Study of K-medium

Applicability of K-medium on $E.\ coli$ Detection, Comparing with That of Deso-medium

HARUKO NOMURA

(Received July 31, 1973)

The data on detecting efficiency of *E. coli* on K-medium were compared with those on *Deso-medium. On K-medium, considerably more *E. coli* colonies were found than on Deso-medium under the same condition. The results suggest the applicability of this new method to estimate the contamination of foods.

Introduction

K-medium is a new type of culture media developed by Katsuya and Yamato in 1956.¹⁾ This organic colloid is made from alkaline silicates, and requires neither heating nor cooling on its turning to a gelatinous form, that is, it can be treated at room temperature. Because of this property, it is considered as more useful medium on studies of bacteriology than Agar-media.

However, very limited attempts have been made to study the applicabilities of this medium. The present paper deals with the application of the K-medium to detection of *Escherichia coli*.

In recent years, *Deso-medium has been regarded to be useful because of its specific for enterobacteriaceae²⁾, so the comparison was also made between the detectability for *E. coli* of K-medium and that of Deso-medium.

Results obtained were as follows:

Experimental

a. Composition of culture media

| 1) K-medium | | (in 1000 ml) | | |
|-------------|---|--------------|--|--|
| | | % | | |
| Solution 1 | ∫ Na ₂ SiO ₃ | 8 | | |
| | Meat Extract | 0.5 | | |
| | Peptone | 0.5 | | |
| Calution 9 | H_3PO_4 | 4 | | |
| Solution 2 | (H ₃ PO ₄ Arabian Gum | 1 | | |

The pH of K-medium was adjusted to be 7.2 ± 0.2 by adding Solution 2 into 10 ml of Solution 1.

^{*} Abbreviation: Desoxycholate-medium

12 H. NOMURA

2) Deso-medium (Eiken manufacture)

| | (in 1000 ml) | | | | | |
|-------------------------------|--------------|--|--|--|--|--|
| | % | | | | | |
| Peptone (Eiken) | 1 | | | | | |
| Lactose | 1 | | | | | |
| NaCl | 0.5 | | | | | |
| Ferric Ammonium Citrate | 0.2 | | | | | |
| K_2HPO_4 | 0.2 | | | | | |
| Neutral Red | 0.0033 | | | | | |
| Desoxycholic Acid Sodium Salt | 0.1 | | | | | |
| Agar (Eiken) | 1.5 | | | | | |
| mIJ 7 9 0 9 | | | | | | |

pH 7.2 ± 0.2

b. Sample of material

E. coli, used as the research material, was Kindly offered by Prof. Suganuma, Department of Bacteriology, Kyoto Prefectural University of Medicine. The culture of this bacteria was diluted with isotonic NaCl solution to a certain concentration. Amount of the material should be taken to give colony counts between 30 and 300 on each plate.

Table 1. Comparison of K-medium with Deso., and calculation for t-test

| Pair No. | medium | Deso- | | | | Number o | | Difference | |
|----------|--------|--------|-----|--------|----------|--------------|-----------------|------------|-----------------|
| | | medium | D | D^2 | Pair No. | K- medium | Deso- medium | D | D^2 |
| | 830 | 150 | 680 | 462400 | 31 | 320 | 50 | 270 | 72900 |
| 2 | 980 | 120 | 860 | 739600 | 32 | 460 | 150 | 310 | 96100 |
| 3 | 380 | 30 | 350 | 122500 | 33 | 980 | 210 | 770 | 592900 |
| 4 | 60 | 20 | 40 | 1600 | 34 | 280 | 100 | 180 | 32400 |
| 5 | 350 | 260 | 90 | 8100 | 35 | 430 | 160 | 270 | 72900 |
| 6 | 510 | 310 | 200 | 40000 | 36 | 360 | 220 | 140 | 19600 |
| 7 | 370 | 70 | 300 | 90000 | 37 | 570 | 80 | 490 | 240100 |
| 8 | 450 | 110 | 340 | 115600 | 38 | 930 | 250 | 680 | 462400 |
| 9 | 70 | 30 | 40 | 1600 | 39 | 180 | 40 | 140 | 19600 |
| 10 | 50 | 40 | 10 | 100 | 40 | 520 | 80 | 440 | 193600 |
| 11 | 620 | 290 | 330 | 108900 | 41 | 100 | 10 | 90 | 8100 |
| 12 | 540 | 240 | 300 | 90000 | 42 | 540 | 40 | 500 | 250000 |
| 13 | 620 | 340 | 280 | 78400 | 43 | 420 | 40 | 380 | 144400 |
| 14 | 100 | 50 | 50 | 2500 | 44 | 170 | 40 | 130 | 16900 |
| 15 | 930 | 210 | 720 | 518400 | 45 | 240 | 40 | 200 | 40000 |
| 16 | 580 | 40 | 540 | 291600 | 46 | 110 | 20 | 90 | 8100 |
| 17 | 750 | 270 | 480 | 230400 | 47 | 200 | 370 | -170 | 28900 |
| 18 | 150 | 30 | 120 | 14400 | 48 | 490 | 120 | 370 | 136900 |
| 19 | 740 | 220 | 520 | 270400 | 49 | 940 | 510 | 430 | 184900 |
| 20 | 130 | 40 | 90 | 8100 | 50 | 190 | 100 | 90 | 8100 |
| 21 | 190 | 30 | 160 | 25600 | 51 | 340 | 50 | 290 | 84100 |
| 22 | 250 | 20 | 230 | 52900 | 52 | 430 | 160 | 270 | 72900 |
| 23 | 880 | 150 | 730 | 532900 | 53 | 370 | 330 | 40 | 1600 |
| 24 | 120 | 20 | 100 | 10000 | 54 | 230 | 80 | 150 | 22500 |
| 25 | 830 | 340 | 490 | 240100 | 55 | 360 | 180 | 180 | 32400 |
| 26 | 220 | 30 | 190 | 36100 | 56 | 450 | 340 | 110 | 12100 |
| 27 | 950 | 350 | 600 | 360000 | 57 | 360 | 190 | 170 | 28900 |
| 28 | 190 | 30 | 160 | 25600 | 58 | 380 | 120 | 260 | 67600 |
| 29 | 420 | 30 | 390 | 152100 | 59 | 400 | 130 | 270 | 72900 |
| 30 | 900 | 690 | 210 | 44100 | 60 | 100 | 20 | 80 | 6400 |
| | | | | | Total | 26010 | 8790 | 17220 | 77042 00 |

Each experimental results was the average of five measurements.

c. Plating and Incubation

One ml. of sample was quickly poured into a petri dish, and incubated at 37° C for 24 ± 3 hrs.

Results and Discussion

The number of colonies of $E.\ coli$, incubated for 24 ± 3 hrs. on K-medium and Desomedium respectively, are shown in Table 1, Fig. 1 and Fig. 2. And it was found that the number of colonies on K-medium was more than that on Deso-medium.

Statistical consideration (t-test): The significance of the mean difference was checked by t-test.

Mean differences are

$$\bar{D} = \bar{X}_D = \frac{\sum D}{N} = \frac{17220}{60} = 287.00 \tag{1}$$

and the variance of the differences are obtained by equation

$$S_D^2 = \frac{N \sum D^2 - (\sum D)^2}{N(N-1)} = \frac{60 \times 7704200 - (17220)^2}{(60)(59)} = 46815$$
 (2)

the variance of the mean is then

$$S_D^{-2} = \frac{S_D^2}{N} = \frac{46815}{60} = 797.00 \tag{3}$$

the standard error of the mean is

$$S_{\bar{D}} = \sqrt{797.00} = 28.23$$
 (4)

then

$$t_0 = \frac{\bar{D}}{\sqrt{\frac{N \sum D^2 - (\sum D)^2}{N(N-1)}}} = \frac{287.00}{28.23} = 10.17$$
 (5)

From the Table of t, the criterion value of t ($|t_{0.01}|=2.617$) is less than the calculated t ($|t_0|=10.20$). Hence, the null hypothesis in this case was rejected at one percent level of significance.

The results above mentioned show that the viable counts of *E. coli* on K-medium are significantly more than those on Deso-medium. This, with facility of its treatment, gives a bright prospect of practical applications to detect *E. coli* indicating the contamination degree of foods.

The auther intends to make a comparative study on the development of red colour produced by *Coliforms-colonies* in the case that the same nutrient is added to K-medium as to Deso-medium.

Acknowledgements

The author wish to thank Prof. Ozaki and Prof. Suganuma for their helpful advice. And the author is indebted to Prof. Katsuya for drawing his attention to this problem. 41 H. Nomura

References

- 1). S. Katsuya, and H. Yamato: Sci. Rep. Saikyo Univ., 2, (2) 103-111 (1956)
- 2). F. Abe, S. Mochinaga and A. Taguchi: Shokuhin Eisei Kenkyu., 11, (4) 37-46 (1961)



Fig. 1. Growth of E, xoli on K-medium.

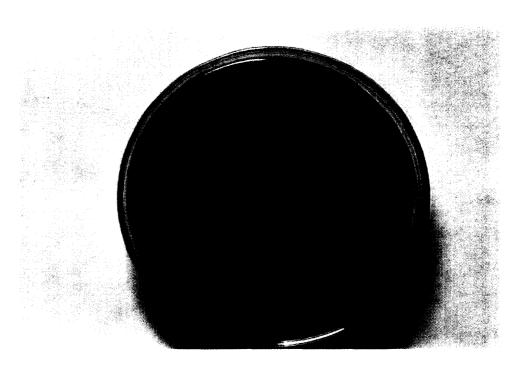


Fig. 2. Growth of E. coll on Desermedium.