<Experimental white paper>

Agar plate growth properties for *Clostridium sporogenes* ATCC 11437

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Test purpose

To identify specific growth requirements for *Clostridium sporogenes* ATCC 11437 when it is cultivated on agar plate

Introduction

For the anaerobic bacteria, anaerobic conditions and media specifications are most important factors to growth and much various conditions, it is depended on the specification of species or strains, facultative or absolute anaerobes and what reduced materials are preferred as a terminal electron accepter (e.g denitrifying reducers and sulfate reducer etc.), are required as well in environmental microbial and ecological studies. However, in pharmaceutical industry, the pharmacopeia in world-wide indicates *Clostridium sporogenes* ATCC 11437 only to use an anaerobic microorganism to execute the growth promotion test and suitability test with anaerobic condition and two kinds of media, reinforced clostridia medium as an enrichment medium and Columbia agar as a detective medium, respectively. In spite of specific mediums and conditions, pharmaceutical microbiologists prefer to use the soybean casein digest agar to recover the test organism, *Clostridium sporogenes* ATCC 11437. Usually prepared SCDA is targeted to aerobic microorganism so that it usually has been high-oxidative, and lack of some nutrients which are essentially required to growth for *Clostridium sporogenes* ATCC 11437.

Through this study, we were tried to figure out what nutrients or supplements are needed, when the SCDA are used to recovery the *Clostridium sporogenes* ATCC 11437.

Test materials and methods

Test organism preparation

Standard test organism, *Clostridium sporogenes* ATCC 11437 was prepared by EZ-CFU One step, 0487Z (MicroBioLogics Inc. USA), Lot No. 487-347-2 according to manufacturer's instruction.

Test media preparation

The basal powder medium was used the soybean casein digest agar (SCDA, Lab M) and modified with agar, yeast extract and glucose (Lab M). Agar (3g L^{-1}) and yeast extract (6g L^{-1}) was added to basal SCDA in order to detect the influence of higher surface hardness and the activity increasement with vitamin and organic peptides supplements, respectively. Glucose (5g L^{-1}) was used to detect that the cell can be utilized it as a carbon source. Detailed information of media modification is listed in table 1.

Table 1. Media modification for *Clostridium sporogenes* ATCC 11437 recovery

	SCDA	SCDA + A	SCDA +AG	SCDA+AGY
Supplements	None	Agar 3g L^{-1}	Agar 3g L^{-1} Glucose 5g L^{-1}	Agar 3g L^{-1} Glucose 5g L^{-1} Yeast extract 6g L^{-1}

To compare with commercial prepared media, the BioMérieux SCDA prepared media was used. Before use it, the media was equivalent at room temperature during 1hours. The fluid thioglycolate medium (FTM, Lab M) was also used for the growth availability of *Clostridium sporogenes* ATCC 11437.

Test organism inoculum

0.1ml of test organism suspension of EZ-CFU One step rehydrated vial was transferred to each media plate via calibrated micropipette and spread-drying it using disposal sterile spreader for 20seconds in triplicate. After spread, plates with cover were left at laminar flow for 15minutes to agar adaption of bacterial cells.

Anaerobic condition generation

Anaerobic condition for agar plates was generated by gas pack system (Anaerogen, Oxoid) and 2.5L anaerobic jar (Oxoid, UK). The gas saturation and jar integrate were checked via anaerobic indicator.

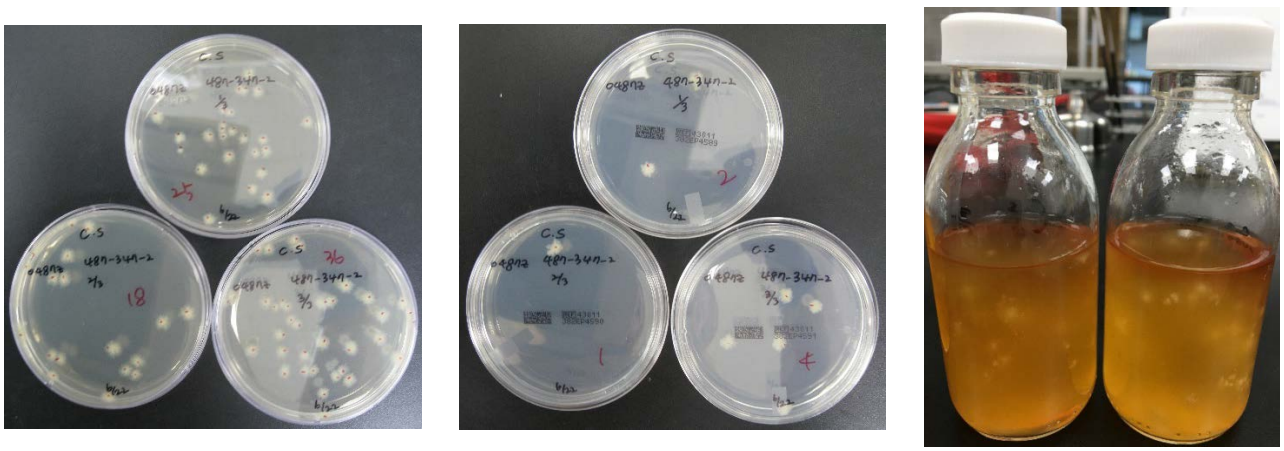
Incubation

Inoculated plates with anaerobic condition were incubated at 35℃ during 3days. Growth and anaerobic indicator were visual checked on every day without its open. The final day during incubation, anaerobic jar was opened and observed the color change of anaerobic indicator, and carried out the colony counting. The FTM bottles were incubated at the same temperature condition.

Results

Comparison of commercial prepared media and fresh in-house media

The growth activity of SCDA medium between commercial prepared media and fresh in-house media is revealed high difference (Table 2). It seems be came from high oxidation of commercial media, which was aged due to continuous contact with oxygen. Whereas, the fresh in-house media was prepared one day before testing, it needs for agar surface drying, and relatively less oxidative than commercial media. It is a similar result that the recovery colonies in FTM, it can be observed within 24hours, was higher than commercial media (Fig 1). By this reason, most critical growth factor for *Clostridium sporogenes* ATCC 11437 is that the redox potential (oxidation / reduction potential) and anti-oxidation.



(A) (B) (C)

Fig1. Pictures of plates and medium for *Clostridium sporogenes* ATCC 11437 recovery
(A) Fresh In-house SCDA; (B) BioMérieux prepared media; (C) FTM (Fluid Thioglycolate Medium)

Table 1. Recovery test results with modified medium for *Clostridium sporogenes* ATCC 11437

Medium	Commercial medium	In-house medium				
	SCDA (BMX)	FTM	SCDA	SCDA + A	SCDA +AG	SCDA+AGY
Recovery Counts CFU / 0.1ml	2	Good growth	25	21	10	14
	1		18	15	8	9
	4		36	14	19	11
Mean	2		26	16	12	11

Manufacturer MAV = 72CFU per 0.1ml

Comparison of commercial prepared media and fresh in-house media

According to the Bergey's manual, the general culture medium for the Genus *Clostridium* is standardized to Peptone-Yeast extract (PY) medium or Peptone-Yeast extract-Glucose (PYG) medium. However, in this study, the test result indicates that two supplements of yeast extract or glucose does not provide good recovery or influences. Indeed, the stronger agar hardness works a negative growth factor for recovery. It is supposed that soft and high moisture are make easy to absorb carbon dioxide and probably works as an anti-oxidant agent.

Discussion

Suitability of SCDA as a recovery medium for *Clostridium sporogenes* ATCC 11437

Even though higher recovery rate of In-house prepared SCDA from commercial prepared SCDA, the recovery rate from manufacturer's MAV (Mean Assay Value) is still low (36.1%). Probably, manufacturer uses the anaerobic blood agar (Columbia agar base), it can be provided much growth factors and reducing agents than SCDA. Therefore, the low recovery rate comes from the recovery medium difference. Besides, in this study, we figure out that the redox potential and reducing are critical factors to growth for *Clostridium sporogenes* ATCC 11437. If the fresh prepared media is used as like as this study or anti-oxidant agent (e.g. L-cysteine·HCl) is added to SCDA, we suppose that the recovery rate will be increased and obviously closer to MAV. And also, it will be another option that the pharmacopeia-recommended medium (e.g. Reinforced clostridial agar or Columbia agar, be contained anti-oxidant and reduced) uses as a recovery medium.

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