Characteristics of New Contact Plate Prepared with Native Gellan Gum

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The contact plate is a medium for simple microbial testing in which microbes are sampled by directly contacting its surface with the test surface. In general, sampling may be difficult when the conventional agar-based contact plate is used for sampling from uneven test surfaces. Additionally, a wet trace of nutrient components may be left on the test surface after sampling. In order to solve these problems, we prepared contact plates by the combined use of native gellan gum and agar as solidifiers. Compared to the conventional agar-based medium, the gellan gum and agar-mixed contact plate exhibited better performance of sampling from uneven test surfaces, owing to its relatively softer and more deformable medium gel. These results indicate that media prepared with native gellan gum and agar are suitable for contact plates.

Keywords: contact plate, medium, gellan gum, agar, microbial

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Introduction

Agar has been widely used as a solidifier for microbial culture media since ca. 1870. Previously, gelatin had been used; however, gelatin-based media are liquefied by microbial digestion and melt at culture temperatures higher than 28°C. In order to solve these problems, the applicability of various materials was investigated, and consequently agar was selected as a suitable solidifier for microbial culture media (Stanier et al., 1986).

Various combinations of nutrient components have also been extensively studied to develop a medium with which desired microbes can be cultured efficiently and selectively. In particular, since great importance was placed on hygiene maintenance in various fields, nutrient components and culture conditions have been optimized for rapid and accurate testing on microbial species and counts. However, solidifiers of culture media have not been studied in depth, and agar has conventionally been used.

The contact plate was initially developed for simple microbial testing on the floor, wall and instrument surfaces of hospitals (Hall and Hartnett, 1964), and enables microbial sampling by directly contacting its surface with the test surface. Currently, it is used to conveniently check the microbial contamination of floors, production facilities, workers, etc. not only in hospitals but also at food and pharmaceutical manufacturing plants.

For the contact plate, various combinations of nutrient components have been studied, but agar has conventionally been used as a solidifier. Recently, test objects unsuitable for the agar-based contact plate were described in detail (Boer, 2006). It is reported that the agar-based contact plate is suitable for sampling from flat surfaces, but not from uneven surfaces.

The performance of sampling from uneven surfaces will be improved by preparing a softer and more elastic contact plate. Therefore, we have focused on native gellan gum (hereafter, NGG) as a solidifier for the contact plate because NGG forms much softer and more elastic gels than agar.

Gellan gum, a polysaccharide produced by Pseudomonas elodea, is classified by functional group into deacylated and native types (Kang et al., 1982). Deacylated gellan gum forms agar-like firm and brittle gels (Moorhouse et al., 1981), and is used as a gelling agent and a stabilizer mainly in the food industry (Morris, 2006), and as a solidifier in culture media for microbes (Shungu et al., 1983; Lin and Castida, 1984) and plant tissue (Shimomura and Kamada, 1986). In contrast, recently developed NGG forms soft and elastic gels (Moorhouse et al., 1981). NGG is used mainly in the food industry (Morris, 2006), with its non-food applications,
such as gelling agents for plant tissue culture media (Shimomura and Omoto, 2006) in development. However, there have been no studies on improving contact plate sampling performance by exploiting the viscoelasticity of NGG gels.

In the present study, we investigated the applicability of NGG as a contact plate solidifier superior to agar for sampling from uneven surfaces.

Materials and Methods

Materials Agar powder for microbial culture (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) and NGG (San-Ei Gen F. F. I., Inc., Osaka, Japan) were used as solidifiers for contact plate preparation. Casein peptone (Polypeptone, Wako Pure Chemical Industries, Ltd., Osaka, Japan) and soybean peptone (Polypeptone-S, Wako Pure Chemical Industries, Ltd., Osaka, Japan) were used as nutrient components to prepare the SCD (soybean casein digest) medium, defined as a medium for microbial detection in the Japanese Pharmacopoeia (15th Ed.).

Preparation of SCD contact plates The SCD medium was prepared as follows. An appropriate amount of a mixture of agar and native gellan gum was added in small portions to 380 g of ion-exchange water at 85°C while stirring to prevent lumping, and dissolved at 85°C by stirring for 10 minutes. In the hot solution, 6.0 g of casein peptone, 2.0 g of soybean peptone and 2.0 g of sodium chloride were dissolved by stirring for about one minute. The solution weight was adjusted to 400 g with ion-exchange water, and the solution was autoclave-sterilized at 121°C for 15 minutes. In a clean bench, 17.0±1.0 g of the solution was poured into a Petri dish for contact plate preparation (diameter: 55 mm; height: 4 mm) to form a convex surface (resulting gel height: 6.9±0.5 mm) by surface tension, and allowed to stand for 30 minutes to solidify. The contact plate was used in various experiments after one-day storage at 5°C.

Measurement of gel properties A texture analyzer, TA-XT2i (Stable Micro Systems Ltd., Surrey, UK) was used to evaluate gel properties of the contact plates. After the one-day storage at 5°C, the contact plate was held at 20°C for one hour before evaluation. A cylinder probe was pressed into the center of the contact plate in a vertical downward direction at a speed of 1 mm/s, and the breaking point and breaking force of the medium were measured. Three types of cylinder probes with different base areas of 7.07, 28.3 and 100 mm² were used.

Ratio of contact with uneven surface The ratio of contact with an uneven surface was evaluated by using the model drawn in Fig. 1. Two wooden rectangular rods (width: 5 mm; height: 1 mm) were fixed on a flat wooden board. The distance between the two rods ($D_{\text{rod}}$) was varied: 5, 10, and 20 mm. Aluminum oxide particles (5 μm, white) were evenly applied to the groove of the model with a spatula and a brush. A contact plate was pressed uniformly and vertically against the model with a force of 5.0, 10.0 and 25.0 N by adding a weight with a base area larger than that of the contact plate. The band width ($w$) of aluminum oxide particles transferred to the medium surface was measured to determine the contact ratio, which is given by 100 \( w/D_{\text{rod}} \) in %.

Performance of sampling from flat and uneven surfaces A flat floor in the laboratory of San-Ei Gen F. F. I., Inc. and a polystyrene mat with bumps (height: ca. 1 mm) were used as test surfaces, and naturally-occurring surface bacteria were sampled. A contact plate was pressed against the test surface with a force of 10 N and incubated at 35°C for 24 h. Colonies on the medium were counted to evaluate the sampling performance of the contact plate.

Results and Discussion

Gel properties A soft and deformable medium is required to improve the performance of sampling from uneven surfaces. As an index of medium softness, the distortion depth of the medium was measured when a force of 0.5 N was applied to the medium with a plunger (base area: 100 mm²) of the texture analyzer. Larger distortion depth indicates a softer and more deformable medium.

In order to elucidate the relationship between agar concentration and medium gel properties, the distortion depth (in mm) under 0.5 N, breaking point (in mm) and breaking force (in N) were measured with decreasing agar concentration from 1.5% (w/w), as defined in the Japanese Pharmacopoeia, to 1.25, 1.0 and 0.75% (w/w).

The experimental results are shown in Figs. 2(a) and 3. When agar concentration decreased from 1.5% to 0.75%, the distortion depth under 0.5 N increased from 0.30 mm to 0.74 mm. In contrast, the breaking point and breaking force decreased from 2.28 mm to 1.80 mm and from 7.80 N to 1.95 N, respectively. These results indicate that decreasing agar concentration makes the medium not only softer and more deformable.
Gel properties of contact plate media. Contact area: 100 mm$^2$; (a) agar medium, (b) agar / NGG medium, 100 mm$^2$; (c) agar / NGG medium, 28.3 mm$^2$; (d) agar / NGG medium, 7.07 mm$^2$.

Fig. 2. Gel strengths of contact plate media. (a) Agar medium, plunger base area: 100 mm$^2$; (b) agar / NGG medium, 100 mm$^2$; (c) agar / NGG medium, 28.3 mm$^2$; (d) agar / NGG medium, 7.07 mm$^2$.

deformable but also less resistant to break -- when a contact plate with a low agar concentration is pressed firmly against a sharp area of an uneven test surface, the medium will most likely break.

Next, the efficacy of NGG for complementing medium strength was investigated as follows. The total concentration of solidifiers was fixed at 1.5% (w/w). As agar concentration was decreased from 1.5 to 1.25, 1.0 and 0.75% (w/w), the difference was replaced by NGG. The gel properties of the four resultant media were measured. Hereafter, the medium prepared with x% (w/w) of agar and y% (w/w) of NGG will be denoted by Medium(A x / NGG y).

The results are shown in Figs. 2(b) and 3. The distortion depth under 0.5 N increased with increasing NGG concentration. The breaking points of Medium(A 1.25 / NGG 0.25) and Medium(A 1.0 / NGG 0.5) were 1.93 mm and 2.23 mm, respectively, slightly shorter than 2.28 mm for Medium(A 1.5 / NGG 0).

A sharp area of an uneven test surface may break the medium, and accordingly, measurements similar to those described above were made by using plungers with base areas of 28.3 mm$^2$ and 7.07 mm$^2$ to examine the gel behavior of each medium when pressed against sharp areas.
The results are shown in Figs. 2(c), 2(d) and 4. When the contact area was 28.3 mm$^2$ or 7.07 mm$^2$, the NGG concentration dependence of each gel property was similar to that for 100 mm$^2$. These results suggest that the addition of NGG enables the preparation of a medium which is more resistant to break when a large force is locally applied.

These gel property measurements revealed that the combined use of NGG and agar as solidifiers enables the preparation of a medium which is softer and stronger than the conventional agar-based medium. When the novel NGG and agar-mixed contact plate is pressed against an uneven test surface, the medium conforms to and establishes contact with the surface without breaking. The NGG and agar-mixed contact plate is therefore more suitable for sampling from all types of test surfaces.

**Ratio of contact with uneven surface** In order to investigate the ratios of contact with a grooved surface for the four types of medium, the evaluation model shown in Fig. 1 was assembled, and the results are shown in Table 1.

When $D_{rods}$ was 5 mm, Medium(A 1.5 / NGG 0) and Medium(A 1.25 / NGG 0.25) did not touch the inner groove surface even under an applied force as strong as 25.0 N, but Medium(A 1.0 / NGG 0.5) established contact without breaking under the same mechanical conditions. Medium(A 0.75 / NGG 0.75) touched the inner groove surface under a force as small as 5.0 N.

When $D_{rods}$ was 10 and 20 mm, Medium(A 1.5 / NGG

<table>
<thead>
<tr>
<th>$D_{rods}$/mm</th>
<th>Force / N</th>
<th>Agar 1.5%</th>
<th>Agar 1.25%</th>
<th>Agar 1.0%</th>
<th>Agar 0.75%</th>
</tr>
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<tbody>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>10</td>
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<tr>
<td>25</td>
<td>0</td>
<td>80</td>
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</table>

* $D_{rods}$: Distance between two rods.
** Ratio of contact / % = Band width of aluminum oxide / $D_{rods} \times 100$. 

![Fig. 4. Gel properties of contact plate media measured with different plungers (base area: 100, 28.3 and 7.07 mm$^2$). 1: Agar 1.5% medium, 2: Agar 1.25% / NGG 0.25% medium, 3: Agar 1.0% / NGG 0.5% medium, 4: Agar 0.75% / NGG 0.75% medium.](image)
and Medium(A 0.75 / NGG 0.75) did not touch the inner groove surface under forces of 5.0 or 10.0 N, but did establish contact under 25.0 N. Medium(A 1.0 / NGG 0.5) and Medium(A 0.75 / NGG 0.75) established contact with the inner groove surface under all applied forces.

These results indicate that Medium(A 1.0 / NGG 0.5) and Medium(A 0.75 / NGG 0.75) are most suitable for microbial sampling from a groove with a depth of 1 mm and a width ranging from 5 mm to 20 mm.

**Performance of sampling from flat and uneven surfaces**

The sampling performances of the four types of medium were tested by using a flat floor and an uneven mat.

The results are shown in Fig. 5 and Table 2. For the flat floor, Medium(A 1.5 / NGG 0), Medium(A 1.25 / NGG 0.25), Medium(A 1.0 / NGG 0.5) and Medium(A 0.75 / NGG 0.75) detected 12.7±2.4, 14.2±2.8, 12.3±2.2 and 10.4±1.2 cfu/25cm² of colonies, respectively. These results revealed that the NGG and agar-mixed medium was equivalent to the agar medium without NGG in the performance of sampling from the flat floor.

When sampling from an uneven mat, the number of detected colonies increased from 14.3±1.7, 24.5±5.1, 42.6±8.4 to 58.0±5.8 cfu/25 cm² with increasing NGG concentration. The sampling performance of Medium(A 0.75 / NGG 0.75) was about four times higher than that of Medium(A 1.5 / NGG 0). However, the accurate determination of colony

![Fig. 5. Sampling test results for contact plates. Incubation conditions: 35°C, 24 h. (a) Flat floor; (b) polystyrene mat with bumps (block width: 10 mm; height: ca. 1 mm). Photographs on the left are close-up views of test objects and contact plates.](image)

<table>
<thead>
<tr>
<th>Sampling location</th>
<th>Agar 1.5%</th>
<th>Agar 1.25%</th>
<th>Agar 1.0%</th>
<th>Agar 0.75%</th>
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<tr>
<td>Flat floor</td>
<td>12.7 ± 2.4</td>
<td>14.2 ± 2.8</td>
<td>12.3 ± 2.2</td>
<td>10.4 ± 1.2</td>
</tr>
<tr>
<td>Uneven polystyrene mat</td>
<td>14.3 ± 1.7</td>
<td>24.5 ± 5.1</td>
<td>42.6 ± 8.4</td>
<td>58.0 ± 5.8</td>
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Incubation conditions: 35 °C, 24 h.
counts was difficult by using Medium(A 0.75 / NGG 0.75) because swarming was observed on part of the medium surface (see Fig. 5).

These results demonstrated that the performance of microbial sampling from uneven surfaces can be improved by the combined use of NGG and agar as solidifiers in culture media.

**Conclusion**

The present study revealed that the combined use of NGG and agar as solidifiers enabled the preparation of a medium which is softer and stronger than the conventional agar-based medium and facilitated the contact of the medium with a grooved test surface. For microbial sampling from a flat floor, there was no difference in sampling performance between the NGG and agar-mixed medium and the conventional agar-based medium. In contrast, for microbial sampling from an uneven mat, the sampling performance of Medium(A 0.75 / NGG 0.75) was over four times higher than that of Medium(A 1.5 / NGG 0). However, Medium(A 0.75 / NGG 0.75) was slightly too soft from a practical viewpoint, making it difficult for the user to feel if the medium contacts closely with a test object. In addition, swarming was observed on part of the medium surface. Accordingly, the combination of 1.0% (w/w) agar and 0.5% (w/w) NGG produces the most practical contact plate. The present study verified that the combined use of NGG and agar as solidifiers in culture media was effective for improving the performance of sampling from uneven surfaces. The combination of these gelling agents might increase the sampling location where simple microbial testing is possible.

**References**