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ANTAGONISM OF YEASTS TO *XANTHOMONAS CAMPESTRIS* PV. *CAMPESTRIS* ON CABBAGE PHYLLOPLANE IN FIELD

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ABSTRACT

Twenty yeast isolates, obtained from cabbage phylloplane, were evaluated for antagonistic activity against *Xanthomonas campestris* pv. *campestris*, in field. Plants of cabbage cv. Midori were pulverized simultaneously with suspensions of antagonists and pathogen. After 10 days, plants were evaluated through percentage of foliar area with lesions. Percentage of disease severity reduction (DSR%) was also calculated. Yeast isolates LR32, LR42 and LR19 showed, respectively, 72, 75 and 79% of DSR. These antagonists were tested in seven different application periods in relation to pathogen inoculation (T_1=4 d before; T_2=simultaneously; T_3=4 d after; T_4=4 d before + simultaneously; T_5=4 d after + simultaneously; T_6=4 d before + 4 d after; T_7=4 d before + simultaneously + 4 d after). The highest DSRs were showed by LR42 (71%), LR42 (67%), LR35 (69%) and LR19 (68%) in the treatments T_7, T_4, T_5 and T_6, which significantly differed from the others. The same yeast antagonists were also tested for black rot control using different cabbage cultivars (Fuyutoyo, Master-325, Matsukaze, Midori, Sekai I and Red Winner). The DSRs varied from 58 to 61%, and there was no significant difference among cultivars.

Key words: *Xanthomonas campestris* pv. *campestris*, cabbage, biocontrol, yeasts

INTRODUCTION

Xanthomonas campestris pv. *campestris* (Pammel) Dowson (Xcc) causes black rot of crucifers, one of the most destructive diseases of cruciferous crops worldwide. It is present in all crucifer-producing regions where yield and quality losses may be very high (31). Black rot can appear on plants at any growth stage. On young plants, margins of cotyledons turn black and may drop off. On mature leaves, symptoms appear along leaf margins as yellow, V-shaped lesions, with the base of the V usually directed along a vein. As the lesions expand toward the base of the leaf, the tissue wilts and eventually becomes necrotic. The infection may move down to the vascular tissue of the petiole and spread up or down the stem of the plant and into roots. The presence of black veins in yellow lesions

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along leaf margins is diagnostic of black rot (13). Disease control usually consists of using resistant cultivars, certified seeds, hot-water treatment of seeds followed by application of antibiotics or protectant fungicides, crop rotation, control of weeds and insects, and destruction of infected plants and debris in the field (13, 24). Nowadays, the use of resistant cultivars is the most economical method of control.

In addition, several microbial antagonists have been studied to improve control alternatives (1, 6). The use of yeasts as control agents of plant diseases is a strategy with great potential (11, 18, 20, 21, 28), mainly because of their ability to compete for nutrients (5, 9) and colonization sites (22).

Despite some research on the biological control of *X. campestris* pathovars in citrus (25, 19), clusterbean (26), cotton (30), mungbean (8,15) rice (14), and soybean (29), very little information exists on the biocontrol of black rot in crucifers (2, 3, 4). This work aims to study the potential of yeasts to control black rot on cabbage *(Brassica oleracea* var. *capitata* L.) in field.

MATERIALS AND METHODS

Yeast isolates

The epiphytic yeast isolates used in this study were obtained from leaves of cabbage collected from three nurseries in the State of Pernambuco, Brazil. From each plant, samples with five leaves were randomly taken. A total of 15 plants were sampled per nursery. The isolation followed the methodology of Melo *et al*. (20) with modifications. Ten discs of 12 mm diameter were removed per leaf, placed in a tube containing 10 ml of sterile tap water (STW), and sonicated for 10 min. Serial dilutions were made in STW and aliquots (0.1 ml) were plated on potato-dextrose-agar medium (PDA) supplemented with 250 mg.L^{-1} tetracycline. Plates were incubated for 48 h at 25°C, and one representative of each yeast colony morphology was transferred to a fresh PDA plate to obtain pure cultures, which were stored on the same medium at 4°C. Suspensions of antagonist yeast candidates were prepared in sterile distilled water (SDW) using 48 hour-old culture grown at 25°C on PDA (0.05% Tween 80 was added to the suspension).

The pathogen strain was obtained from the Bacterial Collection of Plant Bacteriology Laboratory, Agronomy Department/Federal Rural University of Pernambuco. Bacterial suspensions (10^8 CFU.ml^{-1}) were prepared in SDW using 48 hour-old culture grown at 30°C on NYDA medium (27).

Preliminary screening of yeast antagonistic to cabbage black rot

Twenty epiphytic yeast isolates were tested for their ability to reduce the severity of cabbage black rot under field conditions.

Seven week-old cabbage plants (cv. Midori) were simultaneously treated with antagonist candidates (10^6 cell.ml^{-1}) and inoculated with Xcc suspension (10^8 CFU. ml^{-1}). Sprays were performed until run-off. Controls were inoculated with Xcc but not treated with yeasts. Field conditions at the time of the inoculation were: temperature at 32±2°C and 87±3% of relative humidity.

The percentage of disease severity (DS%) was evaluated 10 days after inoculation by estimating the percentage of leafs with lesions areas. The percentage of disease severity reduction (DSR%) was calculated according to Edginton *et al.* (10): DSR(%) = [(DSc - DSt)/DSc)] x 100, where DSc = leaf area with lesions on the control plants and DSt = leaf area with lesions on the treated plants.

Influence of the application period of antagonistic yeasts on the control of cabbage black rot

In order to determine the optimum time for application of the antagonist, the four best black rot biocontrol agents were selected and tested as in the previous trial. Antagonist cell suspensions (10^6 cell.ml^{-1}) were sprayed at seven different periods in relation to inoculation with the pathogen suspension (10^8 CFU.ml^{-1}). The treatments were: T_1 = 4 days before; T_2 = simultaneously; T_3 = 4 days after; T_4 = 4 days before and simultaneously; T_5 = 4 days after and simultaneously; T_6 = 4 days before and 4 days after; T_7 = 4 days before, simultaneously and 4 days after.

Influence of the cabbage cultivar on the control of black rot by antagonistic yeasts

The four best black rot biocontrol agents were also tested as in previous trials, using six cabbage cultivars: Fuyutoyo, Master-325, Matsukaze, Midori, Sekai I and Red Winner. The concentrations of antagonist and pathogen suspensions were 10^6 cell.ml^{-1} and 10^8 CFU.ml^{-1}, respectively.

Statistical Analyses

In all experiments, four leaves per replicate were

used. Each treatment was replicated six times. The results were submitted to analysis of variance (ANOVA), and means were compared by Scott-Knott and Tukey's tests (P=0.05).

RESULTS AND DISCUSSION

As a result of the isolation procedure, 20 yeast isolates were obtained from cabbage. The low number of yeast isolates shows that plants in field are submitted to a high amount and diversity of pesticides, which reduce the epiphytic population of microorganisms. The same fact was observed by Michereff *et al.* (23) when studying yam phylloplane populations. According to Ghini (12), alterations on leaf surface and its environment could be caused by application of pesticides, hormones, fertilizers and also by pollution.

In the initial screening, disease severity reduction ranged from 24.2 to 78.6% for all 20 yeast isolates. Ten isolates had efficiency varying from 24.2 to 35.6%, four from 38.8 to 47.0, three from 57.7 to 60.2 and three from 72.3 to 78.6%. Among the isolates tested, LR19, LR42 and LR35 showed, respectively, 78.6, 75.5 and 72.3% of DSR. These data point the viability of using yeasts for Xcc biocontrol. Few complete studies had shown yeast activity against other plant pathogenic bacteria, such as, *Erwinia carotovora* subsp. *carotovora* on bell pepper fruits (20) and *E. amylovora* on pear flowers (21). The success of an antagonist introduced into the phyllosphere is function of many factors including resistance to an adverse environment, microbiota and plant metabolites, migration ability, competition for space, water and nutrients as well as production of lytic enzymes or antibiotics (1, 5, 16). The resident yeast population has the special ability to compete for nutrients and/or space which enable them to act as biocontrolers (22).

The best period for antagonist application was T_7 (4 days before, simultaneously and 4 days after, in relation to inoculation with the pathogen suspension) however without significantly difference from T_4, T_5 and T_6. The highest DSRs were showed by LR42 in T_7 treatment (71%), LR42 in T_4 treatment (66.7%), LR35 in T_5 treatment (69.5%) and LR19 in T_6 treatment (68.3%), which differed from the others (Fig. 1). The good results obtained with the treatments applied before inoculation are in agreement with Boudreau and Andrews (7), Michereff *et al.* (23) and Reis *et al.* (28). This possibly means that the antagonists are able to inhibit the pathogen establishment probably by colonizing the infection sites and competing for nutrients (5, 9, 23).

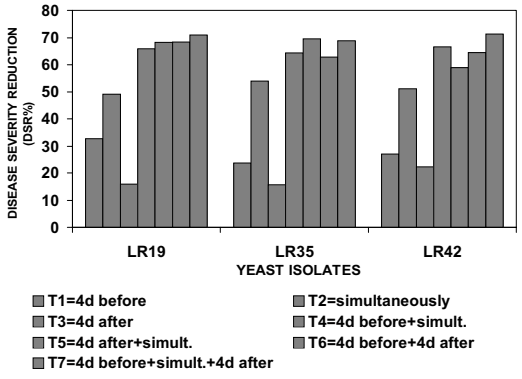

Figure 1: Influence of application period of antagonistic yeasts on the cabbage black rot control.

When the three antagonists were tested against Xcc, using six cabbage cultivars, DSR ranged from 61.3 to 57.9%, without significant difference among cultivars (Fig.2). There was high uniformity among the reaction of the cultivars to Xcc and also to the treatment with antagonists. These cultivars are Japanese hybrids and two of them, "Midori" and "Fuyutoyo", are the most frequent in Pernambuco.

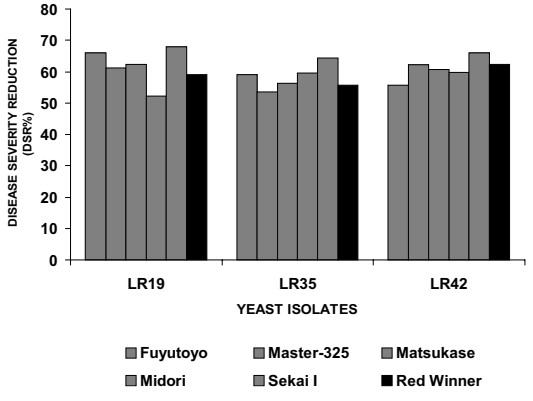

Figure 2: Influence of cabbage cultivar on the black rot control by antagonistic yeasts.

As conclusion, one can say that an integrated control program for the black rot should include resistant cultivar, besides biological control and cultural practices. In the biological control, yeasts should be used due to their efficient mechanisms such

as competition for nutrients and infection sites and, furthermore, because they are easy to grow and formulate.

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RESUMO

Antagonismo de leveduras a *Xanthomonas campestris* pv. *campestris* no filoplano de repolho em condições de campo

Vinte isolados de leveduras, obtidos a partir do filoplano de repolho foram avaliados pela atividade antagônica contra *Xanthomonas campestris* pv. *campestris*, em condições de campo. Plantas de repolho cv. Midori foram pulverizadas simultaneamente com suspensões do antagonista e do patógeno. Após 10 dias, as plantas foram avaliadas através da porcentagem de área foliar infectada. A porcentagem de redução da severidade da doença (DSR%), também foi calculada. Os isolados de leveduras LR32, LR42 e LR19 apresentaram, respectivamente, 72, 75 e 79% de DSR. Estes isolados foram testados em sete diferentes períodos de aplicação dos antagonistas em relação a inoculação do patógeno. (T_1=4d antes; T_2=simultaneamente; T_3=4 d após; T_4=4 d antes + simultaneamente; T_5=4 d após + simultaneamente; T_6=4 d antes + 4 d após; T_7=4 d antes + simultaneamente + 4 d após). As maiores DSRs foram obtidas por LR42 (67%), LR35 (69%) e LR19 (68%), respectivamente nos tratamentos T_7, T_4, T_5 e T_6, que diferiram significativamente dos demais. As melhores estirpes de levedura também foram testadas para controle da podridão negra das crucíferas usando diferentes cultivares de repolho (Fuyutoyo, Master-325, Matsukaze, Midori, Sekai I and Red Winner). As DSRs variaram de 61 to 58%, embora não tenha havido diferença significativa entre as cultivares.

Palavras-chave: *Xanthomonas campestris* pv. *campestris*, repolho, biocontrole, leveduras.

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