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Effect of culture medium on spore production and germination of races of Colletotrichum lindemuthianum

E. DRIJFHOUT and J. JANSEN

Institute for Horticultural Plant Breeding (IVT), P.O. Box 16, 6700 AA Wageningen, the Netherlands

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Abstract

Spore production on the media neopeptone glucose agar (PGA), malt extract agar (MEA), Czapek Dox agar (CDA) and bean pod extract agar (BEA) was determined for 10 races of *Colletotrichum lindemuthianum*, as well as the aftereffects on spore germination on water agar.

Clear race-medium interactions were found for spore production. PGA was a universal medium for races of this fungus, but five races gave a much higher production on MEA. CDA and BEA were less effective for spore production. There was no clear aftereffect of the four media on spore germination. The 10 races could be divided into good, moderate and bad germinators, independent of the medium on which they had been developed.

Additional key words: race-medium interactions.

Over the years we observed considerable differences in spore production between cultures of 10 races of *Colletotrichum lindemuthianum* (Sacc. et Magn.) Scribn. on neopeptone glucose agar. We also found large differences in the infection level between races on sets of differential bean cvs under standardized experimental conditions. The question arose, whether the type of culture medium used in the experiments was suitable for all 10 races, or whether certain races required another medium for a good sporulation. A second question was whether the medium on which the conidia were produced, could affect germination and thereby possibly also the rate of infection of bean plants. To investigate this, experiments were performed to measure spore production of 10 races on four media, and to determine the germination of the conidia, originating from cultures on four media, on water agar.

The following media were used: neopeptone glucose agar (PGA, Mathur et al., 1950) with a small modification (15.0 instead of 20.0 g agar per litre), malt extract agar (MEA, Oxoid), modified Czapek Dox agar (CDA, Oxoid) and bean pod extract agar (BEA). The BEA was prepared as follows: 250 g cut pods of bean cv Topcrop in 800 ml water were autoclaved at 100 °C for 10 min. The soft pod parts were sieved and the pulp was filled up with water to 1 l; 15 g agar was added and the mixture was autoclaved for 30 min at 120 °C.

The *Colletotrichum* races used were: alpha, beta, gamma, delta, epsilon, iota, kappa, lambda, alpha Brazil and C236. Cultures of all races were received from the Research Institute for Plant Protection (IPO), Wageningen, the Netherlands, except C236, which came from the Centro International de Agricultura Tropical (CIAT), Cali, Colombia.

Monospore cultures from each of the 10 races were made and grown as source material. Cultures were grown on four petri plates (9 cm diam.) for each of the four media by spreading 0.1 ml suspension of 2×10^6 spores ml^{-1} over each plate and incubating for 14 days at 21 °C. After incubation 10 ml water was added to each plate for making spore susensions. The concentration of the spores was determined by hemacytometer countings. This experiment was performed on three occasions which were considered as replicates.

Germination of spores was determined on water agar plates (15 g agar/l). Four plates were used for every combination of medium and race. Incubation was done as for spore production. After 3 days of incubation, on each plate 25 spores were observed in four different areas, each covered with a microscope glass cover slip, and germinated spores counted. This experiment was done on two occasions.

For spore production and germination analyses of variance were carried out by Genstat, using a logarithmic transformation for spore production and a probit transformation for spore germination percentages.

Conidial production. Table 1 shows the average spore production for every combination of race and medium, as well as the 95% confidence intervals. Clear race/medium interactions were found ($P < 0.001$). Alpha, beta, epsilon, kappa and lambda had high spore productions on MEA. Delta, iota, alpha Brazil and C236 gave the highest production on PGA, while the production of gamma was about the same on both media. No single race gave the highest production on CDA or BEA. The production of iota and alpha Brazil was very low on CDA and BEA, and that of gamma, delta, kappa and C236 was low on CDA.

Conidial germination. For spore germination no significant interaction was found between media and races, and the media showed no statistically significant differences. However, there were clear differences between races ($P < 0.001$). They could be grouped in (Table 2): 1) well germinating races alpha, gamma, kappa and lambda; 2) moder-

Table 1. Average spore production in all combinations of 10 races and 4 media.

Race	Medium			
	PGA	MEA	CDA	BEA
Alpha	2.3 (1.4- 3,7)[1]	22.7 (14.0-36.8)	0.8 (0.5- 1.2)	3.3 (2.1- 5.4)
Beta	2.2 (1.4- 3.6)	15.2 (9.4-24.7)	1.0 (0.6- 1.6)	1.0 (0.6- 1.6)
Gamma	3.6 (2.2- 5.9)	2.9 (1.8- 4.7)	0.2 (0.1- 0.4)	0.7 (0.4- 1.2)
Delta	5.7 (3.5- 9.3)	2.3 (1.4- 3.8)	0.3 (0.2- 0.5)	1.1 (0.7- 1.8)
Epsilon	4.0 (2.4- 6.4)	22.7 (14.0-36.8)	8.7 (5.4-14.1)	8.1 (5.0-13.1)
Iota	6.4 (4.0-10.4)	0.9 (0.5- 1.4)	0.2 (0.1- 0.3)	0.1 (0.0- 0.1)
Kappa	8.1 (5.0-13.1)	44.7 (27.5-72.5)	0.5 (0.3- 0.8)	2.8 (1.7- 4.6)
Lambda	1.3 (0.8- 2.2)	14.1 (8.7-22.9)	1.3 (0.8- 2.0)	1.1 (0.7- 1.9)
Alpha Brazil	7.8 (4.8-12.7)	0.2 (0.1- 0.3)	0.1 (0.0- 0.1)	0.1 (0.1- 0.2)
C236	4.1 (2.5- 6.7)	2.1 (1.3- 3.4)	0.2 (0.1- 0.4)	1.6 (1.0- 2.6)

[1] 95% confidence intervals are given between parentheses.

Table 2. Average spore germination of 10 races after three days on water agar.

Race	% spore germination
Alpha	100.0 (99.9-100.0)[1]
Beta	36.4 (8.2- 75.7)
Gamma	100.0 (99.3-100.0)
Delta	76.0 (36.7- 96.0)
Epsilon	10.2 (1.0- 40.9)
Iota	61.8 (22.8- 91.0)
Kappa	97.6 (82.3- 99.9)
Lambda	95.2 (73.3- 99.7)
Alpha Brazil	41.5 (10.4- 79.6)
C236	37.2 (8.5- 76.4)

[1] 95% confidence intervals are given between parentheses.

ately germinating races beta, delta, iota, alpha Brazil and C236; 3) badly germinating race epsilon.

MEA gave the highest average spore production, over the ten races considered, followed by PGA, BEA and CDA. Media with natural extracts as from malt and bean pods or with peptone were better for spore production than the synthetic Czapek Dox medium. The stimulants for spore production in neopeptone are vitamins (Mathur et al., 1950), whether or not in combination with other substances. The same might be true for malt and bean pod extracts. PGA has indeed a general suitability for spore production of races of *C. lindemuthianum* (Mathur et al., 1950). However, MEA is to be preferred for races alpha, beta, epsilon, kappa and especially lambda, when large quantities of conidia are needed. PGA is then to be preferred for delta, iota, alpha Brazil and C236. CDA and BEA do not offer any advantage above PGA and MEA.

There was no clear aftereffect on spore germination on water agar from the medium on which the spores had been produced. Agar itself could have a stimulative effect on germination: beta, lambda and kappa had an average germination on water agar of 36, 95 and 98% respectively, while Landes and Hoffmann (1979) found that spore germination of these races in distilled water remained below 10%. They also reported that germination of the conidia in vitro could be stimulated by complex nutrient media like bio malt solution.

Isolates originating from the same monospore culture per race were used in the investigations presented. The question remains whether different, non-monospore originated isolates per race, also may contribute to consistent differences in spore production or germination.

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Samenvatting

Effect van vier cultuurmedia op de sporenproduktie en sporenkieming van fysio's van Colletotrichum lindemuthianum

Onderzoek in vitro werd gedaan naar de invloed van vier cultuurmedia op de sporenproduktie van 10 fysio's van *C. lindemuthianum*. Bovendien werd nagegaan of de kieming van de sporen op water-agar afhankelijk was van het medium waarop de sporen waren gevormd.

Er bleken voor sporenproduktie inderdaad aanwijsbare fysio/medium interacties te zijn. De beste algemene gechiktheid had neopepton-glucose-agar (PGA). Op mout-extract-agar (MEA) werden echter met verschillende fysio's veel hogere sporenproduktie verkregen, terwijl de sporenproduktie van andere fysio's duidelijk lager lag dan op PGA. De resultaten met Czapek-Dox-agar (CDA) en bonepeul-extract-agar (BEA) waren minder goed. Voor de 10 fysio's kan derhalve met PGA en MEA worden volstaan. Media met natuurlijke extracten (mout, bonepeulen) of met pepton, waren beter voor sporenproduktie dan het synthetische medium Czapek-Dox-agar.

De media hadden geen na-effect op de sporenkieming op water-agar. De fysio's konden echter worden ingedeeld in goede, matige en slechte kiemers.

References

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