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Development of Commercial Sterility Detection Method Based on Chromogenic Media Agar in UHT Coconut Cream Liquid Products

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ABSTRACT
Commercial sterility analysis is a crucial step in quality control of Ultra High Temperature (UHT) products such as coconut cream liquid products produced by PT Sasa Inti Minahasa Selatan, to ensure the safety and stability of shelf life. Stability incubation testing takes a long time, which is 7-14 days. Development of commercial sterility methods using Chromogenic Agar selective media is needed to cut the incubation time that is too long. Agar Chromogenic Media is tested for its ability to detect common contaminants in UHT coconut cream products, in particular thermophilic and mesophilic microorganisms that cause spoilage, with a short incubation time. UHT coconut cream samples are prepared by artificial and natural inoculation. Validation was carried out by comparing the results on standard media based on sensitivity, specificity and Time-to-result (TTR) parameters. The results showed a real difference between NA/TGEA media and chromogenic media so that the reading of bacterial colonies growing on chromogenic media was clearer and easier to read due to the change in color and short incubation time where the bacteria causing decay had grown on day 5 with a reading of results that took only 24 hours. Based on the validation that has been carried out, chromogenic selective media can accelerate product release time and improve quality control efficiency in the UHT coconut cream industry.
Keywords*: commercial sterility; chromogenics agar; coconut cream liquid; Ultra High Temperature*

INTRODUCTION

PT Sasa Inti Minahasa Selatan is a company that processes coconut plants as the main product. The market demand for coconut-based products is very high, both locally and for export, so the opportunity to market processed coconut products in Indonesia is very large. In addition, its geographical location on the island of Sulawesi is one of the supporters of PT Sasa Inti to be able to produce high productivity because North Sulawesi is one of the largest coconut producers in Indonesia. PT Sasa Inti is also present to meet this demand by producing practical 65ml and 200ml packaged Sasa Coconut Cream products.

Packaged liquid coconut cream products are processed with *Ultra High Temperature* (UHT) technology where commercial sterile food is processed at high temperatures in a short time to reach *F0* (sterility value) which is enough to destroy pathogenic microorganisms and spoilage. According to Haryadi (2024), the sterilization process is carried out using high temperatures with the aim of inactivating pathogenic microbial spores and heat-resistant decay. UHT coconut cream is then aseptically packaged in hermetically sealed packaging. After the product is packaged, a stability incubation test is carried out which is taken directly at random on the production line. Before being marketed, the product must be free of microorganisms, safe and not easily damaged if left at room temperature. The purpose of the stability incubation test is to ensure that the UHT process has successfully killed bacteria, especially heat-resistant spores, by verifying the quality

of the product and validating the shelflife standards that the Company has determined.

The release of the product takes a very long time, which is carried out on the 14th day, so it is difficult for many customer requests to be fulfilled quickly according to the target customer. This caused complaints from customers which caused the factory to make *an emergency release* to release the product on the 5th day, product incubation was only carried out for 2 days. As a result, the likelihood of product defects is high because bacteria with the potential to cause such defects have not been detected. In addition, the media used is a common medium, and has not been able to detect the potential for bacterial growth early.

Chromogenic media is a new medium used as a rapid culture method that combines the presumptive detection of bacteria that hydrolyze and produce enzymes with the identification of organisms. According to Gazin et al. (2012), chromogenic media combines chromogenic substrates formed as color detectors in bacterial colonies by hydrolyzing enzymes produced by bacteria, making it easier to distinguish pathogens or target bacteria from other microbes. This medium contains synthetic chromogenic substrates that are hydrolyzed by the specific enzymatic activity of certain microorganisms (Manafi, 1996). The substrates present in this chromogenic medium facilitate and improve the accuracy of detection and identification, thereby reducing the need for pure culture isolation and confirmation. The purpose of this study is to develop an analysis method using chromogenic media in order to be an early detection of bacteria that have the potential to cause defects in packaged UHT liquid coconut cream products.

METHODS

The method used in this study is exploratory descriptive, namely by isolating bacteria that cause damage to UHT coconut cream products, incubation testing of *commercial sterility stability* and validation of analysis media by looking at the comparison of results between standard media *Nutrient Agar* (NA) and *Tryptone Glucose Extract Agar* (TGEA) with chromogenic agar *media Bacillus ChromoSelect Agar* (BCSA) based on sensitivity, specificity and *Time-to-Result* (TTR) parameters. The results of the research are presented based on the stages of the research conducted.

Sampling

Samples of UHT packaged liquid coconut cream, before treatment coconut cream and defective coconut cream samples were taken randomly from the production batch at PT Sasa Inti Minahasa Selatan. Furthermore, the samples were directly taken to the Microbiology Laboratory of PT Sasa Inti Minahasa Selatan.

Isolation and Identification of Pure Cultures

Pre-treatment and defective coconut cream samples were aseptically collected and inoculated using the pour plate method on Plate Count Agar (PCA), then incubated at 35°C for 24 hours. Each treatment is repeated twice (duplo). Bacterial colonies that show different morphologies were taken and purified by *the streak plate* method on Nutrient Agar (NA) media to obtain pure culture. Furthermore, pure cultures were carried out gram staining, catalase test, oxidase test and identified using the Vitek-2 Compact tool.

Sensitivity Validation Test (Artificial Inoculation)

Samples from the production line in the same batch are taken and then sanitized the packaging surface using 70% alcohol. Ten samples were aseptically opened using a sterile cutting tool and poured into sterile Erlenmeyer flasks inside a Biosafety Cabinet to prevent contamination. The ten Erlenmeyer flasks containing coconut cream were then re-sterilized using an autoclave for 5 minutes at 121°C. Furthermore, eight erlenmeyer samples were inoculated with pure isolate extracts, including two erlenmeyer *Bacillus cereus* Vitek-2 BCL, two erlenmeyer *Geobacillus caldoxylositycus* Vitek-2 BCL, two erlenmeyer *Bacillus pumilus* Vitek-2 BCL, two erlenmeyer *Bacillus subtilis* Vitek-2 BCL and two other erlenmeyers that were used as negative controls or were not inoculated with bacteria. Samples of coconut cream containing bacteria and negative controls were then incubated in an incubator at 35°C and 55°C temperatures for 0, 3, 5, 7, and 10 days and analyzed in stages.

Conventional Tests

Samples incubated for 0, 3, 5, 7, 10 days are removed gradually then sanitized the surface of the erlenmeyer using 70% alcohol. Each sample was then analyzed aseptically and inoculated by scratching method on *Nutrient Agar* (NA) and *Tryptone Glucose Extract Agar* (TGEA) media. Each of the sampled and negative and positive control media was then incubated in an incubator at a temperature of 35°C for NA media and 55°C for TGEA media. Incubation time is 48-72 hours. Furthermore, observations were made by looking at the presence of bacterial colonies growing in the scratch area, AOAC (2016).

Chromogenic Tests

Samples incubated for 0, 3, 5, 7, 10 days are removed gradually then sanitized the surface of the erlenmeyer using 70% alcohol. Each sample was then opened aseptically and inoculated by scratching on *Bacillus ChromoSelect Agar* (BCSA) media. The sampled scratched media, negative control media and positive control media are then incubated in an incubator at 35°C and 55°C temperatures. Incubation time for 24 hours. Furthermore, observations were made by looking at bacterial colonies growing in the scratch area, AOAC (2016).

RESULTS AND DISCUSSION

Sampling of coconut cream before treatment (before the UHT process) and coconut cream defect samples resulted in colony growth in PCA media. Purification with *streak plates* in NA successfully isolated a number of single cultures. Identification of pure isolates using the Vitek-2 Compact tool confirmed the dominant types of bacteria as the initial contaminants in coconut cream, including the *Bacillus* bacterial group. According to Hariyadi (2024), the bacteria that cause the damage of hermetic (waterproof) packaged food for low-acid foods (pH >4.6) include the *Bacillus* and *Clostridium* bacterial groups. Packaged liquid coconut cream produced by PT Sasa Inti Minahasa Selatan is processed using UHT technology and packaged in hermetic packaging including low-acid foods where the standard pH range of this product is 6.0-6.5 with an aw of 0.88, which is a *highrisk* food category. The food group included in this category is foods with high potential for the growth of pathogenic microbes, especially heat-resistant and spore-

producing microbes. Spores produced by bacteria that have undergone heat treatment require sufficient time, a suitable environment and sufficient nutrients for the survival of the spores and the growth of microbial vegetative cells.

Based on the results obtained by samples containing bacteria and heating treatment, starting to show growth on day 3, there is 1 isolate that has not shown growth, namely *Geobacillus*. This bacterium is the main indicator of the effectiveness of thermal sterilization because of its nature as spore-producing obligate thermophilic bacteria (Hariyadi, 2024). The growth delay of *Geobacillus* is caused by incubation temperature conditions that do not meet its thermophilic requirements, which is around 55°C-75°C. Furthermore, on the 5th to 10th day, the growth of all bacteria was seen through colony growth and color change in BCSA media. This shows that this medium has high sensitivity and good growth support (nutrition) ability for various species of *Bacillus,* so this medium can be used as an effective medium to detect process failures in UHT coconut cream products.

Sensitivity and Specificity Validation Test (Artificial Inoculation)

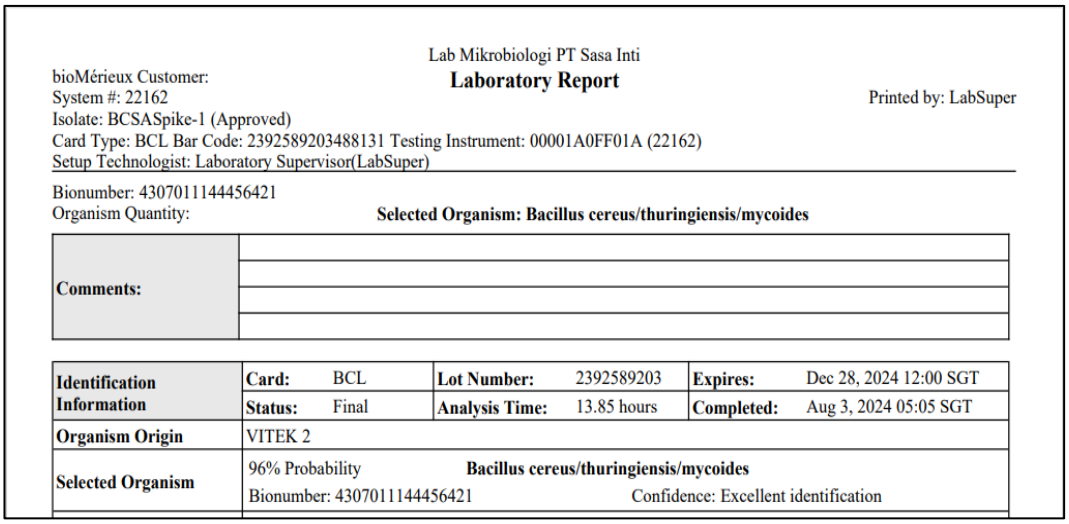
The results of the sensitivity validation test or *True Positive Detection* showed a comparison of the ability of *Bacillus ChromoSelect Agar* chromogenic media with NA and TGEA standard media in detecting the growth of test microorganisms in sterilized and reinoculated coconut cream samples (**Table 1**).

Table 1. Identification of Pure Isolate

Isolation Code	Gram Staining Results	Identification Results of Vitek-2 Compact	Information Probability
Isolate A	Gram Positive, Rod	*Bacillus cereus*	96%
Isolat B	Gram Positive, Rod	*Geobacillus caldoxylositycus*	93%
Isolate C	Gram Positive, Rod	*Bacillus pumilus*	86%
Isolate D	Gram Positive, Rod	*Bacillus subtilis*	93%

The isolates that have been successfully identified, especially the genus *Bacillus* and *Geobacillus*, are spore-forming microorganisms that are commonly known as the main contaminants and spoiling agents in low-acid products such as coconut cream. The presence of Bacillus spp. bacteria (mesophilic/thermophilic) justifies the selection of these isolates for artificial inoculation in sensitivity tests, as they are the most relevant indicator microorganisms to challenge the adequacy of commercial sterilization processes and analytical methods (**Figure 1**).

The sensitivity test is a crucial stage in the method validation, which aims to evaluate the ability of the chromogenic media *Bacillus ChromoSelect Agar* (BCSA) to detect target contaminants (*True Positive*) in sterilized and artificially inoculated UHT samples (**Table 2**).



Lab Mikrobiologi PT Sasa Inti

bioMérieux Customer:

Laboratory Report

System #: 22162 Printed by: LabSuper

Isolate: BCSASpike-1 (Approved)

Card Type: BCL Bar Code: 2392589203488131 Testing Instrument: 00001A0FF01A (22162)

Setup Technologist: Laboratory Supervisor(LabSuper)

Bionumber: 4307011144456421

Organism Quantity: **Selected Organism: Bacillus cereus/thuringiensis/mycoides**

Comments:	

Identification Information	**Card:**	BCL	**Lot Number:**	2392589203	**Expires:**	Dec 28, 2024 12:00 SGT
	Status:	Final	**Analysis Time:**	13.85 hours	**Completed:**	Aug 3, 2024 05:05 SGT
Organism Origin	VITEK 2					
Selected Organism	96% Probability **Bacillus cereus/thuringiensis/mycoides**					
	Bionumber: 4307011144456421 Confidence: Excellent identification					

Figure 1. Test results of Vitek-2 Compact isolate A *Bacillus cereus*

Table 2. Media Sensitivity Test Table

Microorganism Test	Test Media	Time to Result	Final Positive Results (Day 10)	Detection Description
B.cereus	NA/TGEA BCSA	72/48 Jam 24 jam	Positive	White Colony/cream Blue Colony
G. caldoxylositycus	NA/TGEA BCSA	72/48 Jam 24 jam	Positive	Small colonies White Colored Colony, yellow media
B.pumilus	NA/TGEA BCSA	72/48 Jam 24 jam	Positive	White Colony/cream Green/Blue Colony
B. subtilis	NA/TGEA BCSA	72/48 Jam 24 jam	Positive	White Colony/cream Green/Blue Colony

The results of the sensitivity test showed that BCSA Media was significantly faster in detecting the growth of all test microorganisms than standard media (NA/TGEA). The NA and TGEA standard media are non-selective media that are often used for the enumeration of total bacteria, where they provide rich nutrients and allow the growth of all target bacteria including *Bacillus*. However, this media does not provide specific information about the identity of the growing colony. Growing colonies require further confirmation stages such as gram staining and other biochemical tests. These two media are basic culture media used for organism subcultures for maintenance purposes or to check the purity of the subculture of the isolation cup prior to follow-up confirmation tests (Oxoid, 2006).

Time to Result (TTR) data shows that the reading speed of BCSA media results is 24 hours compared to the standard NA/TG media of 48 hours. Chromogenic media such as BCSA is designed to target microorganisms specifically through color reactions. When *the bacillus* grows, the bacteria produce certain enzymes that break down the substrate, breaking down chromophores (dye substances) which then color the colony into a distinctive color of blue, purple, green (Merck, 2018). Testing on bacteria This speed is due to the presence of a chromogenic substrate in BCSA that reacts with specific enzymes produced by the target bacteria (*Bacillus* and *Geobacillus*), resulting in a color that is visible earlier than waiting for the

colony biomass to reach a visible size on a non-selective medium (Fuchs *et al, 2022*) (**Figure 2**).

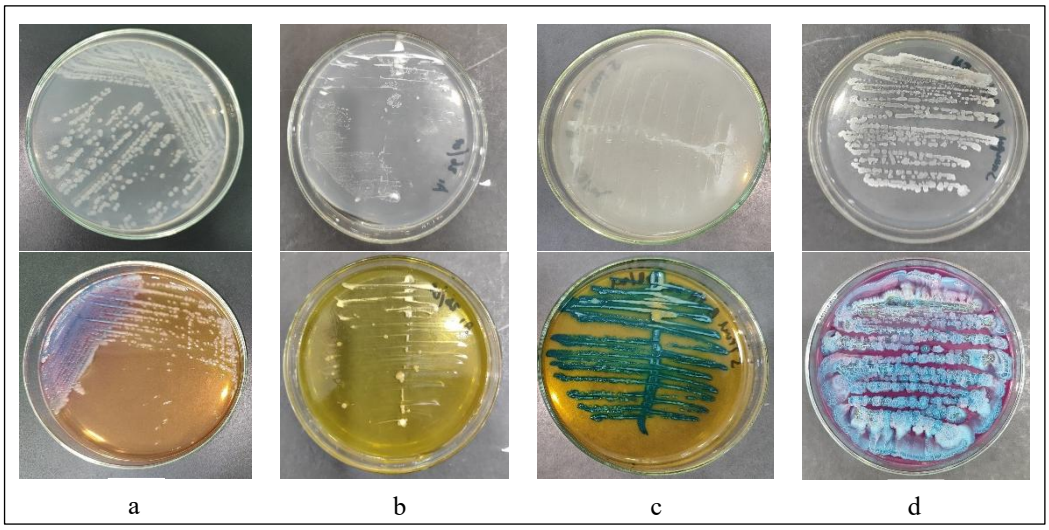


Figure 2. Comparison of commercial sterility test results (a) *Bacillus cereus*, (b) *Geobacillus*, (c) *Bacillus pumilus*, (d) *Bacillus subtilis*.

The negative control results showed 100% specificity (False Negative) for BCSA media. No color reactions or growths were observed in sterile coconut cream samples. This confirms that the components of UHT coconut cream do not cross-react with chromogenic substrates and that the discoloration only occurs due to the metabolic activity of the target microorganism. The development of this analysis method has proven to be valid and superior, especially in terms of time efficiency. The selection of test isolates (*Bacillus* and *Geobacillus*) is particularly relevant because they are the most resistant spore-forming bacteria and a key indicator of commercial sterility (Putri *et al.*, 2020). BCSA's sensitivity in detecting short test isolates demonstrates the ability of this medium to immediately capture the early growth of the most difficult decaying microorganisms. The success of thermophilic detection seen in the discoloration of the medium at 55ºC in 24 hours is critical, as thermophilic microbes often escape rapid detection at conventional incubation temperatures or exhibit slow growth. These results confirm that BCSA meets the sensitivity criteria to detect critical contaminants of concern in UHT coconut cream products.

With much shorter reading results, this chromogenic method validates itself as a sensitive, accurate, and highly efficient method, supporting faster product release decision-making at PT Sasa Inti Minahasa Selatan without sacrificing food safety.

CONCLUSION

The results of this research that developing a commercial sterile method with chromogenic media enables early detection of spoilage bacteria in the product by the fifth day, indicated by color changes in the medium and a short incubation period of 24 hours.

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REFERENCES

AOAC International. (2016). AOAC Official Method 972.44: Microbial Plate Count (Bacterial) Chapter 17. *Official Methods of Analysis of AOAC International* (20th ed.). AOAC International.

Fuchs, E., Raab, C., Brugger, K., Schulz, M., Wagner, M., Stessl, B. (2022). Performance Testing of Bacillus cereus Chromogenic Agar Media for Improved Detection in Cream and Other Food Samples. *Journal Foods* 11 (288) 1-16.

Food and Drug Administration. (2020). Bacteriological Analytical Manual (BAM) Chapter 23: Microbiological Methods for Cosmetics. *Silver Spring*: FDA.

Gazin, M., Paasch, F., Goossens, H., Malhotra-Kumar, S. (2008). Extended-Spectrum Beta-Lactamase Detection with Different Panels for Automated Susceptibility Testing and with a Chromogenic Medium. *Journal of Clinical Microbiology*, 46(11), 3721–3727.

Haryadi, P (2024) Materi 1. Pendahuluan, Pemahaman, Persyaratan & Regulasi Proses Termal. *Proses Termal untuk Industri Pangan*, Batch 22.

Manafi, M. (1996). Fluorogenic and Chromogenic enzyme substrates in culture media and identification test. *Int J Food Microbiol*, 31(1-3): 45-58.

Merck KGaA. (2018). *Bacillus ChromoSelect Agar*: NutriSelect® Plus (dehydrated culture medium) 92325. Darmstadt: MilliporeSigma 1-3.

Oxoid. 2006. *Manual Oxoid*. Edisi 9. Oxoid Limited: Bandung.

Putri, G., Senjawati, M., Erlinanto, Y. (2020) Analisis Penyebab Cacat Produk Santan Kemasan dalam Penetapan Critical Control Point dengan Pendekatan HACCP di PT. X.Inventory: *Industrial Vocational E-Journal on Agroindustry*, 5(02): 64-71

Tetrapak Method (2009). Procedure & Guideline: *Quality Control routines in a Dairy Industry*. QAM-588017-0101: (53-57).