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Identification of bacterial leaf streak of cereals by their phenotypic characteristics and host range in Iran

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Abstract

Forty-four bacterial isolates were obtained from infected wheat, barley and various grasses from different regions of Iran. All isolates were bacteriologically similar to *Xanthomonas campestris* and some of their physiological and biochemical features can be useful for a primary differentiation between them. Depending on their pathogenicity, the isolates were split into two groups; the wheat group isolated from wheat, barley and grasses could infect artificially wheat, barley, rye, *Agropyron elongatum, Bromus inermis*, and *Lolium multiflorum* but not oat, whereas the barley group obtained from cultivated or wild barley was pathogenic to barley only. From their bacteriological characteristics and host range, the barley and the wheat group isolated were identified as *X. campestris* pvs. *hordei* and *cerealis*, respectively. *Aegilops* sp., *Sclerochloa dura*, and *Heteranthelium* sp. were, for the first time, shown to be hosts of *X. c.* pv. *cerealis*.

Introduction

Bacterial leaf streak of cereals has been reported on graminaceous plants in many countries all over the world: in USA, Mexico, Uruguay, Argentina, Brazil, China, India, Japan, Ethiopia, Zambia, Russia, [Duveiller, 1989], Spain [Noval, 1989], Pakistan [Akhtar and Aslam, 1985], Iran [Alizadeh and Rahimian, 1989], Turkey [Demir *et al.*, 1992]. This disease has now become an important problem in many countries. In some areas the losses due to this disease are quite severe [Cunfer, 1988; Schaad and Forster, 1985].

Bacteriological characteristics and host range are employed to identify phytopathogenic bacteria at the pathovar level [Dye *et al.*, 1980]. Five pathovars of *Xanthomonas campestris*, are known as the causal agents of bacterial leaf streak of cereals and grasses [Buchanan *et al.*, 1974]. *X. campestris* pv. *hordei* and *cerealis* were reported as the causal agents of bacterial leaf streak on barley and wheat in south-east of Iran [Alizadeh and Rahimian, 1989].

The aim of the present investigation was to determine the distribution of bacterial leaf streak of cereals, to identify the causal agents at the pathovar level from physiological and biochemical characteristics and host range, and to characterize the natural hosts of these bacteria in Iran.

Materials and methods

The infected cereals and grasses were collected from south-eastern, central and western regions of Iran. Single colonies were isolated from infected leaves and purified as previously described by Alizadeh and Rahimian [1989]. Morphological, biochemical and physiological tests, necessary

for bacterial identification were carried out, as described by Lelliott *et al.* [1987] and Schaad [1980]. The sensitivity to 22 antibiotics was tested using antibiotic discs (Difco) on plates of NA. Hypersensitive reaction of tobacco plant to all isolates were assayed as described by Klement [1964].

Bacterial suspensions of approximately 5×10^6 colony-forming units per mL sterile distilled water (CFU/mL SDW) were infiltrated into the first leaf stage barley (cv. Khazar), wheat (cv. Roshan), rye and oat (French local cultivar) seedlings and 20–25 days-old seedlings of *Agropyron elongatum, Bromus inermis*, and *Lolium multiflorum*, using the partial vacuum pump technique [Boosalis, 1950]. *Sclerochloa dura, Aegilops cilindrica* and *Heteranthelium* sp. were also grown and inoculated with the isolates obtained from these species. About 4 days after inoculation, water-soaking symptoms on the inoculated plant leaves were considered as positive pathogenicity reaction, and chlorosis or no symptoms as negative pathogenicity reaction.

Results and discussion

The symptoms of the disease were observed in barley and wheat fields on barley, wheat, *Bromus* spp., *Hordeum* spp., *Lolium* spp., *Aegilops* spp., *Sclerochloa dura* and *Heteranthelium* sp. The bacteria isolated from infected leaves yielded numerous small, yellow, convex, smooth, circular colonies on NA plates. All isolates had a single polar flagellum and were Gram-negative, oxidase negative, catalase positive, with alkaline reaction on lithmus milk. They were aerobic, grew at 36 °C and produced hydrogen sulphide (SH2) from cystein and peptone and reducing substances from sucrose, giving a positive reaction for gelatin, casein and aesculin hydrolysis. Isolates IBLS1, IBLS8, and IBLS16 weakly hydrolysed gelatin and casein. Arginine dihydrolase, urease, nitrate reduction, acetoin and indole production and MR-VP reaction were negative. The results for acid production from 28 carbohydrates and utilization of 12 organic acids were similar to those previously reported for other *X. campestris* by Alizadeh and Rahimian [1989]. All isolates displayed the tobacco hypersensitive reaction within 24 h.

Table 1 shows that some characteristics of the bacterial isolates, for example lecithin or T80 hydrolysis were variable. All isolates hydrolysed T60 and T20 similarly, but when T80 was hydrolysed, some of them (especially barley isolates) produced a large turbid white zone with small white crystals, whereas the others (wheat and grasses isolates) developed a relatively narrow milky zone of precipitate around the colonies. The tolerance of some isolates of NaCl was higher. All isolates produced slime on the NAS and NAG media, but not similarly. The utilization of α and β-lactose was a discriminative test for separating the wheat isolates from the others. 11 out of the 15 barley isolates hydrolysed starch after three weeks' incubation. The sensitivity of the isolates to 22 antibiotics was studied, but there was no discriminative antibiotic for differentiating the isolates. However, wheat and grasses isolates were more susceptible than the barley isolates to chloramphenicol 30 µg. The susceptibility degree of all isolates to some antibiotics was similar to that reported for other *X. campestris* isolated by Moffet *et al.* [1973] and Alizadeh and Rahimian [1989]. All isolates were moderately susceptible to ticarcillin 75 µg + clavulanic acid 10 µg, amoxicillin 200 µg + clavulanic acid 10 µg. They had very low susceptibility to bacitracine 10 units, carbenicillin 100 µg, gentamycin 10 µg, and vancomycin 30 µg. All isolates were not susceptible to lincomycine 2 µg, cephalothin 30 µg, cefazoline 30 µg and sulfamides 300 µg.

From these results all isolates were identified as *X. campestris* and two groups were characterized from the discriminative tests mentioned above: one group included nearly all barley isolates and the other one almost all wheat and grasses isolates. Bacteriological characteristics alone did not provide decisive criteria in the identification of *X. campestris* pathovars [Dye *et al.*, 1962]. Distinguishing between the different pathovars of *X. campestris* also requires the determination of their host range [Dye *et al.*, 1980].

All isolates from barley (except IBLS11 and IBLS12) and isolate IBLS40 (barley group) were pathogenic only to barley. Within 4–6 days after inoculation, water-soaked spots or stripes appeared on barley leaves inoculated with barley group isolates (Fig. 1a), but no reaction or only small

Table 1. Xanthomonas campestris isolates from cereals and grasses in Iran

Isolates	Original host	Pathovar	Pathogenicity		Lecithine[b]	Lactose[c]	T80[d]	NaCl%	Slime[e]	Starch[f]
			wheat	barley[a]						
IBLS 1	barley	*hordei*	-	+	+	+	++	3	+	-
IBLS 2	barley	*hordei*	-	+	+	+	++	3	+	+
IBLS 3	barley	*hordei*	-	+	+	+	++	3	+	+
IBLS 4	barley	*hordei*	-	+	+	+	++	3.5	+	+
IBLS 5	barley	*hordei*	-	+	+	+	++	3	+	+
IBLS 6	barley	*hordei*	-	+	+	+	++	3	+	-
IBLS 7	barley	*hordei*	-	+	+	+	++	3	+	+
IBLS 8	barley	*hordei*	-	+	+	+	++	3	+	+
IBLS 9	barley	*hordei*	-	+	+	+	++	3	+	+
IBLS 10	barley	*hordei*	-	+	+	+	+	3.5	+	+
IBLS 11	barley	*cerealis*	+	+	-	+	+	3	++	-
IBLS 12	barley	*cerealis*	+	+	+	+	+	2.5	++	-
IBLS 13	barley	*hordei*	-	+	+	+	++	3	+	-
IBLS 14	barley	*hordei*	-	+	+	+	++	3	++	+
IBLS 15	barley	*hordei*	-	+	+	+	++	3.5	++	-
IBLS 16	barley	*hordei*	-	+	+	+	++	3	+	+
IBLS 17	wheat	*cerealis*	+	+	-	-	+	2.5	++	-
IBLS 18	wheat	*cerealis*	+	+	-	-	+	2.5	++	-
IBLS 19	wheat	*cerealis*	+	+	-	-	+	2.5	++	-
IBLS 20	wheat	*cerealis*	+	+	-	-	+	2	++	-
IBLS 21	wheat	*cerealis*	+	+	-	-	+	2	++	-
IBLS 22	wheat	*cerealis*	+	+	-	-	+	2	+	-
IBLS 23	wheat	*cerealis*	+	+	-	-	+	2	++	-
IBLS 24	wheat	*cerealis*	+	+	-	-	+	2.5	++	-
IBLS 25	wheat	*cerealis*	+	+	-	-	+	2.5	++	-
IBLS 26	wheat	*cerealis*	+	+	-	-	+	2.5	+	-
IBLS 27	wheat	*cerealis*	+	+	-	-	+	2.5	++	-
IBLS 28	wheat	*cerealis*	+	+	-	-	+	2.5	++	-
IBLS 29	wheat	*cerealis*	+	+	-	-	+	2.5	++	-
IBLS30	wheat	*cerealis*	+	+	-	-	+	2.5	++	-
IBLS 31	wheat	*cerealis*	+	+	-	+	+	2.5	++	-
IBLS 32	*Aegilops* sp.	*cerealis*	+	+	-	+	+	2.5	++	-
IBLS 33	*A. ventricosa*	*cerealis*	+	+	-	+	+	2.5	++	-
IBLS 34	*A. cylindrica*	*cerealis*	+	+	-	+	+	3	++	-
IBLS 35	*A. cylindrica*	*cerealis*	+	+	-	+	+	2	++	-
IBLS 36	*Bromus* sp.	*cerealis*	+	+	-	+	+	3	++	-
IBLS 37	*B. tectorum*	*cerealis*	+	+	-	+	+	2.5	++	-
IBLS 38	*Hordeum maritimum*	*cerealis*	+	+	-	+	+	2.5	++	-
IBLS 39	*Hordeum maritimum*	*cerealis*	+	+	-	+	+	2.5	++	-
IBLS 40	*Hordeum* sp.	*cerealis*	-	+	+	+	++	3	+	+
IBLS 41	*Lolium strictum*	*hordei*	+	+	-	+	+	2.5	++	-
IBLS 42	*Lolium strictum*	*cerealis*	+	+	-	+	+	2.5	++	-
IBLS 43	*Sclerochloa dura*	*cerealis*	+	+	-	+	+	2	++	-
IBLS 44	*Heteranthelium* sp.	*cerealis*	+	+	-	+	+	2.5	++	-
		cerealis								

IBLS = Iranian Bacterial Leaf Streak of cereals and grasses.

[a] Negative (-) and positive (+) pathogenicity reaction or immune (-) and susceptible (+) reaction.

[b] Absence (-) and presence (+) of lecithinase.

[c] No acid (-) and acid (+) production from α and β-lactose.

[d] Weak T80 (+) hydrolysis [production of a narrow (ca. 1.5 mm wide) zone of precipitate around the colonies], strong (++) T80 hydrolysis [production of a large (2 - 3.5 mm wide) zone of small white crystals of precipitate around the colonies].

[e] Low (+) and high (++) amount slime production.

[f] No hydrolysis (-) and hydrolysis (+) of starch after three weeks' incubation.

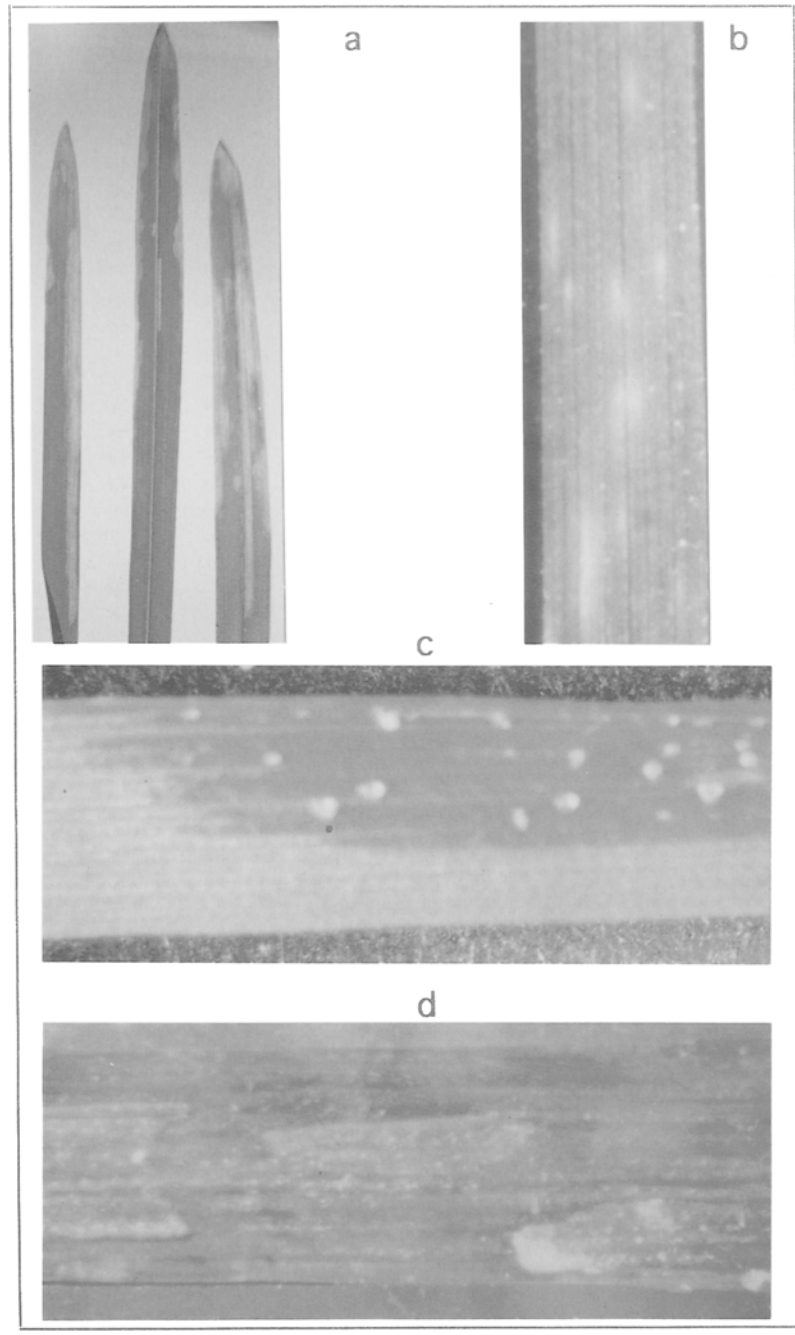

Fig. 1. Symptoms on barley and wheat leaves artificially contaminated with IBLS3 and IBLS22. Water soaked streaks on barley leaves contaminated with IBLS3 (a). Immunity reaction of wheat seedling to IBLS3 (b). Dry yellow granules (c) and translucent layers (d) of bacterial exudates on wheat leaves two weeks after inoculation.

chlorotic spots were observed on wheat (Fig. 1b) and grass leaves inoculated with barley isolates. The water soaking symptom appeared on barley, wheat, rye, *Agropyron elongatum, Bromus inermis*, and *Lolium multiflorum* inoculated with wheat group, including all isolates from wheat, two isolates from barley (IBLS11 and IBLS12), and all isolates from grasses (except IBLS40). Oat

leaves inoculated with wheat and barley isolates showed red chlorosis lesions or no symptoms, indicating that none of the strains could infect oat seedlings. The water-soaked spots on barley and wheat leaves inoculated with barley and wheat group isolates enlarged into translucent streaks with dry and yellow granules (Fig. 1c) or with thin, transparent layers of bacterial exudates on the surface of the leaves within 5 days (Fig. 1d). Isolates IBLS32, IBLS33, IBLS34 and IBLS35 were also pathogenic to *Aegilops cylindrica* and isolates IBLS43 and IBLS44 were pathogenic to *Sclerochloa dura* and *Heteranthelium* sp., respectively.

On the basis of the bacteriological characters and host range data presented above, the isolates of the barley and wheat groups were identified as *X. campestris* pvs. *hordei* and *cerealis*, respectively.

Among the natural hosts reported in this study, three species of graminaceous plants, *Aegilops* sp., *Sclerochloa dura*, and *Heteranthelium* sp. are reported for the first time as hosts of *X. campestris* pv. *cerealis*. According to the literature, the natural and artificial hosts of the different pathovars of *X. campestris* causing bacterial leaf streak in cereals are limited to some species of *Triticum, Hordeum, Bromus, Agropyron, Secale, Avena, Dactylis, Oryza, Echinochloa, Phleum* and triticale (Bradbury, 1986). Since Iran is in the region of the genetic source of graminaceous plants, these bacteria may also attack other grasses which occur within or near the cereal fields in Iran, such as the three genera of grasses presented above as new hosts of the pathovar *cerealis*.

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