Bull. Univ. Osaka Pref., Ser. B, Vol. 26,
大阪府立大学紀要, 農学・生物学, Mar., 1974.

# The Correlation between Coagulase Production and the Other Biochemical Properties of Staphylococci Isolated from Bovine Mastitis

Haruyasu MATSUMOTO, Eiichiro BABA, Tsuneo FUKATA and Sanenori NAKAMA

Department of Veterinary Science, College of Agriculture

In human and animal body, staphylococci are commonly found on the skin and mucous membranes, and in the intestinal content. They are also frequently isolated as a causative agent from suppurative lesions or toxic food poisoning. In the field of dairy farming, staphylococci have been well recognized as a pathogen of bovine mastitis. The coagulase-producing ability of these organisms has been accepted as the most useful criterion to distinguish pathogenic strains from non-pathogenic ones, and at present the classification of these organisms is made on the basis of this property. Furthermore, special attention has recently been paid to some other biochemical reactions closely related to the coagulase production, such as DNase production, phosphatase production or tellurite reduction. In the clinical laboratories, these biochemical properties other than coagulase production are employed in order to estimate easily the pathogenicity of staphylococci on a plate medium. This paper deals with the trial to examine the biochemical properties of staphylococci isolated from bovine mastitis, and also to determine the possible correlation between coagulase-producing property and the other biochemical ones.

## Materials and methods

The organisms employed in this work were 315 strains of staphylococci, which were isolated from 297 cows affected with clinical mastitis in Osaka prefecture. From the results of tube test using rabbit plasma, it was found that they consisted of 122 coagulase-positive and 193 coagulase-negative strains. The hemolysis of each strain was examined on blood agar plate added with washed sheep red cells, and $\beta$ hemolysis was characterized by hot-cold lysis. A part of the strains showing $\alpha$ hemolysis in appearance were tested for $\varepsilon$ hemolysis by the method of ELEK *et al.*[7] and MINAMIMOTO *et al.*[13] One half of the strains employed were also observed for hemolytic reactions on cattle, rabbit, and horse red cells. Tests for DNase production, phosphatase production and tellurite reduction were carried out on the respective plate of DNA Medium[21], Phenol-red Phosphatase Medium[3], and Tellurite Glycine Medium[1,2,24], on which 211 strains were tested. Lecithinase production was tested on Staphylococcus 110 Medium added with one per cent of egg yolk; fibrinolysin production on heart infusion agar added with 20 per cent of dog plasma; caseinase production on nutrient agar added with 2 per cent of casein; lipase production on nutrient agar added with both 0.1 per cent of tween 80 and 0.01 per cent of calcium chloride; lecithinase production on the salt-free medium

by the method described by SPINK *et al.*[20] VP test, MR test, and tests for carbohydrate fermentation were performed on 189 strains.

## Results

The relationship between coagulase production and hemolytic reaction on sheep-blood agar plate in 315 strains of staphylococci is shown in Table 1. All of the coagulase-positive strains were hemolytic, and 9, 23, and 90 strains of them showed $\alpha$, $\beta$, and $\alpha\beta$ hemolysis, respectively, thus the strains showing $\beta$ hemolysis representing as much as 93 per cent of coagulase-positive staphylococci. On the other hand, many strains of coagulase-negative staphylococci were non-hemolytic or $\delta$-hemolytic with the exception of 45 strains which showed $\alpha$ hemolysis. No $\beta$-hemolytic strain was found among the coagulase-negative staphylococci. In comparison of the $\alpha$ hemolysis between 9 coagulase-positive strains and 45 coagulase-negative ones, most of the former and only a few of the latter were found to show somewhat larger zone of hemolysis. Furthermore, 5 coagulase-positive and 12 coagulase-negative strains were selected at random from the $\alpha$-hemolytic strains to examine the $\varepsilon$ hemolysin, and as the result it was found that $\alpha$ hemolysis of coagulase-positive strains represented certainly $\alpha$ type and that of coagulase-negative ones did not represent $\alpha$ type but $\varepsilon$ type.

The results of the hemolytic reactions obtained on cattle-, rabbit-, and horse-blood agar plate are shown in Table 2. The hemolytic reactions on cattle-blood agar plate were entirely similar to that on sheep-blood agar plate. On rabbit- and horse-blood agar plate, however, $\beta$ hemolysis was not shown, and therefore, there may be no correlation between hemolytic type and coagulase production.

The correlation between coagulase production and the other biochemical activities other than hemolysin production is shown in Table 3. It was found that all of coagulase-positive strains showed positive reaction on DNase production, phosphatase production, and tellurite reduction, and that 22 (16 per cent), 61 (45 per cent), and 95 (69 per cent) of coagulase-negative strains also showed positive reaction on the respective test. A comparatively large number of coagulase-positive strains and a few of coagulase-negative ones showed positive reaction on the test

Table 1. Relationship between Coagulase Production and Hemolytic Reaction on Sheep-blood Agar Plate in Staphylococci Isolated from Bovine Mastitis

| Hemolytic type (in appearance) | the number of strains | |
|---|---|---|
| | Coagulase | |
| | positive | negative |
| | 122 | 193 |
| $\alpha$ | 9 | 45 |
| $\beta$ | 23 | 0 |
| $\alpha\beta$ | 90 | 0 |
| $\delta$ or non-hemolytic | 0 | 148 |

Table 2. Comparison of Hemolytic Reaction on Agar Plate Added with Various Blood Cells

| blood cells from: | Coagulase | the number of strains | | | | |
|---|---|---|---|---|---|---|
| | | Hemolytic type (in appearance) | | | | |
| | | α | β | αβ | δ | non-hemolytic |
| Sheep or Cattle | positive | 8 | 9 | 43 | 0 | 0 |
| | negative | 11 | 0 | 0 | 28 | 52 |
| Rabbit | positive | 51 | 0 | 0 | 2 | 7 |
| | negative | 11 | 0 | 0 | 28 | 52 |
| Horse | positive | 2 | 0 | 0 | 33 | 25 |
| | negative | 0 | 0 | 0 | 39 | 52 |

Table 3. Relationship between Coagulase Production and the Other Biochemical Properties

| production, reduction, or fermentation of: | the number of strains | | | |
|---|---|---|---|---|
| | Coagulase-positive | | Coagulase-negative | |
| | positive | negative | positive | negative |
| DNase | 74 (8) | 0 | 22 (5) | 115 (25) |
| Phophatase | 74 (8) | 0 | 61 (8) | 76 (22) |
| Tellurite | 74 (8) | 0 | 95 (16) | 42 (14) |
| Lecithinase* | 55 | 67 | 18 | 175 |
| Fibrinolysin* | 32 | 90 | 23 | 170 |
| Caseinase* | 28 | 94 | 99 | 94 |
| Lipase | 46 | 30 | 34 | 79 |
| Lecithinase* (on salt-free medium) | 0 | 122 (9) | 44 (15) | 149 (30) |
| VP test | 42 | 34 | 29 | 84 |
| MR test | 70 | 6 | 62 | 51 |
| Glucose | 76 | 0 | 113 | 0 |
| Mannose | 76 | 0 | 79 | 34 |
| Maltose | 76 | 0 | 83 | 30 |
| Sucrose | 76 | 0 | 103 | 10 |
| Arabinose | 0 | 76 | 18 | 95 |
| Lactose | 76 | 0 | 97 | 16 |
| Glycerin | 34 | 42 | 53 | 60 |
| Mannitol* | 119 (9) | 3 (0) | 36 (19) | 157 (26) |

Remarks: *; All the strains were employed.
( ); the number of apparently α-hemolytic strains

of lecithinase production. From the results of tests on fibrinolysin, caseinase, and lipase, it was found that coagulase production was not related with each of the three activities. On the salt-free medium, none of coagulase-positive strains produced lecithinase, whereas 23 per cent of coagulase-negative strains produced it. On the test of mannitol fermentation, 98 per cent of coagulase-positive strains and 19 per cent of coagulase-negative ones showed positive reaction. As to the fermentation of the other carbohydrates, the results of coagulase-positive strains were similar to each other, while those of coagulase-negative strains were various.

## Discussion

It has been clarified that there are some differences in biological character among staphylococcal strains from different animal sources, and that samples from the same kind of animals show a similar property. WILLIAMS *et al.*[22] suggested that the hemolytic activity of staphylococci could be used as a guide for detecting pathogenic strains in mixed cultures. As to the strain isolated from bovine udder, there exist a number of papers, such as those of SMITH[18], HIRATO *et al.*[9], NAKAGAWA[14], and others[6,16,17,23], which deal with the relation between coagulase production and hemolytic property. In these reports, it was stated that there was a close correlation between both of the properties, and that most of the hemolytic staphylococci were coagulase-positive strains.

On the basis of the biochemical properties shown by bovine strains, an attempt was made in the present study to confirm the existence of coagulase from hemolytic phenomena induced by the strain. As the result, it was clarified that all of the staphylococci which were $\beta$-hemolytic on the plate added with sheep red cells were coagulase-positive, and $\delta$-hemolytic or non-hemolytic strains were coagulase-negative, and that the strains which showed $\alpha$ hemolysis in appearance were either coagulase-positive or coagulase-negative. In the case of $\alpha$ hemolysis, therefore, it may be impossible to confirm the existence of coagulase in the strain. However, it was also suggested that these $\alpha$-hemolytic strains would be particularly identified by the examination of $\varepsilon$ hemolysin. From these accounts, it was found that if hemolysis was examined on sheep- or cattle-blood agar plate, it would be comparatively easy to confirm the coagulase activity of staphylococci isolated from bovine udder. It has been shown also by the other authors[14,22] that rabbit or horse red blood cells fail to show $\beta$ hemolysis, so that the plate medium added with these cells is unsuitable for that purpose.

HIRATO *et al.*[9], OCHI *et al.*[15], NAKAGAWA[14], and YOSHIMURA[23] stated that coagulase-positive strains lacking in hemolysin or coagulase-negative strains having $\beta$ hemolysin were included in the staphylococcal strains examined by them. Therefore, it is very interesting that none of such strains was found among the isolates from infected cows in this study.

It has been stated that DNase production, phosphatase production, and tellurite reduction of staphylococci are tightly correlated with coagulase production. An attempt has also been made to use these properties in order to detect pathogenic staphylococci. Many reports[5,10,21] were published concerning DNase production and phosphatase production of human staphylococci. It was stated that DNase production was closely correlated with coagulase production. On the strain isolated from bovine mastitis, SHARPE *et al.*[17], FORBES[8], and KUME *et al.*[11] have also stated that most of coagulase-positive strains were able to produce DNase.

In the present study, all of coagulase-positive strains produced DNase, whereas not a few of coagulase-negative strains also produced it. As there may be a number of coagulase-negative strains having DNase-producing activity among the isolates from bovine mastitis, namely, a strain having DNase does not always produce coagulase, it may be reasonable to consider that the test on DNase production is inferior to that on hemolysin production for the purpose of detecting coagulase-positive staphylococci. It may be also reasonable to think that the test on phosphatase production or tellurite reduction is inferior to the test on DNase production for the same purpose, for there existed larger numbers of coagulase-negative strains which showed positive reaction on the former two tests. On the above-mentioned strain which showed $\alpha$ hemolysis in appearance, there was no definite correlation between coagulase production and each of DNase production, phosphatase production and tellurite reduction. As to the present results of lecithinase production, fibrinolysin production, caseinase production, lipase production, VP test, and MR test, there existed no correlation between these biochemical properties and coagulase-producing ability, excepting the fact that many coagulase-positive strains were included in the lecithinase-producing strains. On the test of mannitol fermentation, most of the coagulase-positive strains showed positive reaction, while 81 per cent of coagulase-negative ones showed negative reaction. Therefore, it may be reasonable to consider that the mannitol fermentation is more closely related with coagulase production than with both phosphatase production and tellurite reduction. On the fermentation of mannose, maltose, sucrose, arabinose, and lactose, the results of coagulase-positive strains were uniform, while those of coagulase-negative strains were various.

SPINK *et al.*[20] stated that coagulase-negative strains isolated from clinical sources fermented mannitol and did not produce lecithinase on the salt-free medium. On the test of lecithinase production on the salt-free medium in the present study, all of coagulase-positive strains showed negative reaction, while 77 per cent of coagulase-negative strains also showed negative reaction. It was also found that coagulase-negative strains showing $\alpha$ hemolysis in appearance would be encountered very often in the group of mannitol-positive and lecithinase-negative strains.

## Summary

The ability for staphylococci to coagulate plasma is generally accepted as a marker of pathogenic strains, and it is also one of the major criteria employed to differentiate *S. aureus* from *S. epidermidis*. However, the other properties which are correlated in varying degrees with coagulase production, such as DNase production, phosphatase production or tellurite reduction, are recently being employed by the diagnostic laboratory for the routine identification of a clinical isolate.

The present study was designed to determine the possible correlation between coagulase production and the other biochemical properties of staphylococci isolated from bovine mastitis. The material used consisted of 122 coagulase-positive strains and 193 coagulase-negative ones. The results obtained are summarized as follows:

1) On sheep-blood agar every strain of coagulase-positive staphylococci was hemolytic and the great majority of those strains showed $\beta$ or $\alpha\beta$ hemolysis, whereas many strains of coagulase-negative staphylococci showed $\delta$ hemolysis or

were non-hemolytic with the exception of 23 per cent of the strains which showed $\alpha$ hemolysis in appearance.

2) On the examination of DNase production, phosphatase production and tellurite reduction, it was found that all of the coagulase-positive strains showed positive results and that 16, 45 and 56 per cent of the coagulase-negative strains similarly showed positive results on each examination respectively.

3) The other biochemical properties except those described above did not correlate with the coagulase production.

4) The results of the present study indicate that the hemolysis on sheep-blood or cattle-blood agar has the advantage over the other biochemical reaction for isolation and identification of coagulase-positive staphylococci isolated from bovine mastitis and that the DNase production should be secondly ranked.

## References


1) BAIRD-PARKER, A. C. (1962): J. appl. Bact., 25, 12.
2) BAIRD-PARKER, A. C. (1962): J. appl. Bact., 25, 441.
3) BARBER, M. and KUPER, S. W. A. (1951): J. Path. Bact., 63, 65.
4) COWAN, S. T. (1962): J. appl. Bact., 25, 324.
5) DE WAART, J., MOSSEL, D. A. A., TEN BROEKE, R. and VAN DE MOOSDIJK, A. (1968): J. appl. Bact., 31, 276.
6) EDWARDS, S. J. and RIPPON, J. E. (1957): J. comp. Path., 67, 111.
7) ELEK, S. D. and LEVY, E. (1950): J. Path. Bact., 62, 541.
8) FORBES, D. (1968): J. appl. Bact., 31, 426.
9) HIRATO, K., SHIMIZU, K., KUNISHIGE, T., SHIMIZU, Y., OSAMURA, K., NAKAGAWA, M., NAGAYA, H., FUKUDA, T., SUGAMA, T. and HIGASHINO, Y. (1956): J. Japan vet. med. Ass., 9, 159.
10) JEFFRIES, C. D., HOLMAN, D. F. and GUSE, D. G. (1957): J. Bact., 73, 59.
11) KUME, T. and MURASE, N. (1970): Bull. nat. Inst. anim. Hlth., 61, 56.
12) MARKHAM, N. P. and MARKHAM, J. G. (1966): J. comp. Path., 76, 49.
13) MINAMIMOTO, S., SHIBATA, D. and ISHII, F. (1959): Bull. nat. Inst. anim. Hlth., 37, 85.
14) NAKAGAWA, M. (1958): Jap. J. vet. Res., 6, 191.
15) OCHI, Y. and KATSUBE, Y. (1958): Jap. J. vet. Sci., 20, 159.
16) REID, W. B. and WILSON, J. B. (1959): Amer. J. vet. Res., 20, 825.
17) SHARPE, M. E., NEAVE, F. K. and REITER, B. (1962): J. appl. Bact., 25, 403.
18) SMITH, H. W. (1947): J. comp. Path., 57, 98.
19) SMITH, J. E. (1962): J. comp. Path., 72, 131.
20) SPINK, M. S. and STRONG, S. (1968): J. Path. Bact., 95, 295.
21) WECKMAN, B. G. and CARLIN, B. W. (1957): J. Bact., 73, 747.
22) WILLIAMS, R. E. O. and HARPER, G. J. (1947): J. Path. Bact., 59, 69.
23) YOSHIMURA, H. (1970): Jap. J. vet. Sci., 32, 263.
24) ZEBOVITZ, E., EVANS, J. B. and NIVEN, C. F. Jr. (1955): J. Bact., 70, 686.