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REVISION

PINK DISEASE, A REVIEW OF AN ASYMPTOMATIC BACTERIAL DISEASE IN PINEAPPLE

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ABSTRACT-Pink disease is an asymptomatic pineapple disease in the field and is evidenced with a red-dark coloration when the infected fruit is processed to obtain products such as juice, jam, and preservatives. *Tatumella morbirosei* and *T. ptyseos* (formerly *Pantoea citrea*) have been demonstrated as causal agents. Although *T. morbirosei* and *T. ptyseos* have been well studied, there are currently no cost effective control methods in pineapple cultivation. The purpose of this review is to summarize the significant and updated research on the role of pink disease in pineapple.
Index terms: pineapple, phytopathogen, *gdhB*, biocontrol.

DOENÇA ROSADA, UMA REVISÃO DE UMA DOENÇA ASSIMTOMÁTICA BACTERIANA DO ABACAXI

RESUMO - Doença rosada ou Pink disease é uma doença assintomática do abacaxi no campo, ~~o~~que ~~é~~ evidencia com uma coloração vermelho - escura, quando a fruta infectada é processada para a obtenção de produtos como suco, geléia, e conservantes. *Tatumella morbirosei* e *T. ptyseos* (antigo *Pantoea citrea*) ~~t~~são os agentes causáis dessa doença. Embora T. *morbirosei* e *T. ptyseos* ~~foram~~tenham sido bem estudadas, até o presente não existem métodos eficientes de controle da Pink disease de abacaxi. O objetivo da presente revisão é sumarizar as pesquisas significativas e atualizadas ~~pesquisa~~sobre o papel de Pink disease no cultivo do abacaxizeiro.
Termos para indexação: abacaxi, fitopatógeno, *gdhB*, biocontrole.

INTRODUCTION

Pink disease is an important global threat to pineapple production, especially for the fruit industry. The reported causal agents are *Tatumella morbirosei* (formerly *Pantoea citrea*) and *Tatumella ptyseos*, both belonging to the *Enterobacteriaceae* (CHA et al.,1997a; MARIN-CEVADA et al., 2010). Pink disease has been reported from Australia, Hawaii, Mexico, the Philippines, South Africa, and Taiwan (MARÍN-CEVADA et al., 2006; ROHRBACH, 1983). Cost-effective management strategies for effective control of the disease are still not available. The current schemes consist mainly of insect control, since it has been supposed that insects play a role in the transmission of the pathogen (KADO, 2003). Additionally, plant breeding for obtaining resistant cultivars might be an alternative to overcome the problem (Kado, 2003).

Economic Status of Pineapple

The tropical fruits most cultivated in the world are bananas, mangos, avocados, papayas and pineapples (FAO 2003) with an estimated total worldwide production of about 160 million tons in 2012 (http://faostat3.fao.org/download/Q/QC/E).

About 98% of tropical-fruit production is taking place in developing countries and 80% of the world import trade for these fruits is directed to developed countries (http://fao.org/docrep/006/y5143e/y5143e1a.htm). Pineapple had a worldwide gross production valued at $17 billion in 2012 (http://faostat3.fao.org/download/Q/QV/E).

A large amount of the pineapple trade consists of processed products, such as canned slices and juice (BARTHOLOMEW et al., 2003). Thailand, Brazil, the Philippines, Indonesia, and Costa Rica are the major producers of fresh fruit pineapples, and Costa Rica ranked as the major world pineapple exporter

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in 2011 with 1.75 million metric tons (MMT) (http://faostat3.fao.org/faostat-gateway/go/to/browse/T/TP/E). The world production of canned pineapples has shown a tremendous increase over the last decade or so (http://faostat3.fao.org/faostat-gateway/go/to/browse/Q/QC/E) (Table 1). Similarly, the production of pineapple juice has showed a notable increase in the last years (http://faostat3.fao.org/faostat-gateway/go/to/browse/Q/QC/E) (Table 1).

The pineapple plant [*Ananas comosus* (L.) Merr. var. *comosus*] is widely distributed in the New World and shows a great adaptability to ~~varied~~several habitats. Pineapple is one of the most ancient American crops and was domesticated and dispersed several thousand years ago by Amerindian peoples in the Orinoco and Amazon basins (COLLINS, 1960). This plant is a member of the Bromeliaceae family, which contains about 58 genera and 3352 species (LUTHER, 2012). “Smooth Cayenne” “Red Spanish”, “Queen”, “Abacaxi”, and “MD2” are the principal pineapple grown cultivars (Bartholomew et al., 2003; LOEILLET et al.; 2011 UNCTAD http://www.unctad.info/en/Infocomm/AACP-Products/COMMODITY-PROFILE-Pineapple/).

The productivity of the pineapple depends on the control of factors such as water and nutrient supply (nitrogen, potassium, phosphorus, calcium, iron, magnesium, zinc, boron and other micronutrients), as well as management and control of pests and diseases (ZHANG et al., 1997).

Pink disease of pineapple

The pineapple plant is threatened by several phytopathogenic bacteria that are responsible for diseases like fruit collapse, marbling disease, fruit brown rot, anomalous proliferations and pink disease (Table 2),(JOHNSTON 1957;LIM;LOWINGS 1979; KORRES et al. 2010;SERRANO 1928;DAVIS ET AL. 2005;DAVIS et al. 2006; LYON 1915).

. Among them, pink disease is of great importance because of its potential impact on the postharvest processes (CHA et al., 1997b). Pasteurized fruits affected by this disease produce brownish pigments, so the commodity becomes unmarketable (ROHRBACH, 1989; ROHRBACH; PFEIFFER, 1975) (Fig. 1). The challenge in the control of this disease is that infected plants remain asymptomatic in the field, making difficult to discriminate healthy from affected fruits (Pujol and Kado 1999). Unfortunately, cans of healthy fruit from the same lot of infected fruits are also commonly discarded by quality control, increasing the economic losses (CHA et al., 1997b).

Causative agents of Pink Disease

It has been suggested that this disease is caused by microorganisms of the families *Acetobacteraceae* and *Enterobacteriaceae* (BUDDENHAGEN; DULL,1967; GOSSELE;SWINGS, 1986; KONTAXIS; HAYWARD, 1978; ROHRBACH; PFEIFFER, 1976). Almost four decades ago, an isolate obtained from a diseased fruit, did not show perceptible symptoms when it was inoculated in fresh fruits but induced discoloration after heating the inoculated fruits (Rohrbach and Pfeiffer 1976). The authors identified that strain as belonging to the species *Enterobacter agglomerans* (*Pantoea agglomerans*, GAVINI, et al., 1989) (CHO, et al., 1980; ROHRBACH; PFEIFFER, 1976). Afterwards, *Pantoea citrea* was identified as another causative microorganism (CHA, et al., 1997a), also in the Family *Enterobacteriaceae*. A wide taxonomic analysis of *P. citrea* strains causing pink disease showed their proper classification as the ~~a~~ new species, *Tatumella morbirosei*, in a different G~~g~~enus of *Enterobacteriaceae* and not as a species of *Pantoea* (BRADY et al. 2008; 2010). Furthermore, isolates obtained from pink disease affecting Mexican pineapple fruits were identified as belonging to the species *T. ptyseos* (MARÍN-CEVADA et al. 2010). Hence, at least those two species of the genus *Tatumella* are capable to cause pineapple pink disease.

Epidemiology and other characteristics of pink disease

Seasonality is probably an important factor in the appearance of the disease, since warm and humid environments promote the appearing of the phytopathogen. In our previous research we detected the presence of *T. ptyseos* in 48% of pineapples in the rainy season (MARIN-CEVADA, et al., 2010), and only 3% in the dry season (unpublished results of our group). Besides, the average bacterial number decreased from 10^6 CFU/g of fruit to 10^2 CFU/g of fruit in the rainy and dry season, respectively (MARIN-CEVADA, et al., 2010). In other geographic regions, the incidence of pink disease increases also in the rainy season, February, March and April in Hawaii and Taiwan, and August and September in the Philippines (HINE, 1976; ROHRBACH; JOHNSON, 2003; ROHRBACH; PFEIFFER, 1975). Other biological or environmental factors might be required for the bacterial outbreak, since not all the wet seasons shown higher incidence of pink disease (HINE, 1976). There is no consensus with regard to the entry of the pathogen into the plant. It has been suggested that bacterial cells come into contact with the nectar in the placental regions of flowers (ROHRBACK; PFEIFFER 1975). It has also been proposed that the bacteria involved in pink disease are transmitted to pineapple flowers by insects. The latter scenario is supported by the fact that spraying of crops with insecticide decreases the appearance of the disease (KADO, 2003). In addition, rain splash could disperse the pathogens from infected to healthy plants (HINE, 1976).

The biochemical basis of the pink discolouration in *T. morbirosei* was elucidated by CHA et al., (1997b); PUJOL;KADO (1999; 2000). It is noteworthy that a biochemical basis of pink disease had been suggested by Buddenhagen and Dull since 1967. The pigmentation of affected fruit is initiated by the oxidation of glucose to gluconate by glucose dehydrogenase GdhB. *gdhB* is induced by glucose in the stationary phase (PUJOL;KADO 1999). GdhB oxidizes gluconate to 2-keto-D-gluconate, which is subsequently converted to 2,5–diketogluconate by another dehydrogenase (PUJOL; KADO, 1999; 2000). It has been suggested that 2,5-diketogluconate can form complexes with plant compounds produced at canning temperatures, or may dimerize, producing the decolorization (CHA et al., 1997b; KADO, 2003). Alternatively, 2-keto-D-gluconate present in infected fruits might suffer an accelerated heat oxidation to 2,5-diketogluconate. *gdhA*, a gene for a second glucose dehydrogenase in *T. morbirosei,* is expressed constitutively at low levels and does not participate in the generation of pink disease pigmentation (Pujol and Kado 1999). GdhB possesses a putative binding site for pyrroloquinolinequinone (PQQ). PQQ could be synthesized endogenously in *T. morbirosei* since cell extracts show Gdh activity without adding an exogenous cofactor (Pujol and Kado 1999), and as a matter of fact, the *T. ptyseos* genome possesses at least one locus putatively implicated in PQQ biosynthesis (Acc. Num. WP_025901980).

Control

The application of insecticides in pineapple fields has been suggested to decrease the incidence of pink disease (KADO, 2003). Nevertheless, this practice could damage natural and human ecosystems and it is still unclear how insects and pink disease agents are related. Hence, it would be desirable to find alternative control methods including the production of resistant varieties and the use of biological control. In order to design suitable control and preventive measures, it is essential to get profound knowledge of all the elements of this disease. In combination with an insecticide, the saprophytic bacterium *Bacillus gordonae* have shown promising results for control of pink disease under laboratory and field conditions (KADO, 2003), but its effectiveness and economic benefits are yet to be evaluated.

Recently, our group searched for antagonists against *T. ptyseos*. Among dozens of isolates from pineapple plants, two of them that showed the greatest inhibitory activity in culture media against *T. ptyseos*, where selected for determining their activity in assays in association with plants (MARÍN-CEVADA et al., 2012). The antagonism assays were carried out in double-layer plates, in liquid cultures, and in micropropagated inoculated pineapple plantlets (cv. Smooth Cayenne). The numbers of *T. ptyseos* growing for 96 h in liquid co-culture with the isolate UAPS07070 were three orders of magnitude less thant when growing alone (Marin-CEVADA et al., 2012). The antagonistic bacterium was also able to abate the brownish pigmentation of culture medium produced by the pathogen in co-cultures (MARÍN-CEVADA et al., 2012). It is still unknown if that observation was caused by the drop of *T. ptyseos* population itself, or by an inhibition of the oxidation pathway. In 60 days old inoculated pineapple plantlets, the numbers of *T. ptyseos* were lower in co-inoculation with the isolate UAPS07070 than in lonely inoculation. Particularly, two orders of magnitude less inside the roots and three orders less in the rhizosphere (MARIN-CEVADA et al., 2012).

Bacterial exudates produced by isolate UAPS07070 inhibited the growth of *T. ptyseos* UAPS07007 in rich medium and also on rich solid medium saturated with $FeCl_3$ (Fig. 2). Since siderophores synthesis is inhibited under high Fe^{3+} concentrations, it seems unlikely that such compounds could be responsible for the antagonism between this isolate and *T. ptyseos*. By comparison of partial nucleotide sequences of 16S rRNA and *recA* genes, the antagonistic isolate was identified as *Burkholderia gladioli*, a bacterium included in the potential pathogens phylogenetic clade of this genus (SUÁREZ-MORENO et al., 2012). That cluster also includes the species of the *Burkholderia cepacia* complex, the "*pseudomallei*" group, and *B. glumae* among others. Hence, the likely pathogenic characteristics of *B. gladioli* UAPS07070 hampers its use as a biocontrol agent of pink disease. An alternative to the direct use of *B. gladioli* could consist of the isolated antagonistic substance, or the inoculation of non-pathogenic GMOs designed to synthesize it. Thus, it is crucial to determine the molecular mechanisms responsible for competition between pineapple bacteria.

TABLE 1- Production of canned pineapple and pineapple juice (in metric tons) (Source: FAOSTAT 2014; http://faostat3.fao.org/browse/Q/QC/E).

Canned Pineapple				Pineapple Juice			
Country	**2002**	**2012**	**increase (%)**	**Country**	**2002**	**2012**	**increase (%)**
Thailand	382 689	1 202 739	214.3	Thailand	140 000	440 000	214.3
The Philippines	404 930	827 541	104.4	The Philippines	184 059	153 443	-16.6
Indonesia	138 008	375 069	171.8	Indonesia	46 002	144 257	213.6
Brazil	156 509	190 820	21.9	Kenya	95 537	68 369	-28.4
Kenya	238 842	170 924	-28.4	Brazil	60 196	86 736	44.1
China	125 000	130 000	4.0	Costa Rica	11 700	53 400	356.4
India	64 900	80 083	23.4	China	50 000	52 000	4.0
Malaysia	37 440	62 400	66.7	India	23 600	29 121	23.4
Costa Rica	10 290	53 400	418.9	Angola	4 052	28 000	591.0
Viet Nam	18 690	27 000	44.5	Malaysia	14 400	24 000	66.7
World	1 750 777	3 341 179	90.8	World	741 998	1 197 992	61.5

TABLE 2- Pineapple bacterial diseases.

Disease	Causal agent	Reference
Bacterial heart rot	*Dickeya chrysanthemi*	Johnston 1957
Bacterial fruit collapse	*Dickeya chrysanthemi*	Lim and Lowings 1979
	Klebsiella sp. plus yeasts	Korres et al. 2010
Marbling disease	*Acetobacter peroxydans* and *Pantoea agglomerans*	Rohrbach and Johnson 2003
Fruit brown rot	*Pantoea ananatis*	Serrano 1928
Shoot proliferation	Ca. *Phytoplasma asteris*	Davis et al. 2005
Proliferation of axillary buds	Ca. *Phytoplasma solani*	Davis et al. 2006
Pink disease	*Tatumella morbirosei*, *T. ptyseos*	Lyon 1915; Brady et al. 2008; 2010; Marin-Cevada et al. 2010

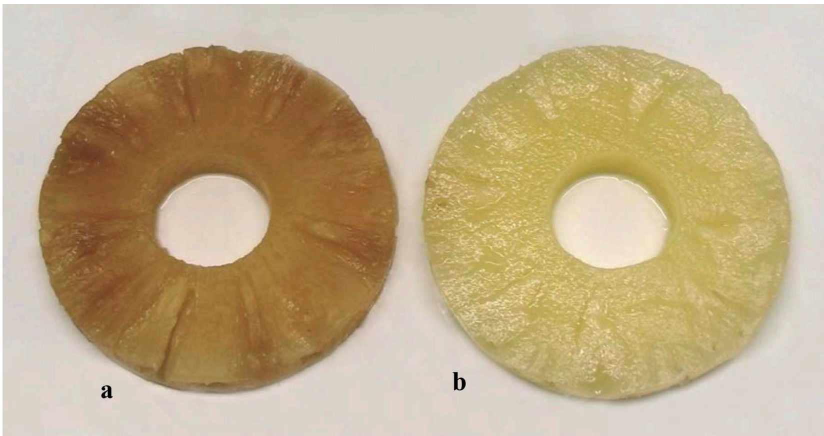

FIGURE 1 - Canned pineapple affected (a) compared to unaffected (b) by pink disease.

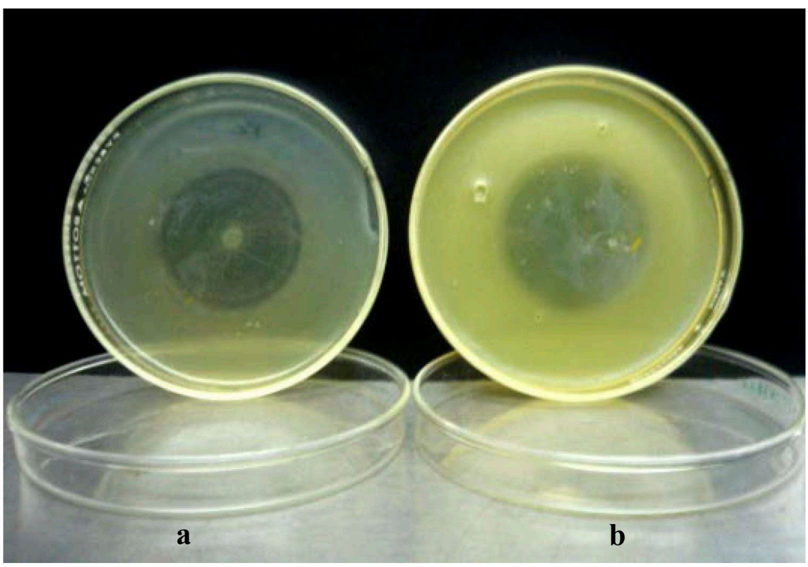

FIGURE 2- Antagonistic activity in solid media.

CONCLUSIONS

The understanding of interactions between pineapple, *Tatumella*, *B. gladioli*, other potential antagonists, and the environment is imperative to design preventive and control strategies for pink disease. Comparative genomic studies of the causal agents and of their antagonists might reveal genes involved in virulence and genes involved in antibiosis. Additionally, the search for new pineapple genotypes that are more resistant to pink disease could greatly reduce the economic impact of this disease in pineapple market.

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