zayıf reaksiyon veren koloni sayısı MYP'e nispeten PEMBA ve HiCromeBCA'da daha fazla sayıda idi.

Öneri: Bu çalışma ile MYP besi yerinin *B. cereus*'un izolasyon ve identifikasyonu için PEMBA ve HiCromeBCA'ya göre daha iyi olduğunu gösterildi. *B. cereus*'un teşhisinde kullanılan kromojenik besi yeri, konvansiyonel besi yerine göre iyi bir seçenek olarak görünmemektedir. Ancak bakteri sayımının yapılmasında, standart besi yerine göre daha üstün olduğu belirlenmiştir.

Anahtar kelimeler: *Bacillus cereus*, kromojenik besi yeri, standart besi yeri, et

Abstract

Tewari A, Singh SP, Singh R, Kumar D. Comparison of a new chromogenic medium with standard media for isolation and identification of *Bacillus cereus*. **Eurasian J Vet Sci, 2013, 29, 1, 39-42**

Aim: The aim of this study was to compare a new chromogenic plating media HiCrome *Bacillus cereus* agar (HIMEDIA®) against two standard selective plating media (PEMBA and MYP), recommended for isolation, identification and enumeration of *Bacillus cereus*.

Material and Method: Twenty nine *B. cereus* PCR confirmed isolates of meat origin were used for evaluation of the three media. All the isolates used in this study were already characterized during research work.

Results: The proportion of isolates with typical colonies was highest in MYP with 27 colonies out of 29, while HiCrome BCA and PEMBA both showed only 25 typical colonies. Isolates with weak reaction were found on all three plating media but the weak reactions were shown by 4, 2 and 4 isolates on PEMBA, MYP and HiCrome BCA, respectively, thus they were more for PEMBA and HiCrome BCA as compared to MYP media.

Conclusions: Our survey showed that the MYP media was better than PEMBA and HiCrome *Bacillus cereus* agar for isolation and identification of *B. cereus*. Chromogenic media did not represent a very good alternative to the conventional standard media for diagnostic of *B. cereus* but it was found superior than standard plating media for enumeration of the bacteria.

Keywords: *Bacillus cereus*, chromogenic plating media, standard media, meat

Introduction

The role of *Bacillus cereus* in outbreaks of food-borne illness is well documented which is associated with emesis and diarrhea (Ahmed et al 1983). The organism produces a large number of potentially pathogenic toxins such as emetic toxin and enterotoxins (TeGiffel et al 1997). Ingestion of toxin-laden food can cause self-limiting emetic and diarrheal syndrome in human being, but occasionally cases with fatal outcomes occur (Fricker et al 2008). The detection and quantification of this emerging pathogen is therefore an important task for microbiological food and clinical diagnostic laboratories.

Most procedures for the isolation and enumeration of *B. cereus* involve direct agar plating. Mannitol Egg Yolk Polymyxin B sulphate (MYP) agar and Polymyxin Pyvurate Egg Yolk Mannitol Bromothymol Blue Agar (PEMBA) are the most commonly used plating medium. The reactions on both plating media are dependent on expression of lecithinase activity by *B. cereus* on the egg yolk incorporated into the medium, lack of fermentation of mannitol, and resistance to polymyxin by these bacteria. Colonies of the organism on the medium are dry and flat, translucent to creamy-white, surrounded by turbid zone of egg yolk precipitate. Despite, some *B. cereus* strains are there which may not give one or more of these key characteristics on the standard media and might therefore may be misidentified or neglected (Szabo et al 1984, Ehling-Schulz et al 2004), besides some other *Bacillus* spp. (e.g. *B. thuringiensis, B. anthracis, B. mycoides*) can grow on MYP and PEMBA and give positive egg-yolk reaction. The major drawback of the standard medium is the tendency of zones from individual colonies to coalesce which often causes difficulty in colony enumeration (Goepfert et al 1972, Harmon et al 1992).

Recently, new chromogenic plating media were developed to overcome these limitations. These media contain synthetic fluorogenic and chromogenic substrates that are cleaved by specific enzymatic activities of certain microorganisms (Manafi 1996). Incorporation of such substrates into selective media facilitates and improves the accuracy of detection and identification, thereby reducing the need for isolation of pure cultures and confirmation of the target organism (Cooke et al 1999, Restaino et al 1999).

The objective of this study was to assess and compare the diagnostic properties of a new chromogenic plating media HiCrome (HIMEDIA®) to that of the conventional MYP and PEMBA plating media, recommended by food authorities for identification of *B. cereus.*

Material and Methods

Bacterial isolates

Twenty nine isolates of *B. cereus* (M-1 to M-29) of meat origin were used for the analysis of plating media. All *B. cereus* strains were already confirmed and characterized by PCR targeting *gyr*B gene (encoding the subunit B protein of DNA gyrase) as described by Park et al (2007) with suitable modifications. All isolates produced PCR product of 475 bp on agarose gel, which is specific to *B. cereus.*

Selective plating media

Polymyxin Pyvurate Egg Yolk Mannitol Bromothymol Blue Agar (PEMBA) and Mannitol Egg Yolk Polymyxin B sulphate (MYP) are standard plating media recommended for the isolation and identification of *B. cereus*. A selective chromogenic media for identification and differentiation of *B. cereus* from other members of the group was procured from HiMedia. HiCrome *Bacillus cereus* Agar (BCA) and both standard media were prepared according to the manufacturer's instructions. A loop full of inoculated BHI broth culture was streaked onto all three plating agar. Then, inoculated plates of PEMBA and MYP were incubated at 35 ^{0}C and HiCrome plates at 37 ^{0}C for 24 h.

Classification of isolates according to type of colonies

The typical colonies of *B. cereus* on PEMBA were crenate to fimbriate peacock blue colored colonies (3-5 mm) surrounded by blue zone of egg yolk hydrolysis against green back ground; whereas, the characteristic colonies on MYP were eosin pink with surrounding zone of egg yolk hydrolysis. The chromogenic mixture present in the HiCrome BCA is cleaved by the enzyme ß-glucosidase found in *B. cereus* which results into the formation of typical light blue large and flat colonies with a blue centre. Weak reaction on PEMBA and MYP was characterized by lack of the typical color of the colony or precipitation zone only underneath and not around the colonies. On HiCrome BCA, a weak reaction designated as very light blue point in the centre of the colonies was observed.

Results

Plating media assessment

Characteristic colonies of *B. cereus* on all the three media are shown in Figures 1-3. Isolates with typical reaction can be easily identified as *B. cereus*, whereas the identification of isolates with weak reaction required more experience and closer observations of the colonies.

The proportion of isolates with typical colonies was highest in MYP (27 typical colonies) while it was same for HiCrome BCA and PEMBA (25 typical colonies on each) as depicted in Table 1. Isolates with weak reaction were found on all three plating media. The weak reactions were shown by 4, 2 and 4 isolates on PEMBA, MYP and HiCrome BCA, respectively, thus they were more for PEMBA (standard media) and HiCrome BCA as compared to MYP media. Isolate M-11 showed weak colony only on HiCrome media and M-18 showed weak colony only on PEMBA

Table 1. Distribution of typical and weak reaction of 29 *B. cereus* isolates on the different selective media.

Media	No. of Typical* colony	No. of Weak colony	Distribution of weak colony of *B. cereus* isolates on tested media
PEMBA	25	4	M-3, M-24, M-15, M-18
MYP	27	2	M-3, M-24
HiCrome BCA	25	4	M-3, M-24, M-15, M-11

*Explanation for typical and weak colony are given in the material and method section.

while both produced typical colony on remaining media.

Discussion

Currently, PEMBA and MYP are two selective plating media which are recommended by food authorities as standards for the detection of *B. cereus* (Rhodehamel and Harmon 1998). The principal of working of these egg-yolk media is the lecithinase activity which is responsible for opaque precipitation zones around suspect colonies. Preparation of egg-yolk containing media is inconvenient; moreover various studies (Szabo et al 1984, Ehling-Schulz et al 2004) admit the occurrence of *B. cereus* strains without lecithinase activity. Besides this, colour and morphology of the colony may also lead to misidentification of some strains. *B. cereus* group organisms (except B. anthracis) generally show a strong expression of degrading enzymes such as proteinases, which leads to increase in pH which causes appearance of the typical peacock blue colonies on PEMBA. However, in present experiments, only 25 presumptive positive isolates showed the expected typical peacock blue colour on PEMBA, similarly 25 typical colonies of *B. cereus* strains were visible distinctly on HiCrome. On MYP, 27 of the presumptive positive strains showed typical reaction which was highest among three tested media. It seems to be the most suitable for identification of meat originated strains because it clearly identifies the strains and also there is low risk of false identity. But Fricker et al (2008) found new chromogenic media i.e. BCM® *B. cereus* group plating medium as a good alternative to the conventional standard media (PEMBA and MYP) in their study.

Out of 29, total 5 isolates (M-3, M-11, M-15, M-18, M-24) showed weak reaction on at least one of the tested plating media, whereas M-3 and M-24 showed weak reaction on all three plating media. It might be explained by variances in the gene which regulates the production of enzymes responsible for working of these media. Similarly Fricker et al (2008) also revealed a significant correlation between atypical colony appearance and specific variances within the plcR gene sequences of tested strains of *B. cereus* on standard and chromogenic media. Besides this, both PEMBA and MYP plating media have the same principle for identification of *B. cereus*, that's why M-3 and M-11 isolates showed weak reaction on them.

Three isolates (M11, M15 and M18) produced weak colonies on PEMBA and HiCrome which required expertise for identification. M11 isolate showed weak colony only on HiCrome agar but gave typical reaction on MYP and PEMBA. The HiCrome BCA media tested in this study is based on the activity of β-D-glucosidase which is not present in other two standard media. The expression of β-D-glucosidase in *B. cereus* strains grown on HiCrome was quite variable most probably that's why this isolate produced weak colonies on HiCrome BCA.

On PEMBA and HiCrome, 5 of the presumptive positive strains showed weak reactions, making identification difficult. It clearly indicates that MYP has better detection capability as compare to PEMBA and HiCrome. In agreement to our study, Nemeckova et al (2011) also found MYPA more suitable as compare to PEMBA, BrillianceTM agar and HiCrome *Bacillus* agar for dairy plant laboratories for isolation and identification of *B. cereus* from raw milk.

As such, chromogenic plating medium did not seem to be an appropriate alternative to the conventional standard plating media in our study. However, colony enumeration and isolation were easier on HiCrome than on MYP and PEMBA. Because *B. cereus* has tendency to form wide zone of turbidity surrounding each colony, and these colonies coalesce on PEMBA and MYP agar plates which further obstruct the counting of true positives. While HiCrome has higher selectivity and there is formation of discrete, non-coalescing colonies of *B. cereus*. But Peng et al (2001) found BCM *B. cereus/B. thuringiensis* chromogenic plating agar (based on PI-PLC activity of *B. cereus*) more selective and differential for isolation and identification of *B. cereus* from foods as compared to MYP.

Conclusions

Thus the study indicates that MYPA seems to be the most suitable standard plating media because there is only low risk of

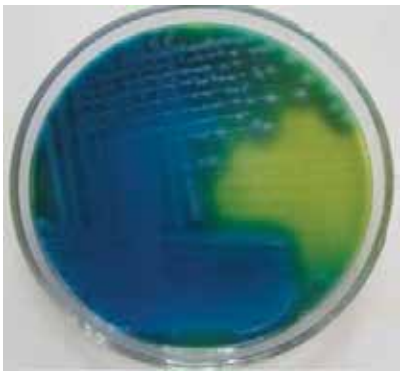

Figure 1. Typical Growth of Bacillus cereus on PEMBA showing fimbriate peacock blue colored colonies surrounded by blue zone of egg yolk hydrolysis.

Figure 2.Typical Growth of Bacillus cereus on MYP agar pink colonies surrounded by zone of egg yolk hydrolysis.

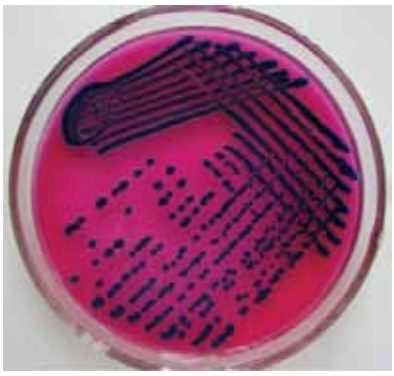

Figure 3.Growth of Bacillus cereus on HiCrome agar typical blue colored, flat, large, blue centered colonies.

false identification. Chromogenic media can improve and facilitate *B. cereus* enumeration, because it suppresses the background flora as well as this media is based on a single enzymatic reaction resulting in less ambiguous results.

References

Ahmed AAH, Moustafa MK, Marth EH, 1983. Incidence of Bacillus cereus in milk and some milk products. J Food Prot, 46, 126-128.

Cooke VM, Miles RJ, Price RG, Richardson AC, 1999. A novel chromogenic ester agar medium for detection of salmonellae. Appl Environ Microbiol, 65, 807-812.

Ehling-Schulz M, Fricker M, Scherer S, 2004. Bacillus cereus, the causative agent of an emetic type of food-borne illness. Mol Nutr Food Res, 48, 479-487.

Fricker M, Reissbrodt R, Ehling-Schulz M, 2008. Evaluation of standard and new chromogenic selective plating media for isolation and identification of Bacilluscereus. Int J Food Microbiol, 121, 27-34.

Goepfert, JM, Spira WM, Kim HU, 1972. Bacilluscereus: food poisoning organism. A review. J Milk Food Technol, 35, 213-227.

Harmon SM, Goepfert JM, Bennett RW, 1992. Bacilluscereus, in; Compendium of Methods for the Microbiological Examination of Foods, 3rd edition, Eds; Vanderzant C, Splittstoesser DF; Washington, APHA, USA, pp: 593-604.

Manafi M, 1996. Fluorogenic and chromogenic enzyme substrates in culture media and identification tests. Int J Food Microbiol, 31, 45-58.

Nemeckova I, Solichova K, Roubal P, Uhrova B, Svirakova E, 2011. Methods for detection of Bacillus sp., B. cereus, and B. licheniformis in raw milk. Czech J Food Sci, 29, 55-60.

Park S, Kim H, Kim J, Kim T, Kim H, 2007. Simultaneous detection and identification of Bacillus cereus group bacteria using multiplex PCR. J Microbiol Biotechnol, 17, 1177-1182.

Peng H, Ford V, Frampton EW, Restaino L, Shelef LA, Spitz H, 2001. Isolation and enumeration of Bacillus cereus from foods on a novel chromogenic plating medium. Food Microbiol, 18, 231-238.

Restaino L, Frampton EW, Irbe RM, Schabert G, Spitz H, 1999. Isolation and detection of Listeria monocytogenes using £uorogenic and chromogenic substrates for phosphatidylinositol-specific phospholipase. C J Food Prot, 62, 244-251.

Rhodehamel EJ, Harmon SM, 1998. Bacillus cereus, in; Food and Drug Administration Bacteriological Analytical Manual, 8th edition, (revision A), (CD-ROM version. R.L. Merker (Ed.). AOAC International, Gaithersburg, MD, Accessed at: 06.11.2012

Szabo RA, Todd ECD, Rayman MK, 1984. Twenty-four hour isolation and confirmation of Bacillus cereus in foods. J Food Prot, 47, 856-860.

TeGiffel MC, Beumer RR, Granum PE, Rombouts FM, 1997. Isolation and characterisation of Bacillus cereus from pasteurised milk in household refrigerators in the Netherlands. Int J Food Microbiol, 34, 307-318.