

# The evaluation of chromogenic medium for quantitative analysis of B.cereus in Korean traditional sauce.

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## ABSTRACT

The comparative evaluation of B.cereus chromogenic medium (CHROMagar™, France) and MYP medium were performed, reference strains compared with the typical form of colonization using two medium, B.cereus and B.thuringiensis did not distinguish between both. Using reference strains liquid and food spiked contamination test results, the typical colonies on CHROMagar plates was detected somewhat less than MYP agar, it took incubation time for approximately 48 hours on CHROMagar, but it was easy to enumerate colonies compared to MYP agar. B.cereus quantitative results of two medium for actual traditional Korean sauce, soybean paste, ssamjang (a processed soybean paste mixed with red pepper paste, garlic and sesame), fermented red pepper sauce were variable. We isolated 30 suspicious colonies from 3 of tested samples using two medium and identified the colonies using 16S rRNA sequencing.

As a result, B. Subtilis and B.amyloliquefaciens other than B.cereus were identified from suspicious colonies on MYP agar and all of suspicious colonies on CHROMagar were identified 100% B.cereus.

## OBJECTIVES

B.cereus widely distribute in nature, in particular, is found in Korean traditional sauce frequently and is foodborne pathogen causing vomiting and diarrhea. The current Korean regulation level of traditional sauces (except fermented soybean lump) is below 10,000 per g. Korean public method recommend to use MYP(mannitol-egg yolk-polymixin) agar as selective medium for quantitative analysis of B.cereus. B.cereus produce phospholipase so that forms a zone of white precipitate around the colonies and can't use mannitol so that appear phenol red color. However, misidentification problems of B.cereus may often arise in standard selective medium. Many chromogenic media have been developed in order to improve its problems. In this study, we performed comparative evaluation of a new B.cereus chromogenic medium (CHROMagar™, France) and MYP medium.

## MATERIALS & METHODS

### ➤ The experimental material

Food samples: red pepper paste (gochujang) 3 kinds, soybean paste (doenjang) 3 kinds, a processed soybean paste mixed with red pepper paste, garlic and sesame(ssamjang) 2 kinds, sour red pepper paste (chogochujang) 2 kinds.

Medium: B.cereus chromogenic medium (CHROMagar™, France), MYP medium

Strains: 12 kinds

| B.cereus(KCCM 40152)        | B.cereus(KCCM 12142)        | B.cereus(KCCM 11774)        | B.cereus(KCCM 11204)        |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| B.thuringiensis(KCCM 40030) | B.thuringiensis(KCCM 11808) | B.thuringiensis(KCCM 11579) | B.thuringiensis(KCCM 11429) |
| B.subtilis(KCCM 11314)      | B.pumilus(KCCM 12509)       | B.licheniformis(KCCM 12676) | B.coagulans(KCCM 11712)     |

### ➤ Experimental methods

- Respectively reference strain of B.cereus (4 strains), B.thuringiensis (4 strains), B.subtilis (1 strain), B.pumilus (1 strain), B.licheniformis (1 strain), B.coagulans (1 strain), and then inoculated on CHROMagar and MYP agar

After incubation at 30 °C for 48 hours, a typical colony form observed.

- Three strains (KCCM 11204, 11774, 40152) of B.C isolates suspended in saline and spreaded in MYP medium and Chromagar and incubated for 24 hours at 30 °C. After then counted created colonies. (In case of Chromagar medium, we counted colonies after 48 hours because the colony did not clear)

- Red pepper paste, soybean paste, a processed soybean paste mixed with red pepper paste, garlic and sesame sterilized by radiation(30 kGy) and spiked 2 strains of B.C(KCCM 11204,11774) . Spiked samples spreaded in Chromagar and MYP agar and incubated for 24 hours at 30 °C and then counted typical colonies (In case of Chromagar medium, counted after 48 hours)

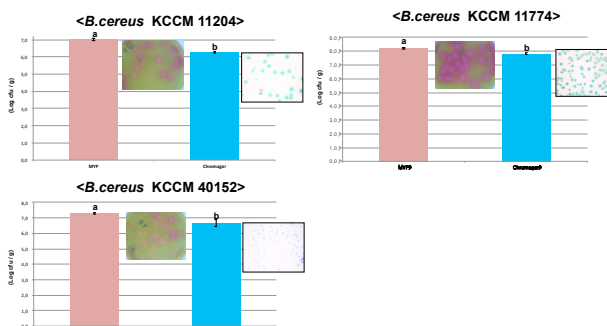
- 10 kinds of traditional sauces (three kinds of soybean paste, two kinds of a processed soybean paste mixed with red pepper paste, garlic and sesame, bean paste, red pepper 5 kinds) added diluent and homogenised, and then spreaded Chromagar and MYP medium. After incubated for 24 hours at 30 °C, counted typical colonies (In case of Chromagar medium, counted after 48 hours)

- 2 red pepper paste and 1 soybean paste samples analyzed using MYP medium and Chromagar and isolated 30 B.C suspicious colonies and identified 16S rRNA sequencing

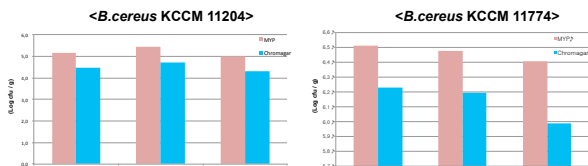
### ➤ Statistical Methods

Using MINITAB all the experimental results were conducted test of significant difference by t-test. We accepted to exist significant difference in case of p <0.05 between groups.

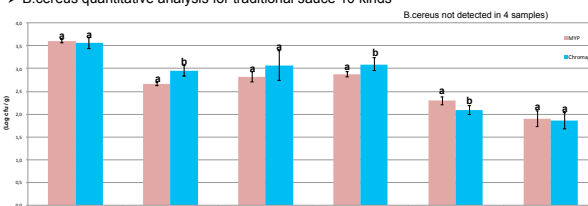
### ➤ Test Results of liquid samples



### ➤ Test Results of spiked samples



### ➤ B.cereus quantitative analysis for traditional sauce 10 kinds



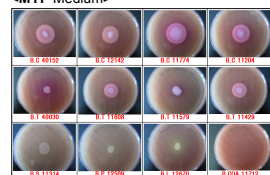
### ➤ 16S rRNA sequencing results of presumptive B.cereus colonies

| 시료    | MYP 배지                         | Chromagar 배지        |
|-------|--------------------------------|---------------------|
| 고추장 1 | Bacillus amyloliquefaciens 99% | Bacillus cereus 99% |
|       | Bacillus cereus 99%            | Bacillus cereus 99% |
|       | Bacillus cereus 99%            | Bacillus cereus 99% |
|       | Bacillus amyloliquefaciens 99% | Bacillus cereus 99% |
|       | Bacillus cereus 99%            | Bacillus cereus 99% |
| 고추장 2 | Bacillus subtilis 99%          | Bacillus cereus 99% |
|       | Bacillus subtilis 99%          | Bacillus cereus 99% |
|       | Bacillus subtilis 99%          | Bacillus cereus 98% |
|       | Bacillus cereus 99%            | Bacillus cereus 99% |
|       | Bacillus subtilis 99%          | Bacillus cereus 99% |
| 된장 2  | Bacillus subtilis 99%          | Bacillus cereus 99% |
|       | Bacillus subtilis 99%          | Bacillus cereus 99% |
|       | Bacillus subtilis 99%          | Bacillus cereus 99% |
|       | Bacillus cereus 99%            | Bacillus cereus 98% |
|       | Bacillus subtilis 99%          | Bacillus cereus 99% |

## RESULTS

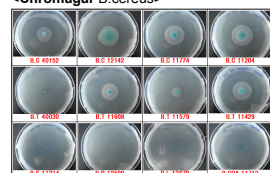
### ➤ The colony pattern of reference strains in MYP medium and CHROMagar

#### <MYP Medium>



- ❖ A typical colony: Pink colonies with turbid-halo
- ❖ All of 4 B.cereus were typical colonies
- ❖ B.thuringiensis: 2 strains were typical colonies and 2 strains were atypical colonies
- ❖ B.coagulans did not grow and B.subtilis, B.pumilus, B.licheniformis formed atypical colonies

#### <Chromagar B.cereus>



- ❖ A typical colony: Blue colonies with turbid-halo
- ❖ All of 4 B.cereus were typical colonies
- ❖ 3 strains of B.thuringiensis were typical colonies and 1 strain of B.T were atypical colony.
- ❖ Other strains couldn't grow

## CONCLUSION

1. MYP agar and CHROMagar B.cereus didn't distinguish B.cereus and B.thuringiensis, typical colony of B.cereus grew pink colony with white halo and other Bacillus spp. Could grow in MYP agar and typical colony of B.cereus grew blue colony with white halo and other Bacillus spp. could not grow in CHROMagar B.cereus.
2. MYP Agar could enumerate colony within 24 hrs but it was difficult to enumerate colony after 24 hrs. CHROMagar B.cereus could enumerate colony after 48 hrs because typical color of B.cereus appeared after 48 hrs incubation, 30°C.
3. According to the results of liquid sample(soy sauce) test and spiked sample test, typical colonies detected fewer in CHROMagar B.cereus than MYP agar.
4. According to the results of 10 fermented Soybean paste samples, typical colonies of B.cereus detected more in CHROMagar B.cereus than MYP agar.
5. According to the result of 16S rRNA sequencing for suspicious B.cereus colony, B.subtilis, B.amyloliquefaciens other than B.cereus were identified in MYP agar and all of colonies were identified as B.cereus in CHROMagar B.cereus.