

## RESULTS

### **Bacterial strains**

*X. smithii* subsp. *citri* strains of Thailand were isolated from different kinds of *Citrus* spp. namely mandarin (*C. reticulata*), lime (*C. aurantifolia*), pummelo (*C. grandis*) and sweet orange (*C. sinensis*) from major citrus producing provinces of Thailand (Table 5). Total *X. smithii* subsp. *citri* strains in this study were 19 strains from Thailand, 2 strains from Japan, and 2 strains from Saudi Arabia. Other xanthomonads included in this study were consisted of 10 strains of *X. fuscans* subsp. *aurantifolii*, 2 strains of *X. alfalfae* subsp. *citrumelo*, 1 strain of *X. campestris* pv. *campestris*, 11 strains of *X. campestris* pv. *glycines*, 9 strains of *X. smithii* subsp. *smithii* and 1 strain of *X. fuscans* subsp. *fuscans*.

### **Pathogenicity tests**

All Xsc strains that isolated from Thailand were pathogenic on lime (*C. aurantifolia*) leaves. The typical symptoms of citrus canker usually associated with hyperplasia lesions on upper and lower leaf surface and surrounded with yellow halo within 2-3 weeks after inoculation by using a blunt end syringe to infiltrate  $10^6$  cfu/ml of the bacterial suspension into leaves (Fig. 3)

### **Development of new semi-selective medium for *Xanthomonas smithii* subsp. *citri***

The new semi-selective medium (XSC) was developed based on NA and supplemented selectivity based on SX and FS media. XSC medium contained (per liter): NA 23 g, potato starch 10 g, casein hydrolysate 2 g, dextrose 2 g, 1% methyl green 1.5 ml, 1% methyl violet 2B 0.25 ml, 1% cycloheximide 5.0 ml, 1% cephalixin 2.5 ml and 0.1% D-methionine 3.0 ml. All Xsc isolates grew on XSC medium after incubated at 30°C for 2 days. Colonies were round, convex, pale green and surround by clear zone due to starch hydrolysis (Fig. 4D).

### **Recovery of *Xanthomonas smithii* subsp. *citri* strains on new semi-selective medium**

Plating efficiencies of XSC, FS and SX media in recovery of 8 isolates of Xsc were 76.28%, 93.63% and 47.23% respectively (Table 6). FS medium was the highest efficiency in recovery of pure culture but Xsc colonies grew slower than XSC medium. All strains of Xsc grew up very fast on XSC as compared to NA medium in which just 2 days after plating pin point colony size (0.5-1.0 mm.) showed up and able to determine by eyes. On the other hand, pin point colony size of Xsc on SX and FS media were observed after 3 days and 5 days (Table 7) after plating, respectively.

### **Recovery of *Xanthomonas smithii* subsp. *citri* form naturally infested materials on new semi-selective medium**

In recovery of Xsc from citrus samples, XSC medium provided the highest efficiency than SX and FS media. It was able to isolate Xsc from all tested samples (Table 8) in the experiment and numbers of colony were as high as NA medium whereas SX and FS media were able to isolate Xsc only 1 out of 7 samples with low number of colony, 1-2 colonies per plate.

**Table 5** Geographical origin, host and year of isolation of strains of *Xanthomonas* species used in this study

Strain	Geographical origin		Host	Year
<i>X. smithii</i> subsp. <i>citri</i>				
T1	Kamphaeng Phet	Thailand	<i>Citrus sinensis</i>	2003
T3	Chiang Mai	Thailand	<i>Citrus grandis</i>	2003
T4	Chiang Mai	Thailand	<i>Citrus reticulata</i>	2003
T5	Kamphaeng Phet	Thailand	<i>Citrus aurantifolia</i>	2003
T7	Chiang Mai	Thailand	<i>Citrus reticulata</i>	2003
T8	Chiang Mai	Thailand	<i>Citrus reticulata</i>	2003
T10	Chiang Mai	Thailand	<i>Citrus grandis</i>	2003
T13	Kamphaeng Phet	Thailand	<i>Citrus sinensis</i>	2003
NT14	Kamphaeng Phet	Thailand	<i>Citrus reticulata</i>	2003
NT18	Chiang Mai	Thailand	<i>Citrus aurantifolia</i>	2003
NT20	Sukhothai	Thailand	<i>Citrus aurantifolia</i>	2003
NT22	Chiang Mai	Thailand	<i>Citrus grandis</i>	2003
NT25	Kamphaeng Phet	Thailand	<i>Citrus reticulata</i>	2003
OCr1.1	Chiang Rai	Thailand	<i>Citrus reticulata</i>	2002
OCr1.2	Chiang Rai	Thailand	<i>Citrus reticulata</i>	2002
LCp2.1	Chumphon	Thailand	<i>Citrus aurantifolia</i>	2002
LCp2.2	Chumphon	Thailand	<i>Citrus aurantifolia</i>	2002
SWRb	Ratchaburi	Thailand	<i>Citrus sinensis</i>	2003
Fp1-2	Chiang Rai	Thailand	<i>Citrus grandis</i>	2003
XCC-32	Shimizu	Japan	<i>Citrus natsudaidai</i>	1998
XCC-131	Yui	Japan	<i>Citrus unshiu</i>	1998
1258 (Hartung, Xc-322)		Saudi Arabia	<i>Citrus</i> sp.	ND
1270 (Hartung, Xc-328)		Saudi Arabia	<i>Citrus</i> sp.	ND
<i>X. fuscans</i> subsp. <i>aurantifolii</i>				
1415 (IBSBF 392)		Brazil	<i>Citrus limon</i>	1981
1416 (IBSBF 423)		Uruguay	<i>Citrus limon</i>	1981
1417 (IBSBF 1583)		Argentina	<i>Citrus limon</i>	1990
1418 (IBSBF 380)		Brazil	<i>Citrus aurantifolia</i>	1981
1419 (IBSBF 434)		Brazil	<i>Citrus aurantifolia</i>	1982

Table 5 (continued)

Strain	Geographical origin	Host	Year	
<i>X. fuscans</i> subsp. <i>aurantifolii</i>				
1420 (IBSBF 1473)	Brazil	<i>Citrus aurantifolia</i>	1999	
1421 (IBSBF 1495)	Brazil	<i>Citrus aurantifolia</i>	2000	
1460	ND	ND	ND	
1461	ND	ND	ND	
1463	ND	ND	ND	
<i>X. alfalfae</i> subsp. <i>citrumelo</i>				
1267 (X-85, J. Miller)	Florida	<i>Citrus</i> sp.	1985	
1274 (4600, D. Gabriel)	Florida	<i>Citrus</i> sp.	ND	
<i>X. smithii</i> subsp. <i>smithii</i>				
1318 (ATCC 14982)	Uganda	<i>Gossypium hirsutum</i>	ND	
317	Sukhothai	Thailand	<i>Gossypium hirsutum</i>	1984
579	ND	Thailand	<i>Morus</i> sp.	1986
584	Sukhothai	Thailand	<i>Gossypium hirsutum</i>	1986
1034	Nakhon Sawan	Thailand	<i>Gossypium hirsutum</i>	1990
1035	Nakhon Sawan	Thailand	<i>Gossypium hirsutum</i>	1990
1037	Lop Buri	Thailand	<i>Gossypium hirsutum</i>	1990
1051	Loei	Thailand	<i>Gossypium hirsutum</i>	1990
1232	Prachin Buri	Thailand	<i>Gossypium hirsutum</i>	1993
<i>X. fuscans</i> subsp. <i>fuscans</i>				
1316 (NCPB 381)	Canada	<i>Phaseolus vulgaris</i>	ND	
<i>X. campestris</i> pv. <i>campestris</i>				
657	Phetchaburi	Thailand	<i>Brassica oleracea</i>	2004
<i>X. campestris</i> pv. <i>glycines</i>				
NKR21	Nakhon Ratchasima	Thailand	<i>Glycine max</i>	2001
CM 60-1	Nakhon Ratchasima	Thailand	<i>Glycine max</i>	2002
No.21-1	Chiang Mai	Thailand	<i>Glycine max</i>	2002
RE 07	Khon Kaen	Thailand	<i>Glycine max</i>	2002
239	Chachoengsao	Thailand	<i>Glycine max</i>	1983
241	Phitsanulok	Thailand	<i>Glycine max</i>	1982
281	Phitsanulok	Thailand	<i>Glycine max</i>	ND
285	Phitsanulok	Thailand	<i>Glycine max</i>	ND

Table 5 (continued)

Strain	Geographical origin		Host	Year
<i>X. campestris</i> pv. <i>glycines</i>				
728	Chiang Rai	Thailand	<i>Glycine max</i>	1987
1204	Songkhla	Thailand	ND	1992
1324	Songkhla	Thailand	<i>Vigna radiata</i>	1994

Abbreviations: IBSBF, Phytophthora Culture Collection of Instituto Biológico, Campinas, Brazil; ATCC, American Type Culture Collection, Manassas, VA; NCPPB, National Collection Plant Pathogenic Bacteria, York, England; ND, not determined.

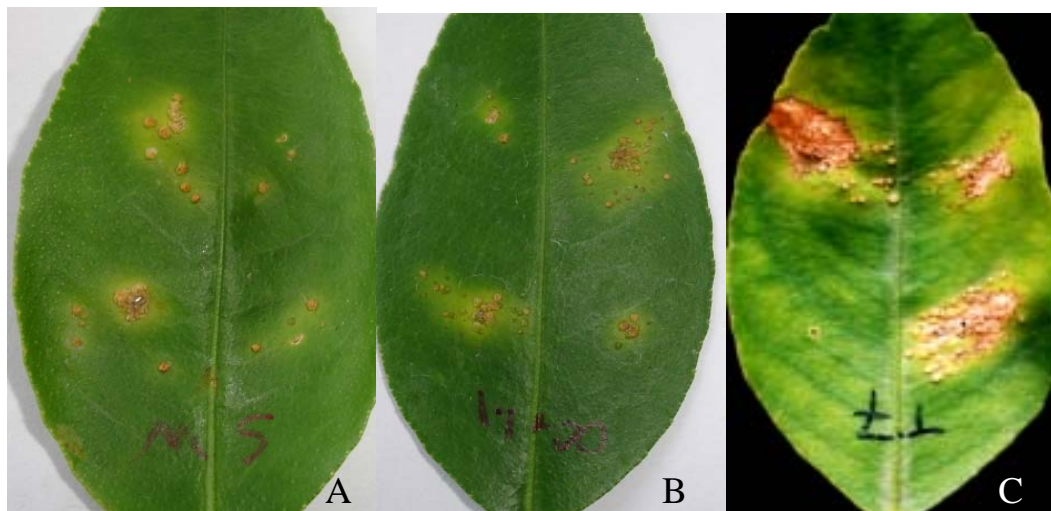
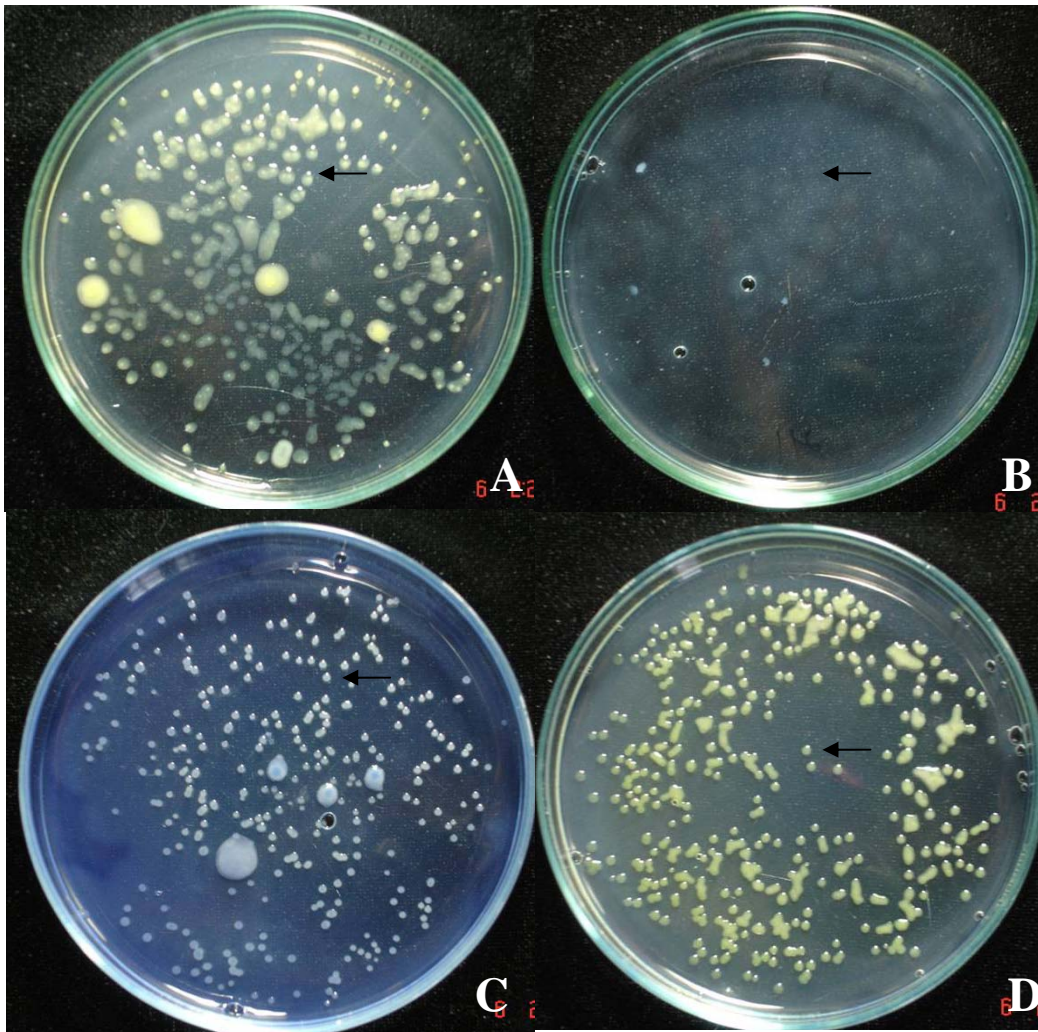


Figure 3 Pathogenicity test of *Xanthomonas smithii* subsp. *citri* strain SW (A), OCr1.1 (B), and T7 (C) on lime (*C. aurantifolia*) leaves. Typical canker symptoms with hyperplasia lesions were observed on upper and lower leaf surface surrounded with yellow halo within 2-3 weeks after inoculation using a blunt end syringe to infiltrate  $10^6$  colony forming units (cfu/ml) of the bacterial suspension.



**Figure 4** Recovery colonies of *Xanthomonas smithii* subsp. *citri* (Xsc) from lesions of lime leaf on NA, SX, FS and XSC media. Typical colonies of Xsc (marked with arrows) after plating 4 days on media: A) nutrient agar, B) SX = Schaad and White's semi-selective agar; C) FS = Fieldhouse and Sasser semi-selective agar and D) XSC = new semi-selective agar for Xsc.