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Symbiosis of *Salmonella* and *Escherichia coli* by MY Phenomenon

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Abstract

Background: The symbiotic relationship between *E. coli* and *Salmonella* was demonstrated using hydrogen sulfide production named MY phenomenon, which is a feature of *Salmonella*. **Methods:** In order to confirm the hydrogen sulfide production of *Salmonella* by iron sulfide formation, deoxycholate-hydrogen sulfate-lactose agar medium having both a sulfur source and an iron source was used. The case where *Salmonella* was cultured alone was compared with the case co-cultured with *E. coli*. In the case of culture with *Salmonella* alone, the MY phenomenon has not occurred. When *Salmonella* was co-cultured with *E. coli*, *Salmonella* cultured near *E. coli* existing place showed a black color named MY phenomenon. When *E. coli* was cultured alone, it turned red due to the organic acid produced. However, when *E. coli* was cultured in the part where *Salmonella* is inoculated on the surface of the medium, the MY phenomenon appeared there. Furthermore, hydrogen sulfide production was more active in *Salmonella* co-existing with *E. coli*. **Conclusion:** The study shows an important relation that *E. coli* is a promoting factor for *Salmonella* culture by NY phenomenon. The relation suggests that bacterial symbiosis relation exist in bacterial flora.

Keywords

Symbiosis of *Salmonella*, *Escherichia coli*, Bacterial Symbiosis, Bacterial Flora

1. Introduction

Non-typhoidal *Salmonella* has been associated with many food-borne diseases all over the world [1]. Not only developing country, especially in the United States, it is the cause of an estimated 1.4 million illnesses annually [2]. Various

foods, such as chicken, beef, and pork, have been implicated in outbreaks caused by *Salmonella* spp. [3] [4]. A characteristic of *Salmonella*e, which is a food poisoning-causing bacterium, is that it produces hydrogen sulfide (H_2S) when cultured with using a medium containing a sulfur source. H_2S is toxic to the human body [5].

So, it can be said that *Salmonella* is not a probiotic or beneficial bacterium. In other words, *Salmonella* is a bacterium rather harmful to humans.

H_2S produced by *Salmonella* reduces the iron source contained in the medium, thereby forming iron sulfide, and a color change to black is observed. We have defined this as visualization of H_2S production, and we have continued research that will lead to the development of new methods for detecting *Salmonella*e and testing the antimicrobial properties of materials [6].

First of all, when slices of lemon placed and cultured on which the *Salmonella* was densely inoculated, we discovered that black rings are formed around the lemon (Named as MY phenomenon) [7]. Next, experiments were continued to identify substances that significantly visualize the H_2S production produced by *Salmonella*. As a result, it was revealed that H_2S production of *Salmonella* is remarkably visualized in black under the influence of organic acids which are components of lemon, such as citric acid and ascorbic acid. Other citrus and acerola, kiwi fruit, strawberries, green peppers or paprika showed same [8] [9], **Figure 1**.

By the same phenomenon, a method for detecting *Salmonella* was devised by analyzing the expression state of iron sulfide (II) on the agar medium and/or the color change of the agar medium.

Next, it was also discovered that the H_2S production of *Salmonella* is inhibited by the influence of salt content and antibacterial substances. Therefore, it became clear that visualization of H_2S production may be an indicator of active growth of *Salmonella* [10] [11], **Figure 2**.

In addition, we discovered that H_2S by *Salmonella* can be visualized by making the medium surface to anaerobic, and developed a new antibacterial test method. That is, when a substance that does not inhibit the growth of bacteria is

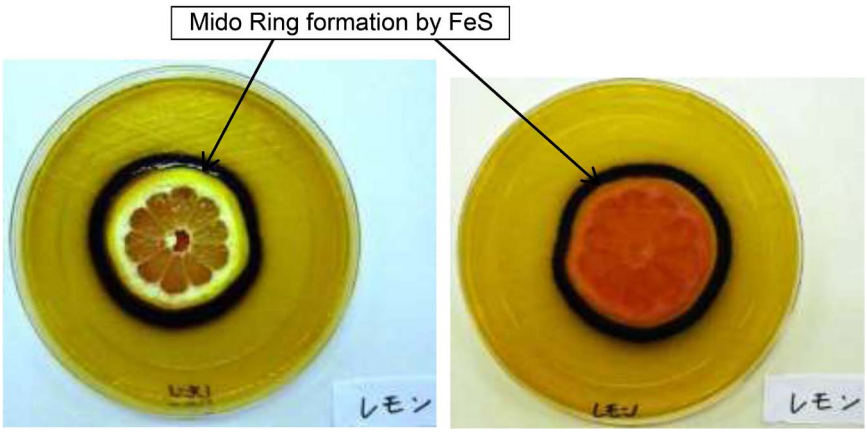

Figure 1. Effects of lemon slice on H_2S production of *Salmonella*. [7].

placed on the medium and cultured for a predetermined time, then only the medium below the sample turns black. On the other hand, in the culture medium under the sample having the antimicrobial property, the phenomenon of black change is not observed [13], **Figure 3**. Thus, we have discovered a new antimicrobial test that will prove the antimicrobial properties of the material if *Salmonella* shows no visualization of H_2S production [14].

The developed antibacterial test method has made it possible to test the antibacterial property of water-insoluble substances as well as water-soluble substances more quickly and more accurately than the current method [15] [16].

The above is the result of our research using the MY phenomenon of *Salmonella*. Furthermore, since it discovered that the organic acid which *E. coli* etc. produce also exhibits the MY phenomenon to on *Salmonella*, shows the knowledge below.

2. Materials and Methods

Media used: Deoxycholate-hydrogen sulfate-lactose (DHL) agar medium (Eiken Chemical Co., Ltd.). The characteristic of the medium is that of each of 1 L of solution contains 1 g of ammonium ferric citrate as a ferric source 2.3 g of sodium thiosulfate source as a sulfur source.

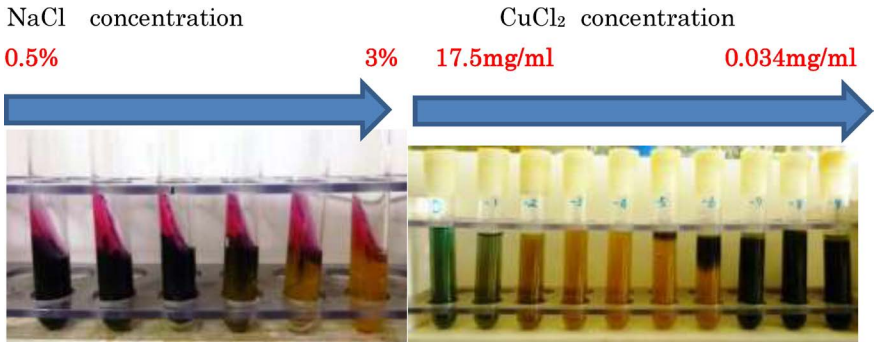

Figure 2. Effects of antimicrobial substances sodium chloride and copper ion on H_2S production of *Salmonella* [11] [12].

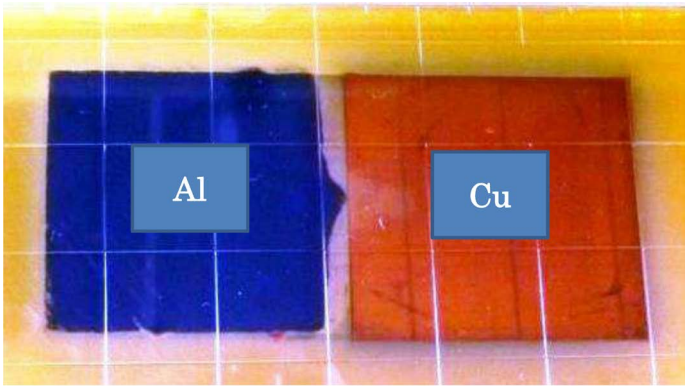

Figure 3. After 24 hours of culture, observe from the back side of the medium in petri dish. Since aluminum is not antibacterial, H_2S production is not observed beneath the medium. On the other hand, H_2S is not produced because copper is antibacterial [14].

Used strain of *E. coli*: Growth at 44.5˚C in *Escherichia coli* Medium (EC Medium Eiken Kagaku Japan) from environmental isolates. The IMViC test with the characteristics of indole reaction (I), methyl red reaction (M), Voges-Proskauer reaction (Vi) and Simmons' citrate availability (C) shows a pattern of "++−−" [17] [18].

***Salmonella* strain:** non typhoid, serotype Derby, isolated from the fresh market of Vientiane Lao P.D.R. and H_2S producing strain.

Sterile Cotton swab (Eiken. Japan): used when smearing and inoculating *Salmonella*.

Sterile Toothpick (Daiso Japan): Use a pointed tip when inoculating *E. coli*.

The following three experiments were tried using the above materials.

The case not contacting *E. coli* and *Salmonella* directly:

Inoculate *Salmonella* and *E. coli* to two petri dishes of the same culture medium as follows.

A: The *Salmonella* was inoculated on half of the dish with a sterile cotton swab. Nothing was inoculated on the other half.

B: Similarly, *Salmonella* was smeared on half of the dish. Then *E. coli* was inoculated on the other half using a toothpick tip.

After incubation at 37˚C for 24 h in an incubator, observed the results.

The case *Salmonella* was contaminated with *E. coli*

The following procedure of C and D was performed on 2 sheets of DHL agar medium.

C: Using toothpick tip, inoculate *E. coli* into 5 spots on the medium of one dish at intervals like 5 in the dice.

D: The other dish was smeared with *Salmonella* and then inoculated *E. coli* 5 places as above.

Then, these were cultured at 37˚C for 24 hours.

From a petri dish in which *E. coli* was cultured with *Salmonella*, isolation of *Salmonella* was done as below E and F.

E: Portion of *Salmonella* only.

F: Coexistence of *Salmonella* and *E. coli*.

3. Results

The case not contacting *E. coli* and *Salmonella* directly: (Figure 4)

- **Result of A:** After cultivation, In the case of smear inoculation only with *Salmonella*e on one side of the petri dish, no MY phenomenon was shown that *Salmonella* produces H_2S. Because no blackened is observed. This means iron sulfide is not formed (Figure 4). Repeat this experiment 10 times or more and get the same result.
- **Result of B:** However, in the petri dishes in which *E. coli* are inoculated on the other side which had not been coated with *Salmonella*e, a black line, which is a visualization of H_2S production, is produced at the boundary where the *Salmonella* bacteria are cultured (Figure 4).

Repeat this experiment 10 times or more and get the same result.

The case *E. coli* is contaminated on smear *Salmonella* (Figure 5)

- **Result of C**: Where only *E. coli* is inoculated into the medium, all five locations turn red at the *E. coli* growth point (**Figure 5** Left Panel). Repeat this experiment 10 times or more and get the same result.
- **Result of D:** The case *E. coli* contaminate on *Salmonella* (The fact is shown in **Figure 6**), black spots are observed in all five places where *E. coli* and *Salmonella* is coexist (**Figure 5**). Repeat this experiment 10 times or more and get the same result.
- **Result of E:** Only, *Salmonella* is isolated (**Figure 6**). Repeat this experiment 10 times or more and get the same result.

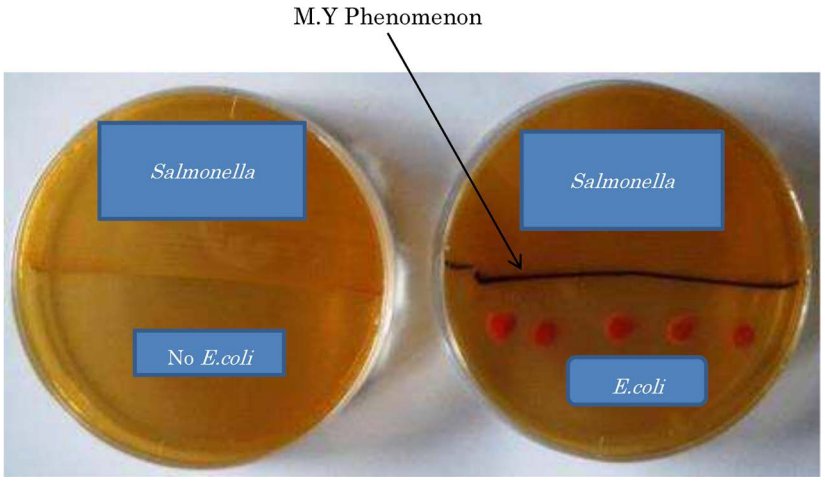

Figure 4. Left: Result of A. *Salmonella* cultured on one side. No M.Y. Phenomenon occurs. Right: Result of B. When culturing *E. coli* 1 cm away from the border of *Salmonella*, Black line appears. The M.Y. Phenomenon occurs.

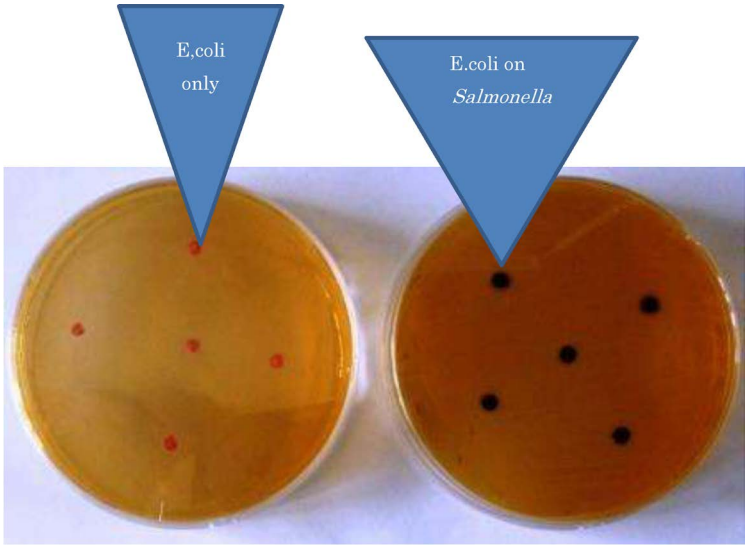

Figure 5. Left: Result of C. Colonies of *E. coli* on DHL agar show red color. Right: Result of D. *E. coli* grown on inoculated *Salmonella* turned black. *E. coli* was identified by isolation from black part (see next **Figure 6**).

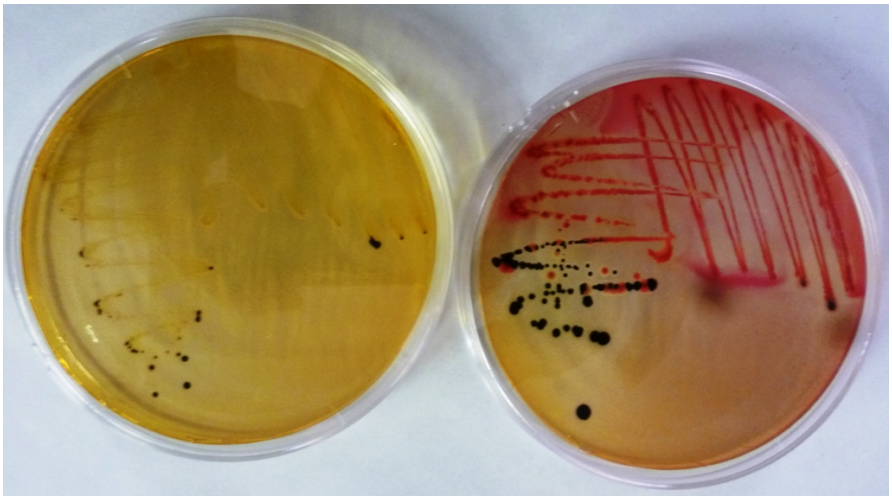

Figure 6. Left: Result of E. *Salmonella* colonies isolated from the part without *E. coli*. Right: Result of F. *Salmonella* colonies (black color) isolate from the part contaminated with *E. coli* (red color).

- **Result of F:** Both *Salmonella* and *E. coli* are isolated. The black color of *Salmonella* colonies is clearer than case E (**Figure 6**). Repeat this experiment 10 times or more and get the same result.

4. Discussion

The phenomenon of visualization of H_2S production by *Salmonella* in a medium containing a sulfur source and an iron source has existed as a previously known fact [19] before we have discovered the MY phenomenon of forming Mido Ring around a sliced lemon.

In the case of using the DHL medium, the colony isolated alone is black in the center, but when *Salmonella* grows densely, H_2S produced by bacteria is not visualized. This fact is already understood as a matter of course for those who have been testing for *Salmonella*. However, we are the first to document this fact.

The fact that we discovered next is that even under conditions where *Salmonella* is growing densely, if the surface is covered with a device that contains citric acid, ascorbic acid, etc. as well as sliced lemons, can visualize H_2S production by *Salmonella*. For example, other sliced citrus fruits and other fruits showed same results [7].

These are the fact that author has made clear about this as MY Phenomenon. In this present study, it has been confirmed from the following three points that citrus fruit and other organic acids as well as *E. coli* promote the H_2S production of *Salmonella*.

1) Compare the results of A and B in **Figure 4**. Only when *E. coli* is cultured on the other side, M.Y phenomenon, this time as a black line appearance, occurs at the place where *Salmonella* is densely grown and the borderline where the E. coli is cultured. The black line is considered to appear as a result of H_2S produced by metabolism of sulfur source contained in the culture medium reacting

with the iron source to form iron sulfide. It is suggested that *Salmonella* is active in H_2S production near cultured *E. coli*. It can say that *E. coli* acts positively on *Salmonella* growth.

2) As the result of C, *E. coli* ferments lactose to produce acid and gas, when inoculated alone and cultured, it turns red. This is because in the DHL medium, the pH indicator turns red due to the organic acid produced by the *E. coli.* This fact is known.

However, in the result D, when *E. coli* is inoculated on densely inoculated *Salmonella*e, the M.Y phenomenon of black visualization occurs at the places where *E. coli* was inoculated. That is, the active H_2S production of *Salmonella* is observed in the part inoculated with *E. coli.* Previous our studies have shown that *Salmonella* active H_2S production shows that it has activated the growth of *Salmonella* itself [11]. Therefore, it is suggested that the contaminating *E. coli* has an effect of promoting the growth activity of *Salmonella* itself.

3) Similarly, black part from which both *Salmonella* and *E. coli* are isolated, it can be determined that the black part is a coexistence of *Salmonella* and *E. coli*. In the case result F from where *E. coli* and *Salmonella* co-exist, the black color is obviously remarkable as compared with the case where it is isolated from the part *Salmonella* alone sown in Result E. Therefore, for *Salmonella*, existence of *E. coli* is symbiosis. So, by what mechanism does *E. coli* activate *Salmonella* H_2S and growth? As one of the reasons, it can be mentioned that the organic acid which is a metabolite produced from *E. coli*, may also be an energy source of *Salmonella* like the citrus component. Of course, in addition to *E. coli*, bacteria having similar effects on *Salmonella* are also considered to exist. However, this is a future research topic. In addition, it would be further research that whether the same phenomenon can be find as this research or not even in the intestines of humans or animals.

5. Conclusion

The following facts show that *E. coli* is assisting *Salmonella* growth.

- When *E. coli* is cultured near *Salmonella*e, visualization of hydrogen sulfide (MY phenomenon) production of *Salmonella* occurs near *E. coli*.
- When *E. coli* is cultured in DHL medium, it turns red due to the organic acid produced.
- When *E. coli* contaminates on densely cultured *Salmonella*, the MY phenomenon appears and therefore blackens.
- When *Salmonella* coexists with *Escherichia coli*, hydrogen sulfide production becomes significant rather than isolating *Salmonella* alone.

The relations suggest that bacterial symbiosis relation exist in bacterial flora.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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