BIOLOGIA PLANTARUM 36 (4): 633-635, 1994

BRIEF COMMUNICATION

The effect of nutrient medium and age of *Erwinia amylovora* and *Erwinia herbicola* cultures on their reactivity in agglutination test

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Abstract

The influence of the type of nutrient medium (meat-peptone nutrient agar, KING B medium, YDC medium) and the age of bacterial cultures of *Erwinia amylovora* and *E. herbicola* on reactivity of their antigens with homologous antisera were examined. In the case of *E. amylovora*, the best agglutination reactions were observed with isolates cultivated on the YDC nutrient medium. After 192 h of cultivation, seven outof ten isolates reacted positively. The reactivity of *E. herbicola* antigen decreased in dependence on culture age more rapidly than with *E. amylovora*. The highest rates of positive agglutination reactions were observed with *E. herbicola* cultivated on the YDC and KING B nutrient media. After 144 h of cultivation on both these nutrient media eight out of ten isolates reacted positively.

Erwinia amylovora (Burrill) Winslow, Buchanan, Krumwiede, Rogers et Smith 1920 is considered to be a dangerous phytopathogenic microorganism. This pathogen evokes the fire blight of rosaceous plants and has a high epidemiological potential with a large range of harmful effects on a wide range of host plants (Van der Zwet and Keil 1979). *E. amylovora* belongs to the quarantine bacterial organisms.

The agglutination reaction of *E. amylovora* antigen with homologous antiserum is the most rapid and simultaneously reliable determination method. *E. amylovora* isolates have a relatively high level of homogeneity. Polyclonal *E. amylovora* antisera react only exceptionally with other species of bacteria (Vantomme *et al.* 1982). The age of the bacterial culture in the agglutination test of phytobacteria should be respected to obtain reliable results. The type of nutrient medium used for the cultivation of bacteria is of importance, too.

Received 28 December 1993, *accepted* 23 February 1994.

Ten isolates of phytopathogenic *E. amylovora* bacteria and ten isolates of saprophytic *Erwinia herbicola* (Löhnis) Dye 1964 bacteria were selected. *E. herbicola* is the most studied bacterium of all potential antagonists of *E. amylovora*. For the investigation into which type of nutrient medium is the most suitable for the cultivation of *E. amylovora* and *E. herbicola* bacteria they were both inoculated on meat-peptone nutrient agar (*Imuna*, Šarišské Michalany, Slovakia), medium KING B (King *et al.* 1954) and medium YDC (yeast extract-dextrose-$CaCO_3$, Davis 1979). *E. amylovora* isolates originated from North, West and Central Bohemia from hawthorn, pear and cotoneaster. *E. herbicola* isolates originated from North, West, Central and South Bohemia from hawthorn, pear, apple tree, service tree, cotoneaster and quince. The bacterial cultures were cultivated at 27 °C (Lelliott and Dickey 1984) after inoculation on the nutrient media.

Agglutination reactions of *E. amylovora* and *E. herbicola* isolates with their homologous antiserum were evaluated at 24 h intervals for 264 h. The bacterial cultures were used at a dilution of 10^9 cells per cm^3 in the agglutination reaction. *E. amylovora* antiserum was prepared against the isolate Ea 531 (locality Louny, host plant hawthorn) and *E. herbicola* antiserum was prepared against the antigen Eh HMA 9 (locality Praha, host plant hawthorn). The rabbits were injected once subcutaneously with 1 cm^3 and twice intravenously with 2 cm^3 of *E. amylovora* antigen 531 killed by formaldehyde. Two-component adjuvant (Al-span-oil) was used in subcutaneous injections (Dickey *et al.* 1984, Mráz 1992). *E. herbicola* HMA 9 isolate was killed by formaldehyde and it was injected twice subcutaneously in doses of 1 cm^3 and 2 cm^3 without adjuvant (Calzolari *et al.* 1982).

The agglutination reactions were performed as long as at least one antigen of the whole tested group of isolates reacted positively. Every *E. amylovora* and *E. herbicola* isolate was simultaneously tested with antiserum from nonimmunized rabbits as the control.

Table 1. Comparison of the number of positive reactions of *Erwinia amylovora* and *Erwinia herbicola* isolates in agglutination test in dependence on their age and on the type of nutrient medium (10 isolates were tested).

	Nutrient medium	Age of bacterial culture [h] 24	48	72	96	120	144	168	192	216	240	264
E. amylovora	meat-pept.	10	10	10	9	8	8	6	4	3	2	0
	KING B	10	10	10	9	8	7	6	4	4	0	0
	YDC	10	10	10	9	9	9	8	7	5	0	0
E. herbicola	meat-pept.	10	10	10	10	9	3	1	0	0	0	0
	KING B	10	10	10	10	10	8	5	3	0	0	0
	YDC	10	10	10	10	10	8	6	4	2	0	0

In the case of *E. amylovora*, the best agglutination reactions were observed with isolates cultivated on the YDC nutrient medium. Even after 192 h of cultivation, seven of ten isolates reacted positively (Table 1). Worse reactivity was recorded with cultures cultivated on meat-peptone and KING B nutrient media. After 192 h of

cultivation, four of ten isolates reacted on each of the two latter media (Table 1).

These results show that YDC nutrient medium is the most suitable for the cultivation of *E. amylovora*. Isolates cultivated on this medium can still be used for reliable determination in the agglutination test after 192 h of cultivation.

In the case of *E. herbicola*, antigen reactivity decreased in dependence on culture age more rapidly than with *E. amylovora*. The best positive agglutination reactions were observed with bacteria cultivated on the YDC and KING B nutrient media. On the KING B medium, three of ten isolates reacted after 192 h positively, on the YDC medium four of ten isolates reacted positively. But on the meat-peptone nutrient agar only one isolate showed a very weak reaction after a 168 h cultivation (Table 1).

For the cultivation of *E. herbicola*, YDC and KING B nutrient media are most suitable. Isolates cultivated on these media can be used for a reliable determination in the agglutination test for at least 144 h of cultivation.

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