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## CONIDIAL PRODUCTION OF *CERCOSPORA BETICOLA* SACC. ON TOMATO JUICE AGAR

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羅瑯俊 : 토마도주 寒天培養基上에서의 사탕무 褐斑病 分生孢子形成

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### ABSTRACT

LA, Yong Joon (Coll. of Agr., Seoul National Univ.) Conidial production of *Cercospora beticola* Sacc. on tomato juice agar. Kor. Jour. Bot. VI (1): 8—10, 1963.

Agar media containing various amount of tomato juice were tested to determine the degree of conidial production of *Cercospora beticola*.

Non-sporulating culture on potato dextrose agar readily sporulated on agar media containing various amount of tomato juice 48 hours after transfer.

Considerably small amount of sporulation occurred on agar media containing 10% of tomato juice. Sporulation increased considerably on media containing more than 20% of tomato juice; the higher the proportion of tomato juice in a medium, the greater the number of spores produced.

### INTRODUCTION

In the studies on the pathogenicity of *Cercospora beticola* Sacc., which causes leaf spot disease on sugar beets, it is desirable to obtain abundant conidia readily on artificial media for use as inoculum.

Although studies by various investigators<sup>(1,2,3,4,5,6)</sup> resulted in procedures that can be used to obtain conidia of *C. beticola*, it is, nevertheless, still difficult to obtain them in sufficient number for creation of artificial epidemics on test materials. Thus the lack of precise and efficient method of obtaining abundant conidia of *C. beticola* on artificial media required a method and/or medium which would facilitate abundant spore production. The author<sup>(5)</sup>, during his previous studies on *Cercospora* leaf spot disease of sugar beets, was able to obtain abundant conidia of *C. beticola* on both of the agar and liquid medium of the V-8 juice. However, V-8 juice is not easily accessible at Korean market at present and it was necessary to develop a method which can be used in place of V-8 juice. Tomato juice is not only one of the major components of the V-8 juice, but also it has advantages of being readily available at Korean market and less expensive than V-8 juice. Thus in an attempt to develop an economical method which would facilitate abundant spore production, agar media containing various amount of tomato juice were tested to determine the degree of conidial production of *C. beticola*.

### MATERIALS AND METHODS

Commercially canned tomato juice (Trade mark: Sammi) was used in this study. Six different

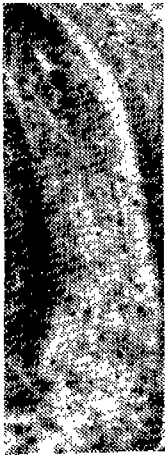


Fig. 7



Fig. 8

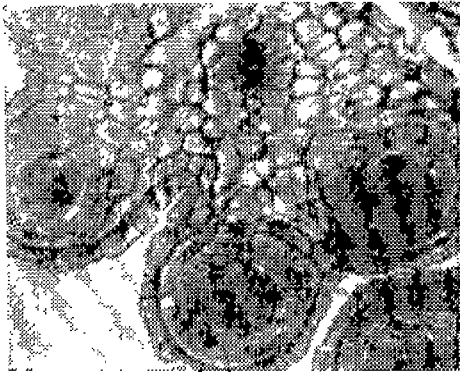
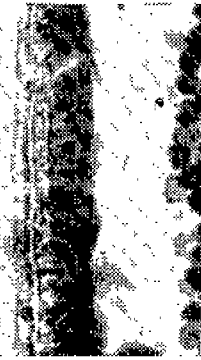


Fig. 9



a



b

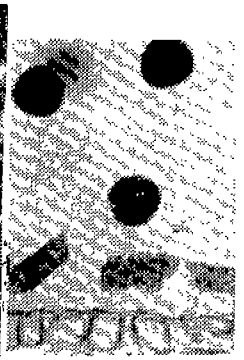


Fig. 10

Fig. 11

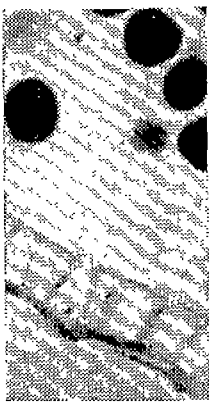


Fig. 12



Fig. 13

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Fig. 1—5. *Brassica*: Fig. 1. Protuberance of integumental outgrowth. Fig. 2. Development of integument.

Fig. 3. Megaspore mother cell. Fig. 4 Enlarging chalazal megaspore. Fig. 5. 2-nucleate embryo sac.

Fig. 6. Mature embryo sac immediately before blooming. a,b,c, *Brassica*; d, long and curved nucellus of *Raphanus*. Fig. 7 Abortive embryo sac and elongated nucellus of F<sub>1</sub> at blooming stage. Fig.8 Abortive embryo sac of F<sub>1</sub> at blooming stage.

Fig. 9 Young anther of *Raphanus*. Fig. 10 Stretched and flattened epidermis of *Brassica*: a.

tetrad stage; b. immediately after microspore formation.

Fig. 11—12 *Brassica*: Fig.11 Disintegration of tapetal cell before blooming stage. Fig. 12 Endothecium at blooming stage. Fig. 13 Tapetal tissue of F<sub>1</sub> at blooming stage.

concentrations of tomato juice agar were prepared by adding the following proportions of tomato juice on to the given quantity of medium: 10, 20, 40, 60, 80, and 100%. Difco-Bacto agar was added to each in the amount of 20 g per liter. The media were sterilized in an autoclave at 15 lbs pressure for 20 minutes and the pH of the media was adjusted to 5.5-6.0 by addition of calcium carbonate. Twenty ml of each medium was placed in petri plates.

Conidia of *C. beticola* were isolated from the diseased lesions of the sugar beet leaves and grown on potato dextrose agar in petri plate on which no conidia were produced but only mycelial growth resulted. When the mycelial mat on potato dextrose agar had shown sufficient growth (about 6 cm in diameter), round disks (5 mm diam) of agar were cut from the margins of the mycelial mat by using biscuit cutter and each of these disks was placed on the center of agar plates containing different amount of tomato juice and incubated at room temperature.

After 10 days of growth, a disk of agar (5 mm diam) was cut from the margin of the culture and placed on a concave watch glass on which 1 ml of sterile distilled water was added and the agar piece thoroughly macerated by using a piece of wooden rod. Two agar disks were cut from each of the 5 replicate plates and two counts from each agar disk were made, making the total of 20 counts for each medium containing different amount of tomato juice.

Spore counts were made by taking the conidial suspension from the concave watch glass and flooding the surface of a haemocytometer. Thus each medium containing different amount of tomato juice was evaluated as to the efficiency of conidial production by determining the number of spores produced per unit surface area of the medium.

## RESULTS AND DISCUSSION

Non-sporulating mycelial culture of *C. beticola* on potato dextrose agar produced conidia on tomato juice agar 48 hours after transfer and the light colored mycelial growth from the potato dextrose agar slowly became dark green in color. Transfer of a sporulating culture from tomato juice agar to fresh tomato juice agar always gave abundant sporulation.

Sporulation hardly occurred on media containing 10% of tomato juice, whereas more than 20% times as many spores were produced on media containing more than 20% of tomato juice, and the higher the proportion of tomato juice in a medium, the greater the number of spores was produced.

The comparative yield of conidia expressed as number of spores produced per unit surface area on each of the 6 different concentrations of tomato juice agar is shown in Table 1.

**Table 1.** Number of conidia of *C. beticola* produced per unit area on each of the agar media containing different amount of tomato juice,

Percentage of tomato juice in agar medium	Number of conidia
10	1.2*
20	23.9
40	29.3
60	40.3
80	49.9
100	54.5
L.S.D. at 5% level	8.7

\* Each datum represents the average of twenty 9-mm<sup>2</sup> haemocytometer counts made on the spore suspension obtained from each of 5 replicate plates.

The results indicate that agar media containing more than 20% of tomato juice are highly efficient in obtaining abundant conidia, whereas agar media containing less than 10% of tomato juice are not. The fact that the non-sporulating cultures of *C. beticola* on potato dextrose agar produced conidia on tomato juice agar 48 hours after transfer and that the greater number of spores were produced on agar media containing greater amount of tomato juice indicates that the sporulation of *C. beticola* on artificial media is affected by nutritional factors that are qualitative as well as quantitative.

### 摘 要

1. 寒天培養基中の 토마도주含量과 사탕무 褐斑病菌 *Cercospora beticola* 의 胞子形成度와의 關係를 調査하였다.
2. 감자주寒天培養基上에서 胞子を 形成하지 않는 培養菌 *C. beticola* 는 토마도주를 10% 以上 含有하고 있는 寒天培養基上에서 移植后 48 時間만에 胞子を 形成하였다.
3. 토마도주를 10% 含有하고 있는, 寒天培養基上에서는 極히 少數의 胞子が 形成되었으나, 토마도주 含量이 20% 以上인 寒天培養基上에서는 多數의 胞子が 形成되었고, 寒天培養基中の 토마도주 含量이 높을수록 더 많은 胞子が 形成되었다.

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