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NEW MICROBIAL ANTAGONISTS TO CONTROL POST HARVEST DISEASES

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Abstract

An investigation was undertaken to identify new antagoinstic microorganisms, on the surface of fruits and vegetables, against post harvest pathogens. Washings from apple, orange, banana, lemon, avocado, date, kiwi fruit, cucumber, cauliflower, and broccoli surface were placed in fresh apple wounds and subsequently challenged with *Botrytis cinerea* and *Penicillium expansum*. Subsequently, individual isolations were made from frequently occurring microbial colonies in wounds where rot didn't develop. Three of these yeast isolates (SE-243, SE-244, SE-245) obtained from cucumber, date, and avocado proved to be potentially effective antagonists against post harvest pathogens. Those isolates were identified as *Candida oleophila (SE 244)* Montrocher and *Candida gulliemondii* (Castellani) Langerson and Guerra (SE-243 and SE-245).

INTRODUCTION

A continuing and critical needs exist for safe and effective alternatives to synthetic fungicides for the control of post harvest diseases of fruits and vegetables. A selection strategy for isolating naturally occurring antagonistic microorganisms from the surface of fruits and vegetables by utilizing wounds in fruit as a selective screen has been reported (Wilson etal. 1993). Three antagonistic yeast were identified utilizing this Procedure : *Candida sake* (subsequently identified as *Candida oleophila*), *Cryptococcus laurentii* and *C.tenuis* .

Other antagonists have been isolated from the surface of fruit such as *Pichia* *guillermondii* Wickerham (US-7) which was isolated from washings from lemon (Wilson and Chalutz 1989). This antagonist exhibited antagonistic activity against a wide spectrum of post harvest pathogens of fruit and vegetables (Chalutz and Wilson 1990 and Droby *et al.* 1991). *Cryptococcus laurentii* (Kufferant) Skinner has been reported as an effective antagoist against apple rots (Roberts 1990).

This paper reports some non-antibiotic producing antagonists which are effective in controlling post harvest rots of apple. The *in vivo* screening strategy described previously by Wilson *et al.* 1993 was employed.

MATERIALS AND METHODS

Apple, orange, banana, lemons, avocados, dates, kiwi fruit, cucumbers, cauliflower and broccoli were obtained from an organic source (Fredrick Produce, Fredrick, MD 20705, USA). Individual fruit and vegetable were thoroughly washed in 150 ml sterile deionized water in polyethylene containers to dislodge organisms from their surfaces. Each fruit was washed twice in separate containers. These washings were used as inoculum for screening potential antagonists .

Apples (C.V. Golden Delicious) were placed in plastic trays and wounded (3 mm x 5 mm deep). Nine apples were used per treatment and two wounds were made on each apple. Fifty ul of the two separate washings were pipetted in one wound of each fruit.After 2 hr the treated and non-treated wounds were inoculated with 20 ul of a spore suspension of *Penicillium expansum* Link (4x105 cfu/ml). Fruits were then stored at room temperature (25 ± 2C) for 7 days.

Tissue was scraped from wounds not showing rot after 7 days and suspended in 100 ml of 1% peptone water and mixed in a laboratory blender (Stomacher, 400 Tekmar) for 60 sec. Ten ml of each suspension was removed and diluted to 10^{-3} and spread on yeast maltose agar plates.

After one day, single colonies with different morphological appearance were streaked on new plates of yeast maltose agar. After colonies developed, each was checked microscopically to distinguish the yeasts from bacteria.

Potential yeast antagonists were grown in a liquid yeast maltose medium for 24 hr. Nine apples were used for each selected isolate and two wounds were made per each apple. Then, 50 ul portions of $1x10^8$ CFU suspension of washed cells was pipetted into apple wounds. After 2 hr, the wounds were inoculated with 20 ul of *p.expansum* spores (4x105 spores /ml.). The fruits were examined for rot development after 9,12, and 14 days. Only those antgonists which reduced rot by at least 60% were selected for further study.

Discs of agar containing the effective isolates were inoculated onto yeast maltose agar plates flooded with a spray of 4 x 10^5 spores/ml of *penicillium expansum* to test for antibiotic production. The plates were incubated for 24 hr and the isolates which did not exhibit antibioses were selected.

Among the 25 isolates initially obtained from the fruit washings, three showed potential for controlling *penicillium expansum* in apple wounds. The isolates SE-243, SE-245 were identified as *Candida guilliermondii* and isolate SE-244 was identified as *Candida oleophila*. The isolates that belong to *C.guilliermondii* were discarded because of possible medical implications (Mok and Baffeto 1984, Booth *et al.* 1988, Nagy *et al.* 1984) and *C.oleophila* was tested further.

Further tests of *Candida oleophila* against *Botrytis cinerea* and *Monilinia fructicola*

To investigate the effect of *Candida oleophila* against *Botrytis cinerea* and *Monilinia fructicola*, three apple fruits were randomly chosen. The antagonistic isolates were grown for 24 hr in a liquid yeast-maltose medium and two wounds were made in each fruit (3 mm x 5 mm deep). On each fruit one wound received 50 ul of 1x10^8 CFU /ml suspension of the isolate *C. oleophila*. The other wound received 50 ul of filter-sterilized water as a check. After 2 hr, all wounds were inoculated with 20 ul of either *B.cinerea* or *Monilinia fructicola* spores (4x10^5 spores/ml). After 12 days, the wounds were examined for rot incidence .

RESULTS

Twenty five yeast isolates were harvested by selecting antagonists from large populations in washings from the surface of fruits and vegetables.

This approach using fruit wounds as a selective medium, was confirmed as an effective means of identifying potential antagonists for biological control. Among the twenty five selected isolates, the eleven isolates which reduced rot by at least 60% after 7 days at room temperature (25±2°C), were tested further (Table 1). Among

Table 1. Percentage rot 5 and 7 days after apple wounds were inoculated with 11 possible antagonists and challenged with *Penicillium expansum*

Isolate Number	Percentage Rot *	
	Day 5	Day 7
2 A2	11.1	55.6
4 Cu.	00.0	22.2
9 O	00.0	00.0
10 K	33.31	55.6
12 K	00.0	55.6
13 L	11.1	33.3
17 L	22.2	55.6
18 L	00.0	00.6
19 L	00.0	55.6
20 L	00.0	44.4
23 V	00.0	00.0
Water	88.9	100
1 A. 3 A, 5C, 6-8D, 11 K, 14-16 L, 21-22 O, 24-25 V		over 60

* n = 9; A= apple; C=cauliflower; Cu=cucumber; D= date; K=kiwi fruit; L = lemon; Or=orange.

Table 2. Percentage rot 9 and 12, and 14 days after apple wounds were inoculated with the most possible antagonists and challenged with *Penicillium expansum*

Isolate Number	Percentage Rot *		
	Day 5	Day 12	Day 14
Cu 4.	33.3	55.6	55.6
9 D	44.4	55.6	55.6
18 L	55.6	77.8	88.9
23 V	11.1	55.6	88.9
Water	100	100	100

* n=9; Cu = cucumber; L = lemon; D=date; V=avocado.

these eleven isolates 4 were superior in reducing the percentage of rot by *P.expansum* 14 days following incidence inoclum. Three isolates out of the four initially isolated from avocado, cucumber, and date remained the most promising antagonists against *Penicillium expansum* twelve days following inoculation (Table 2).

The three superior antagonists (SE-243, SE-244, and SE-245) were found to show no antibiotic activity when tested *in vitro.* (Fig.1).

Identification of the three isolates was made by the CBS yeast Division, Delft, The Netherlands, using an extended identification process. Two of the isolates (ES-243, SE-245) were identified as *C.guilliermondii* which has already been demonstrated as an effective antagonist, but has relatives which are reported associated with human disease (Mok and Beffetto 1994, Booth *et al.* 1988, Nagy *et al.* 1984). The third isolate (SE-244) was identified as *C.okophila* Montrocher (Table 3).

Table 3. Identification of the most effective isolates 12 days after challenging against *Penicillium expansum* in apple wounds.

Isolate	Name
SE-243	*Candida guilliermondii* (Castellani) Langerson and Guerra
SE-244	*Candida oleophila* Montrocher
SE-245	*Candida gulliermondii* (Castellani) Legerson and Guerra

Since the isolate *Candida oleophila* was found to be highly effective against *Penicillium expansum* in apple wounds and has no relatives with a human medical history (Wilson *et al.* 1993) an expanded test was conducted to determine its effectiveness against other pathogens of apple fruit, *Botrytis cinerea* Pers ex Fr. and *Moilinia fructicola* in apple wounds.

The data in Table 4 demonstrated that *Candida oleophila* inhibits both *B.cinerea* and *M.fructicola* up to 9 days in apple wounds. The inhibition of *P.expansum* was comparable to that of *B.cinerea* .

DISCUSSION

An urgent need exists for alternative to synthetic fungicides for the control of

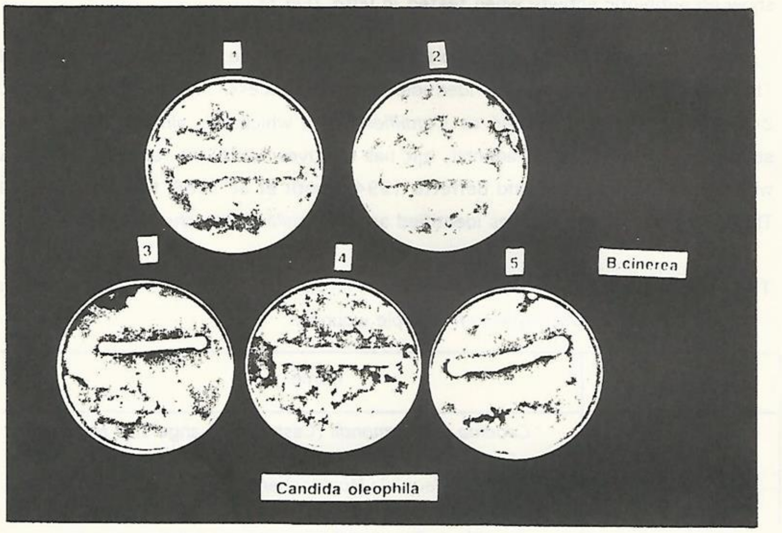

Fig.1. Antibiotic effect of *C.oleophila* against *B.cinerea* (5 isolates)

post harvest diseases. Antagonistic microorganisms hold promise of providing such alternatives (Wisniewski and Wilson 1992).

Table 4. Evaluation of *Candida oleophila* against *Penicillium expansum, Botrytis cinerea* and *Monilinia fructiola* in apple wounds for 7.9, and 12 days at (23+2C).

Days after Inoculation	Candida oleophila + Pathogen	Percent Rot*
Day 7	*Candida oleophila + P.expansum*	8.33 b
	Candida oleophila + B.cinerea	33.33 ab
	Candida oleophila + M.fructicola	20.83 ab
	Water (control)	70.83 a
Day 9	*Candida oleophila + P.expansum*	33.33 c
	Candida oleophila + B.cinerea	58.4 b
	Candida oleophila + M.fructicola	33.3 d
	Water (control)	87.5 a
Day 12	*Candida oleophila + P.expansum*	45.83 d
	Candida oleophila + B.cinerea	79.16 b
	Candida oleophila + M.fructicola	58.4 c
	Water (control)	100.

The selection of the most effective antagonists as biocontrol agents is dependent upon a screening method which will select the best organism from a large population of potential antagonists.

The strategy described by Wilson *et al.* (1993) allows the rapid scneening and selection of potential antagonists in an individual wound from large, diverse populations of microorganisms. In our studies, employing this procedure, we were able to isolate and identify three new antagonistic yeast strains from avocado, cucumber, and date which were effective in the control of post harvest rots of apple. It is interesting that other isolates of these species have previously been identified from other fruit surfaces-*C.oleophila* (from tomato) (Wilson *et al.*, 1993); and *P.guilliermondii* from lemon fruit (Wilson and chalutz, 1989). This indicates that these organisms are widespread in their distribution on fruit surfaces. These yeast isolates also appear to be effective in controlling a wide range of post harvest pathogens.

In our screening procedures, an arbitrary decision was made to select only yeast antagonists which do not produce antibiotic. We are assuming that there will be objection to the commercial application of antibiotic producing antagonist and bacteria to food. When antibiotics are placed on food they may provide the potential for human pathogens to develop resistance to them thus diminishing their effectiveness in human disease therapy .

It is important in the selection of antagonistic yeasts that those associated with human pathology be eliminated. For these reasons, research toward the commercialization of *P.guillimondii* which has relatives associated with human pathology has discontinued .

Among the isolates identified in this study, the isolate SE-244 (*C.oleophila*) holds the greatest promise for future development .

At this point, only a small sampling of potential antagonists for the control of post harvest diseases has been made. The wound selection strategy in this study should be utilized for more extensive screening for potential antagonists. In order to discover the most effective antagonists possible, more extensive screening is needed of microbial populations from a variety of micro environments. It would be profitable to look at a wider array of plant surfaces, as well as soil microflora.

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REFERENCES

1. Booth, Lv., AL. Collins, A. Lowes, and M.Radford. 1989. Skin rash associated with *Candida guilliermondii.* University of southampton, Dept. of Microbiology and Public Health, Southampton General Hospital, England. Med. 1988 : 16 (4) : 295-296.

2. Chalutz, E. and C.L. Wilson. 1990. Biological control of green and blue mold and sour rot of citrus by *Debaryomyces hansenii.* Plant Dis., 74 : 134-137 .

3. Droby, S., E. Chalutz, and C.L. Wilson. 1991. Antagonistic microorganisms as biological control agents of post harvest diseases of fruits and vegetables. Post harvest News inform., 2 : 169-173 .

4. Mok, W.Y. and M.S. Baffeto dasilva. 1984. Mycoflora of the human dermal surface. Can. J. Microbiol, 30 (10) : 1205-1209 .

5. Nagy, B., P. Sutka, M. Zeine-el- Abidine, Kovacsl, V. Forgace, J. Pulag, R. Gimes, F. Paulin, F.Lindeisz, J.C sepli *et al.* 1989. *Candida guilliermondii* var. *guilliermondii* infection in infertile women. Mycoses, 1989, 32 (9) : 4463-4468 .

6. Robert, R.G. 1990. Post harvest biological control of gray mold of apple by Cryptococcucus Laurentii. Phytopathotegy, 80 : 526-530 .

7. Wilson, C.L. and E.Chalutz. 1989. Post harvest biological control of Penicillium rots of citrus with antagonistic yeasts and bacteria. Scientia Hortic., 40 : 105-112 .

8. Wilson, C.L., M.E. Wisniewski, M.E., S. Droby, and E. Chalutz. 1993. A selection strategy for microbial antagonists to control post harvest disease of fruits and vegetables. Scientia Horticulture, 53 : 183-189 .

9. Wisniewski, M.E. and C.L. Wilson. 1993. Biological control of post harvest disease of fruits and vegetables. Recent Advances. Hort. Science., 27 : 49-98 .

اكتشاف ميكروبات مضادة جديدة لمقاومة أمراض ما بعد الحصاد

سنية محمد النشوى

قسم بحوث امراض ما بعد الحصاد ، معهد بحوث امراض النباتات - مركز البحوث الزراعية - الجيزة .

إجريت دراسة لتعريف كائنات حية مضادة جديدة من الميكروبات الكائنة على اسطح ثمار الفاكهة والخضر لاستخدامها ضد مسببات امراض ما بعد الحصاد.

عند استخدام غسول ثمار كل من التفاح - البرتقال - الموز - الليمون - الافوكادو- البلح - الكيوى - الخيار - القرنبيط و البروكلى للحقن فى مواقع الجروح على ثمار التفاح الطازجة ، ثم اخضاع كل من فطرى البوترياتس سينيريا والبنسيليوم اكسبانسم . للمنافسة مع الميكروبات المحتملة فى غسول الثمار فى نفس مواقع الجروح.

بعد الحصول على العديد من العزلات المنفردة من بين المستعمرات المكروبية النامية فى مواقع الجروح والتى لم ينتج عن وجودها اعفان، اثبتت الدراسة وجود ثلاث عزلات الخميرة المعزولة من ثمار الخيار والبلح والافوكادو واثبتت قدرة تنافسة كامنة ضد مسببات امراض ما بعد الحصاد وقد عرفت هذه العزلات الثلاثة على انها كانديدا اوليوفيلا (٢٤٤) كانديدا جلير مونديى (٢٤٣ ، ٢٤٥).