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Below are reasons why a general designation for *Xanthomonas* *oryzae* (all pathovars) should NOT be included in the USDA select agent list

Despite the likelihood that they have been introduced into the USA previously, *Xanthomonas oryzae* pathovars from other countries have NOT established in the USA. Rice was introduced for cultivation into the USA (North Carolina) more than 200 years ago and has been cultivated in other parts of the US for over 100 years. Although many rice diseases have either been introduced or developed on rice during the history of its cultivation in the US, the two well-characterized pathovars of *X.* oryzae, *X. o.* pv. *oryzae* and *X. o.* pv. *oryzicola,* have **not established** in the US. There is a report that a rice bacterial disease occurred in Texas and Louisiana, and that the causal agent was a low virulence strain of *X. oryzae* pv. *oryzae* (formerly called *X. campestris* pv. *oryzae*) (Jones 1989). However, later reports suggested that the US strains are likely related to *X. oryzae,* but are NOT pv. *oryzae* or pv. *oryzicola*. Furthermore, my laboratory used high throughput genome sequencing to compare sequences of two US strains with the sequences of the Asian and African *X. oryzae* (see point B below). **We have found that the US strains, although grouping into *X. oryzae,* are genetically distinct from the other *X. oryzae* pathovars *(X. o.* pv. *oryzae* and *oryzicola)*. Based on this comparative genome information and the fact that the US strains cause very little disease on rice, we are proposing that the US strains be grouped into a new subspecies (Triplett et al., in preparation).**

A. There are likely several reasons for the lack of establishment of bacterial blight and bacterial leaf streak diseases of rice in the US, and these reasons are powerful arguments why constraining research on *X. oryzae* pathovars, in general, is not reasonable:

1) **The climates of rice producing areas in the US are not conducive to long-term survival of *X. o.* pvs. *oryzae* and *oryzicola*.** The diseases caused by these pathovars are favored by warm temperatures (25-35C), high humidity, and frequent rainfall (Ou 1985). In fact, bacterial leaf streak (caused by *X. oryzae* pv. *oryzicola*) is NOT found in temperate climates; it is only found in tropical Asia (Ou 1985; Webster 1992). Within the US, California conditions are too dry (low humidity and low rainfall) for disease development by either pathogen. Conditions in the south-central US rice production areas may be conducive within the growing season, but the cold winters in combination with commonly used rotation and tillage practices (see below) would prevent season

to season survival. **Risk of long term survival of *X. oryzae* pvs. *oryzae* and *oryzicola*: low to moderate**

2) **Persistence of the pathogens would be negligible in the US rice systems.** *X. oryzae* pvs. *oryzae* and *oryzicola* do not survive well in soil or irrigation water. Given that rice in the US is rotated and that only one crop per year is grown, the persistence of the pathogen in fields would be low. Although the pathogen can survive in weed hosts located in the field (*X. oryzae* pv. *oryzae* infects a limited number of weed hosts), winter temperatures would hinder survival, particularly if rotation or conservation tillage practices are applied (Mizukami 1969). Conservation tillage practices in the southern US growing regions, which involve preparation of soil in the autumn prior to spring planting, controlling weeds and volunteer rice with herbicides, then drilling in the rice seed in the spring, would prevent establishment of the pathogen. In sum, tillage, crop rotation, and winter temperatures would greatly reduce survival of inoculum in the field. **Risk of persistence: low**

3) **Potential spread of the *X. oryzae* pvs. *oryzae* and *oryzicola* would be limited.** The seedling stage of rice is the most vulnerable to infection (Ou 1985). In Asia, where rice seedlings are transplanted from seed beds to the field, the pathogens can be efficiently introduced into wounds created during the transplant process. Field to field spread of the pathogens in Asia can occur by strong winds/driving rains produced in typhoons and through distribution on seed. Movement between paddies can occur in irrigation waters, but survival for extended times in irrigation water is low. **US rice cultivation practices and environments are not conducive to spread of and infection of rice by the bacterial blight or bacterial leaf streak pathogens.** In the US, rice is directly seeded, which avoids the damage during transplanting. For example, in California rice is seeded by airplane with pre-germinated seeds dropped directly into the water. The water temperature is low, which is not favorable for bacterial blight development. If an infestation occurred, hurricanes in the southern US production areas could move the pathogens between plants and fields in a single season. However, the lack of a continuous crop, conservation tillage practices (above) and the winter climates would drastically reduce the likelihood of establishment of the pathogen. **Risk: low to moderate**.

Conclusion to A: *X. oryzae* pathovars are of low risk to US agriculture, and do not warrant raising research containment levels beyond those imposed for quarantine research. Risk of escape of sufficient inoculum from research laboratories to allow even a single season disease problem is minimal. Containment beyond that already required for research with this pathogen is unwarranted.

B. **A bacteria related to *Xanthomonas oryzae*, but distinct from pvs. *oryzae* and *oryzicola* already exists in the USA, and does not cause significant disease problems.** In 1989, Jones et al reported the occurrence of *X. oryzae* (at that time called *X. campestris* pv.*oryzae*) in rice in Texas and Louisiana (Jones 1989). This same pathogen was detected in symptomless plants of the perennial weed species *Leersia hexandra* (clubhead cutgrass), suggesting that the weed could act as an alternate or even the primary host (Gonzalez, Xu et al. 1991). Strains of *X. c. oryzae* isolated from symptomless *L. hexandra* caused very mild blight symptoms in rice. In artificially inoculated *L. hexandra,* the pathogen multiplied without evidence of disease. When asked if the disease/pathogen were still a problem in Texas, Dr. Carlos Gonzalez (Texas A&M University) replied, "*No, we have not seen the disease since they stopped planting Lemont and related susceptible cultivars*." Because the weed species still harbors the pathogen, the bacterium is present, but there is no susceptible rice host planted, so no problem. Additionally, there are no further reports on the occurrence of the disease in rice after the initial reports.

In the above and subsequent studies, the US strains were shown to be distinct from Asian strains in several ways. First, the virulence was low compared to Asian strains (Jones 1989). Second, restriction fragment length polymorphism analysis using repetitive DNA elements common to *Xanthomonas* as probes showed that the US strains had many fewer copies of the repetitive elements than the Asian strains(Jones 1989). Third, a set of monoclonal antibodies generated to Asian strains *X. o.* pv. *oryzae* distinguished between the US and Asian isolates (Benedict, Alvarez et al. 1989). Fourth, based on later studies using Restriction Fragment Length Polymorphism analyses, the US strains were shown to be **distinct from *X. o.* pvs. *oryzae or oryzicola***, and were suggested to be a **new pathovar of *X. oryzae*** (Leach 1991; Ryba-White, Notteghem et al. 1995).

Whole genome comparisons of all *Xanthomonas* sequences available in public databases, including three strains of *X. oryzae* pv. *oryzae* and one strain of *X. o.* pv. *oryzicola*, were used to develop a set of PCR primers that distinguish (1) *X. oryzae* from other *Xanthomonas* species, (2) *X. o.* pv. *oryzae* from *X. o.* pv. *oryzicola*, and (3) *X. o.* pv. *oryzicola* from *X. o.* pv. *oryzae* (Lang, Hamilton et al. 2010). **No other pathovars of *Xanthomonas*** amplify with the primer sets chosen. The US strains amplify with only one of four robust *X. oryzae* species-specific primers, suggesting they **are** related to *X. oryzae.* The US strains did **not** amplify with four *X. o. oryzicola* specific primer pairs; thus, they are not *X. o. oryzicola*. US strains amplify with one of four primer pairs specific to *X. oryzae* pv. *oryzae.* This evidence is consistent with earlier speculations that the **US strains are very different from Asian and African strains of *X. oryzae* pv. *oryzae* or *oryzicola*.**

Most recently, my laboratory used high throughput genome sequencing to compare sequences of two US strains with the sequences of the Asian and African *X. oryzae*. **We have found that the US strains, although grouping into *X. oryzae,* are genetically distinct from *X. oryzae* pathovars *oryzae* and *oryzicola* (Triplett et al., in preparation).** There are many key differences: as examples, the US strains do NOT have TAL effectors (virulence genes common to both pvs. *oryzae* and *oryzicola)*, and they have different complements of mobile genetic elements. Based on this comparative genome information and the fact that the US strains cause very

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little disease on rice, **we are proposing that the US strains be grouped into a new subspecies.**

Given all of the above information, to list and exclude 'all pathovars' of *X. oryzae* from the US would be impossible.

C. **Selection of *X. oryzae* pathovars, which are fairly fastidious and mutable, for malicious intentions is unlikely.** Both pathogens are fairly fastidious, particularly in liquid cultures. The pathogens do not survive well in water and are fairly highly mutable, meaning that if caution is not taken, pathogenicity is lost in continuous culture. **Risk of use for malicious intent: low**

D. If we eliminate the study of pathogen/host systems that are not in the US at present, our ability to implement crop protection means in response to disease outbreaks will be severely limited. Therefore, continued public research into the interaction of *X. oryzae* pv. *oryzae* and *X. oryzae* pv. *oryzicola* with rice is a critical component of US biosecurity.

Overall Conclusion: *X. oryzae* pathovars are of low risk to US agriculture, and do not warrant raising research containment levels beyond those imposed for quarantine research. Risk of escape of sufficient inoculum from research laboratories to allow even a single season disease problem is minimal. Containment beyond that already required for research with this pathogen is unwarranted.

Case studies of relevance:

Although we have not had the ability to study the persistence and spread of *X. oryzae* strains in the US, we can learn from a case study in the Philippines. In the late 1970's, race 6 (type strain PXO99) was first found in one location in Los Baños, Philippines (Mew, Vera Cruz et al. 1992). The race 6 strains were virulent to all of the bacterial blight resistance genes known at that time, and, importantly, were virulent to the predominantly used resistance gene in the Philippines at that time, Xa4 (Mew 1987; Mew, Vera Cruz et al. 1992; Adhikari, Vera Cruz et al. 1995). The race 6 strains were as aggressive to the rice as any of the Philippine isolates. Interestingly, race 6 has not been isolated from field sites again despite comprehensive sampling in the area where the strains were originally found and throughout the island of Luzon (Nelson, Baraoidan et al. 1994; Ardales, Leung et al. 1996; Vera Cruz, Ardales et al. 1996)(C. Vera Cruz, personal communication).

Adhikari et al. (Adhikari, Vera Cruz et al. 1995) used RFLP analysis with multiple probes to show genome relationships of *X. oryzae* pv. *oryzae* strains isolated throughout Asia. They demonstrated that Philippine race 6 strains were different from other Philippine strains, and they did not cluster with other Philippine strains. Instead, the race 6 strains grouped with strains from India and Nepal. Given that the race 6 strains are virulent to most bacterial blight resistance genes (Mew 1987), and that they are aggressive to the genetic background of rice used in the Philippines (Mew, Vera Cruz et al. 1992), it is

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intriguing that the race never emerged as important in the Philippines, and did not spread beyond the initial site. This case study shows that an isolate may be aggressive to local germplasm and virulent to various resistance genes, but this does not mean it is fit to establish in any environment.

This influence on other fitness factors in the establishment of pathogens is further supported by the maintenance of distinct lineages of *X. o.* pv. *oryzae* in distinct geographic environments (Leung, Nelson et al. 1993; Adhikari, Vera Cruz et al. 1995; Leach, Leung et al. 1995). Furthermore, the distribution of lineages in different environments on the island of Luzon in the Philippines is consistent with particular lineages of *X. o.* pv. *oryzae* being constrained by environment. Ardales and colleagues (Ardales, Leung et al. 1996) sampled 13 sites along a 310 KM transect spanning indigenous rice growing areas in the mountainous region and the improved irrigated agroecosystems in the lowlands of the island of Luzon. They demonstrated geographic differentiation between pathogen populations in different agroecosystems. For example, race 5 (cluster A) was found only in the highland areas of Ifugao.

These two cases support our contention that *X. oryzae* pathovars introduced from other parts of the world would not likely establish readily in the US agroecosystem. They point out that many factors influence the survival, persistence, and ability to spread of a pathogen, including the environment (temperature, humidity, etc), the cultivation practices, the genetic background of the host, nutrient and water management, cultural practices, and presence of alternate host.

Admittedly, there are many gaps in our knowledge about the epidemiology of *X. oryzae*, particularly in the US temperate agroecosystems. We do not know much about the native, very weakly virulent *X. oryzae* that persists on weed hosts common to the rice paddies. However, these **native** *X. oryzae* strains have been around for over 15 years, and have not poised a risk to US rice production.

Sincerely,

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University Distinguished Professor

LITERATURE CITED

Department of Bioagricultural
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Department of Bioagricultural
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Department of Bioagricultural
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