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Full Length Research Paper

Profiling the transcriptome of *Sclerotium cepivorum* Berk related to white rot on garlic (*Allium sativum* Linnaeus)

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One concern for garlic (*Allium sativum* Linnaeus) producers is the damage caused by the pathogen *Sclerotium cepivorum* Berk. Unfortunately, the genetic and molecular mechanisms that participate during *S. cepivorum* Berk pathogenesis are currently unknown. In order to identify and isolate genes that are differentially expressed by the fungus during garlic white rot pathogenesis, PCR-based suppression subtractive hybridization (SSH) was used. Combining SSH and cDNA arrays hybridization techniques, 120 ESTs whose expression is restricted to the pathogenic stage were identified and isolated. Fourteen ESTs showing higher expression in cDNA arrays were sequenced, these included homologues to oxaloacetate acetylhydrolase (Oah), cysteine desulfurase (Nfs 1p), regulator of drug sensitivity (Rds 1p), outer membrane protein (Omp 1) and cell wall adhesion (Fig2p). One selected EST with high homology to Oah gene, a putative virulence factor, was analyzed by RT-PCR. The possible role of Oah gene in the pathogenesis of this fungus toward garlic is discussed. The combined applications of SSH and cDNA arrays permitted global analysis of gene expression patterns in *S. cepivorum* Berk, as an initial stage to improve the knowledge at molecular level from fungal pathogenesis.

Key words: Pathogenesis, differential gene expression, suppression subtractive hybridization, cDNA arrays, Oah gene.

INTRODUCTION

White rot, caused by *Sclerotium cepivorum* Berk, is the predominant disease of garlic crops worldwide. Once introduced into the field, the sclerotia can survive for over 20 years without the presence of an *Allium sativum* Linnaeus host. The sclerotia are specifically induced to germinate by *Allium* root exudates, in particular alkyl cysteine sulfoxides (ACSOS) (Coley et al., 1990; Davies et al., 2007; Ulacio-Osorio et al., 2006). Current control methods for white rot involve intensive fungicide

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application and crop production on uninfected land (Miñambres et al., 2010; Melero-Vara et al., 2000). However, this approach is becoming less practical as soil microbes become more efficient at degrading the active fungicidal chemicals thus reducing the efficacy of many fungicides. Several alternative strategies for white rot control are now being utilized as part of an integrated control program that includes cultural practices such as soil solarisation and biological control (Davies et al., 2007). Unfortunately, fungicide application is still required for effective management with these systems.

The molecular mechanisms that participate in the regulation of the interaction fungal-garlic are still unclear. Studies of gene expression profiles during fungal-garlic interaction can provide clues about pathogenesis regulation and may lead to the discovery of molecular targets for novel antifungal drugs. Suppression subtractive hybridization (SSH) technique is now well known and has been used by many laboratories (Diatchenko et al., 1996; Yang et al., 1999; Guevara-Olvera and Acosta-Garcia, 2011). It also permits the identification of differentially expressed genes without the need to obtain previously cloned cDNA.

The aim of the present study was to use a genomic approach to identify and clone all the fragments of genes differentially expressed in *S. cepivorum* Berk during pathogenesis on garlic using SSH method and cDNA arrays.

MATERIALS AND METHODS

Fungus and plant material

S. cepivorum Berk, strain C2 an isolated from Cortazar, Guanajuato, México and *Allium sativum* Linnaeus Texcoco provided by the Instituto Nacional de Investigaciones Forestales Agricolas y Pecuarias (INIFAP) was used for experimental work.

Fungal-garlic interaction

One square centimeter of *S. cepivorum* Berk young mycelium grown in potato dextrose agar (PDA) was used to inoculate garlic clove during 72 h in a 50 ml glass flask containing 20 ml of double distilled water. As control, the fungus was inoculated in absence of the garlic.

Isolation of total RNA

RNeasy plant mini kit (Qiagen, Hilden, Germany) was used to extract the total RNA from mycelium in absence (driver) and presence (tester) of garlic. RNA purified by RNeasy column was analyzed for integrity and size by formaldehyde agarose gel electrophoresis, and quantification and purity of RNA by $A_{260/280}$ value, using a Jenway 6405UV/vis spectrophotometer (Jenway, Dunmow, UK).

Synthesis, amplification and purification of cDNA

One micro-gram of total RNA of each conditions of fungal development was used as template to synthesize the first strand of cDNA using the Superscript II Reverse Transcriptase (Life Technologies, Rockville, MD, USA) and the SMART™ PCR cDNA synthesis kit (Switch Mechanism At the 5´ end of RNA Transcript) (Clontech, Palo Alto, CA, USA), then amplified by LD-PCR with 15, 18, 21, and 24 cycles separately (Davies et al., 2007) and analyzed through 1.2% agarose gel electrophoresis in order to identify the optimal cycle number for which a suitable amount of PCR product is obtained rather than to build the SSH library. For SSH control, 1 µg placental total RNA was used to synthesize cDNA driver and placental cDNA with *Hae* III-digest øX174 DNA was used as cDNA tester. CROMA-SPIN 1000 column (CLONTECH, Palo Alto, CA, USA) was used to purify cDNA.

Isolation of differentially expressed cDNA fragments

Suppression subtractive hybridization was conducted using the CLONTECH PCR-Select™ cDNA subtraction kit (CLONTECH, Palo Alto, CA, USA). The tester (garlic presence) and driver (garlic absence) cDNAs were partially digested with *Rsa* I, a four base-cutting restriction enzyme that yields blunt ends. The tester cDNA fragments were divided into two aliquots, and each was ligated separately with adapter 1 and adapter 2 resulting in two populations of tester cDNA. A small amount of each tester population (600 ng) and driver in excess (2 µg) were mixed, heat-denatured, and allowed to anneal 8 h at 68°C. The two samples from the first hybridization were combined and annealed with additional freshly denatured driver cDNA (1 µg), overnight at 68°C. A primary PCR was conducted to amplify those cDNAs that represented differentially expressed genes. A secondary PCR amplification was conducted using nested primers 1 and 2R to reduce background levels (CLONTECH PCR-Select™ cDNA subtraction kit). The secondary PCR amplification products were electrophoresed and fragments longer than 500 bp were sliced using a scalpel and purified by QIAEXII Gel extraction kit (QIAGEN, Hilden, Germany).

Cloning and screening of subtraction fragments

PCR fragments (0.2 µg) were ligated to the pCR2.1–TOPO cloning vector (1 µl) according to the manufacturer´s instructions (INVITROGEN, Carlsbad, CA, USA). 2 µl ligation reaction solutions were transformed into 50 µl of *Escherichia coli* chemically competent cells strain TOP 10. The transformation culture was plated on Petri dishes containing LB/kamamycin/IPTG/X-gal, and white colonies were screened for insert fragment. Individual white bacterial transformants were cultured into LB/ampicillin/kanamycin medium and then it was shaken at 37°C overnight, and plasmid was screened for inserts presence using the restriction enzyme *Eco*RI (Invitrogen, Carlsbad, CA, USA).

Storage of library

Selected white colonies containing recombinant plasmid were inoculated separately into 5 ml LB/ampicillin/kanamycin solution, and it was shaken at 37°C overnight. Then 500 µl of each culture were added into 2 ml cryogenic vial (Corning, Acton, MA, USA) containing 500 µl 100% glycerol and kept at -80°C.

Construction of cDNA arrays

Five micro-grams of each recombinant plasmid were spotted into 7X10 cm BrightStar™-Plus positively charged nylon membrane (Ambion Inc, Austin, TX, USA) to construct 6X4 (usually 12X4) clones array using a Slot Blot Manifold Hoefer PR 648 (Amersham

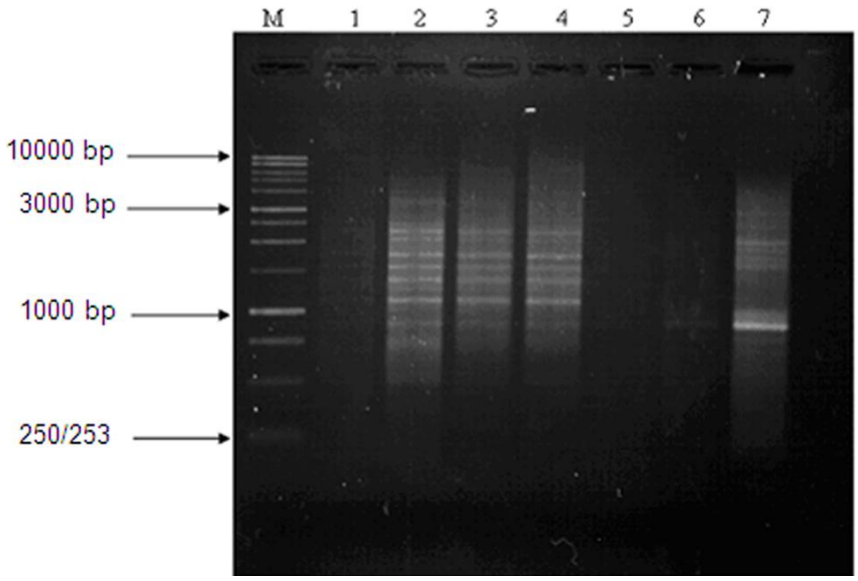

Figure 1. Optimization of PCR cycles. Lane M, 1 kb DNA marker; lanes 1, 2, 3, and 4, cDNA amplified; 15, 18, 21, and 24 cycles respectively, RNA tester; lanes 5, 6, and 7, cDNA amplified; 15, 18, and 21 cycles respectively, human placental total RNA.

Biosciences, Buckinhamshire, UK). As negative control, 5 µg of pCR2.1–TOPO cloning vector and 5 µg of recombinant plasmid containing an internal fragment of ScGpdh gene (369 bp; Accession number DQ522162) from *S. cepivorum* Berk as internal control were spotted, respectively. 100 ng for each driver and tester cDNA were added as positive controls.

Preparation and labeling of cDNA probes and membrane hybridization

Replicates of the SSH library were hybridized by Southern analysis (Sambrook et al., 1989) with the driver or tester cDNA probes. These probes were generated by incorporating fluorescein-11-dUTP using Gene Images CDP-Star random prime labeling module according to the manufacturer´s instructions (Amersham Pharmacia Biotech Inc, Piscataway, NJ, USA). Detection of fluorescein-labelled probes in Southern dot blots was performed using Gene Images CDP-star detection module (Amersham Pharmacia Biotech Inc, Piscataway, NJ, USA), employing anti-fluorescein alkaline phosphates conjugate and CDP-Star detection reagent.

DNA sequencing and database comparison

The nucleotide sequences of differentially expressed fragments were determined using the ABI PRISM 310 genetic analyzer (Perkin Elmer, Norwalk, CT, USA). On-line database comparisons were performed using blastx algorithm (Altschul et al., 1990) from National Center for Biotechnology Information (NCBI).

Differential expression of ScOah

Total RNA isolation from *S. cepivorum* Berk mycelium in absence (driver) and presence (tester) of garlic followed of reverse transcription were conducted as described above. PCR amplification of the ScOah cDNA (175 bp) was performed using specific sense and antisense primers whose sequences were as follows: 5´-CTCTTGAATAGCCAACATAGCCG-3´ and 5´-AAAGGAGATGGCTGCGAAGACT-3´, respectively. As internal control, PCR amplification of a constitutive gene ScGdph cDNA of 369 bp (Accession number DQ522162) was obtained using specific sense and antisense primers whose sequence were as follows: 5´-GGTGTCAACAACGAGACCTACA-3´ and 5´-GCGGACAGTCAAGTCAACAAC-3´, respectively. One hundred nano-grams of each driver and tester cDNA were used as a template. PCR products were separated by agarose gel electrophoresis, (1.2%) and the optical density of the EtBr-stained bands was recorded using a Vilber Lourmat gel documentation system (Marne-La-Valée Cedex, France) equipped with an ultraviolet light transiluminator.

RESULTS

RNA isolation and cDNA synthesis for SSH

To detect genes involved in *S. cepivorum* Berk pathogenesis, fungal mycelium was harvested 72 h post inoculation and before any adhesion to the garlic tissues surface (not shown) for RNA isolation. The amount of RNA extracted from mycelium in absence (driver) and mycelium in presence (tester) of garlic was 15 and 20 µg, respectively. The first strand and double-stranded cDNA were obtained. For cDNA subtraction, optimization of the number of PCR cycles was done to ensure that cDNA will remain in exponential phase of amplification. Over-cycled cDNA is a very poor template, on the other hand, undercycling results in a lower yield of PCR product. The products obtained with 15, 18, 21, and 24 cycles were separated on 1.2% agarose gel electrophoresis. For tester (Figure 1) and driver (not shown), the optimal

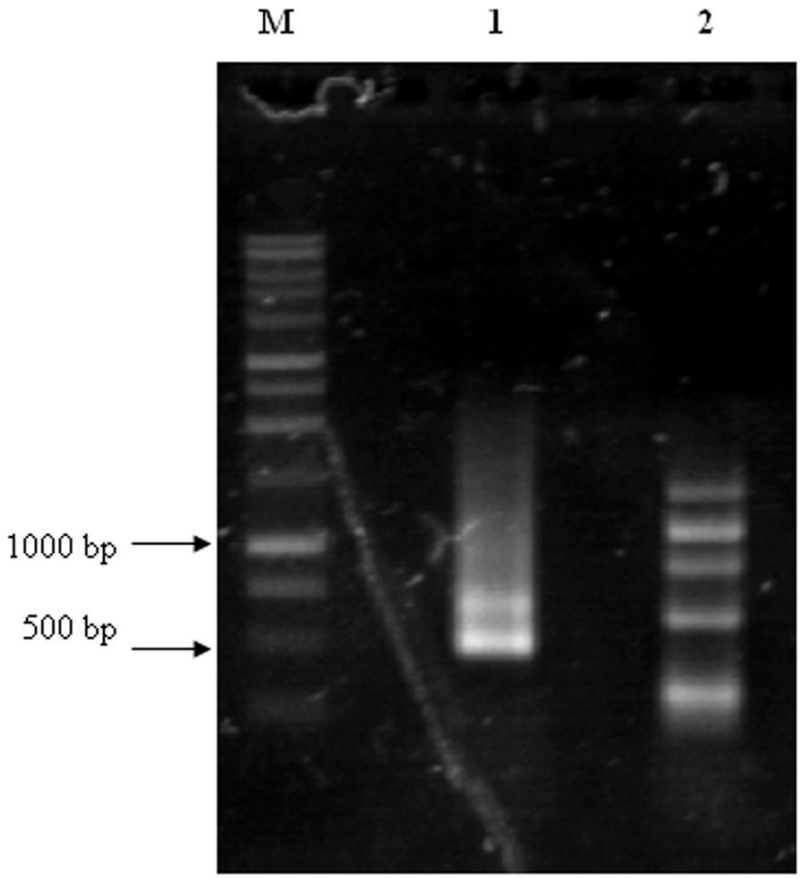

Figure 2. Secondary PCR of subtracted samples and screening for inserts. (A) PCR using nested 1 and 2R primers. Lane M, 1 kb DNA marker; lane 1, SSH from tester and driver; lane 2, SSH from tester and driver human placental total RNA.

cycles number were 18 while for placental RNA, the 21 cycle sample was selected (Figure 1).

PCR-selected cDNA subtraction

cDNA before digestion with *Rsa* I, appeared as a smear of 0.5 to 10 kb on 1% agarose gel electrophoresis, and after digestion, the cDNA size was smaller (0.1 to 2 kb) (not shown). After SSH, a primary PCR was conducted to amplify cDNAs, which represented differentially expressed genes. A secondary PCR amplification was performed using nested PCR primers 1 and 2R to reduce background, and several bands could be clearly seen among these smears (Figure 2, Lane 1), while for SSH control øX174/Hae III DNA fragments are seen (Figure 2, Lane 2). PCR products were isolated and cloned into pCR 2.1TOPO TA vector. 120 White colonies were selected for plasmid DNA isolation and analyzed for inserts presence using *Eco*RI restriction enzyme. 96 Recombinant plasmids, containing fragments with estimated size around 500 bp, were selected.

Differential expression of genes identified by SSH

Differential expression was assayed by Southern blot analysis. Ninety six clones were spotted in duplicate on nylon membrane and hybridized with both tester and driver probes. Forty six clones hybridized exclusively with tester probe. Screen from 20 clones are shown (Figure 3). Clone HR4, HR35, HR46, HR49 and HR57 are not included in Table 1 because they are redundant in sequence. Only clones HR9 and HR40 were expressed at a slightly increased level in tester cDNA, while 18 clones were expressed at a much higher level in tester cDNA, confirming the differential gene expression during pathogenesis of *S. cepivorum* Berk.

Sequence and homology analyses of SSH clones

Among 96 gene fragments screened, 46 clones differentially expressed (ESTs) were sequenced and analyzed for DNA homology. Fourteen ESTs sequenced were found more than once when compared to the GenBank

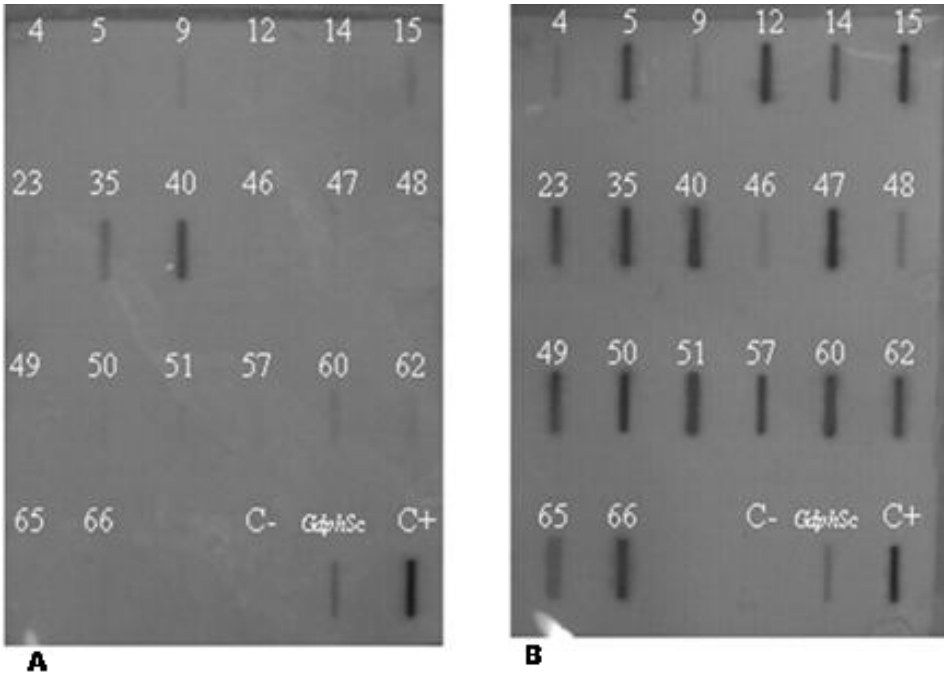

Figure 3. Differential screening of clones from subtracted library generated using SSH by Southern blot analysis with driver; (A) and tester (B) cDNA as probes. Each number indicates HR clones. One hundred nano-grams of cDNA were added as positive controls (C+): **A**, driver; **B**, Tester.

non-redundant TRANSLATED query-PROTEIN database (blastx). The identity of these 14 sequences are listed in Table 1 and were arbitrarily classified into five groups of proteins: I, Cell wall; II, Cell membrane; III, Host response avoidance; IV, Pathogenicity factors and V, Others. Two from 14 gene fragments were expressed during *S. cepivorum* Berk pathogenesis on garlic exhibit homology to cell wall proteins, and they could be involved in host recognition: Fig2p (Zhang et al., 2002; Blumwald et al., 1998) dBap (Arrizubieta et al., 2004; Cucarella et al., 2001). Four sequences encode membrane proteins, 3 of them could be involved in host recognition: Rds1p (Wolfe et al., 1999), Omp1 (Emelyanov and Demyanova, 1999) and Mp (Monteagudo et al., 2004), and 1 involved in signal transduction Pcm4 (Chang et al., 1999). Two sequences are related to putative proteins involved in host response avoidance: Nsf1p (Lu et al., 2004) and Glnd (Garcia and Rhee, 1983; Jiang et al., 1998).Three sequences exhibited homology to putative pathogenicity factors: Oah (Cessna et al., 2000), Adhy (Vaneechoutte et al., 2004) and Geh-1 (Sayari et al., 2001; Simons et al., 1998). Furthermore, clone HR62 had a very strong homology (Score, 297; E-value, 2e-79) to a putative pathogenicity factor oxaloacetate acetylhydrolase of *Botryotinia fuckeliana* Whetz (the teleomorph of *Botrytis cinerea*), a haploid, filamentous, heterothallic ascomycete (Table 1, Figure 4A). Others gene fragments had homology to a positive regulator of telomerase Est1 (Evans and Lundblad, 2002), a carboxyphosphonoenolpyruvate phosphonomutase PrpB (Horswill and Escalante-Semerena, 1997) and a hyphotethical protein (Guevara-Olvera et al., 2006).

Differential expression of ScOah transcript during pathogenesis of *S. cepivorum* Berk

ScOah is expressed at a much higher level during the compatible reaction with garlic than when grown in absence of a host (Figure 4B). Although non-quantitative, this technique did provide evidence for differential expression of ScOah transcript during pathogenic stage of *S. cepivorum* Berk.

DISCUSSION

White rot disease of onion, garlic and other *Allium* spp. results from the attack by the soilborne fungus *S. cepivorum* Berk and is a continuing concern for worldwide garlic production. Garlic producers are really concerned about the big losses caused by *S. cepivorum* Berk pathogenesis. Several control methods have been employed; however, these become less effective as the pathogen is able to degrade the fungicides. One alternative still unexplored is the genetic engineering. In order to identify genes involved in *S. cepivorum* Berk pathogenesis on garlic, Suppression subtractive

Table 1. Functional classification of gene products activated during .*S cepivorum* Berk pathogenesis toward garlic.

Clone	Gene with high homology	I/P*	Score	E-value	Accession No.
I - Cell wall					
HR48	*Fig2P*, Cell wall adhesin (*Saccharomyces cerevisiae*)	35/50	31.6	8.8	DR774659
HR47	*Bap*, Biofilm-associated surface protein (*Staphylococcus aureus*)	26/43	37.4	0.14	DQ054537
II - Cell membrane					
HR23	*Rds1p*, Regulator of drug sensitivity (*Saccharomyces cerevisiae*)	45/60	32.3	4.1	DR774654
HR12	*Omp1*, Outer membrane protein (*Rickettsia prowazekii* str. Madrid E)	42/51	25.4	6.3	DR774656
HR65	*Mp*, Potential transmembrane protein (*Candida albicans* SC5314)	35/51	33.5	1.8	DR774658
HR5	*Pcm4*, Calcium/calmodulin-binding membrane protein (*Paramecium tetraurelia)*	28/50	31.6	8.4	DR774661
III - Host response avoidance					
HR51	*Nsf 1p*, Cysteine desulfurase (*Magnaporthe grisea*)	86/94	250	1.00E-76	DR774652
HR9	*Glnd*, PII Uridylyl transferase protein (*Escherichia coli*)	27/44	33.1	2.2	DR774663
IV - Pathogenicity factors					
HR62	*Oah*, Oxaloacetate acetylhydrolase (*Botryotinia fuckeliana*)	90/97	297	2.00E-79	DR774651
HR14	*Adhy*, Acyl dehydratase (*Ralstonia metallidurans* CH34)	30/56	31.6	7	DR774660
HR66	*Geh-1*, Lipase (*Staphylococcus epidermidis* RP62A)	28/45	35	0.74	DR774662
V - Others					
HR50	*Est1*, Positive regulator of telomerase (*Saccharomyces cerevisiae*)	45/60	35.8	0.58	DR774655
HR15	hypothetical protein SsuiDRAFT_1635 (*Streptococcus suis* 89/1591)	36/44	42	0.008	DR774657
HR60	*Prp B*, Carboxyphosphonoenolpyruvate phosphonomutase (*Pyrobaculum aerophilum*)	35/54	76.3	2.00E-13	DQ054537

*Percentage of amino acids: **I,** Identities; **P,** Positives.

hybridization method was used. Blast analysis indicated that thirteen of the reported above fourteen sequences had strong homology to genes of known function or sequences present in GenBank database and represent at least 5 classes of putative genes. Blast analysis showed that clone HR51 had strong homology to Nsf 1p, a cysteine desulfurase of *Magnaporthe grisea* (Score, 250.00; E-value, 1e-76) (Lu et al., 2004), which could be involved in alkyl cysteine sulfoxides cleavage contained in *Allium sativum* Linnaeus exudates In addition, clone HR23 is related to Rds1p, a regulator of drug sensitivity from *Saccharomyces cerevisiae* (Score, 32.30; E-value 4.10) (Wolfe et al., 1999). It is a putative zing-finger transcriptional activator of genes involved in multistress response, so both genes could play a pivotal role avoiding host response. On the other hand, clone HR12 exhibited homology to Omp1, an outer membrane protein from *Rickettsia prowazekii* str. Madrid E (Score, 25.40; E-value 6.30) (Emelyanov and Demyanova, 1999) involved in virulence, while clone HR48 showed homology to Fig2P, a cell wall adhesin from *S. cerevisiae* (Score, 31.60; E-value 8.80) (Zhang et al., 2002) and a serine-threonine-rich secreted glycoprotein. It has been

A

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HR62: 1      YMRIRAAKGAKDAMNSDIVLIARTDALQQLGYDECIKRLKVARELGADVGLLEGYTSKEM 60
             YMRIRAAK  AK+ AMNSDIVLIARTDALQQLGYDECIKRLKVARE+GADVGLLEGYTSKEM
BfOAH: 159   YMRIRAAKAAKEAMNSDIVLIARTDALQQLGYDECIKRLKVAREMGADVGLLEGYTSKEM 218

HR62: 61     AAKTVKEFAPWPILLNMVENGATPIITTKEAHEMGFRIMIFSFAALAPAMLAIQETFLRLKKE 123
             AAKTVKEFAPWPILLNMVEN G+TPIITTKEA  EMGFRIMIFSFAALAPA  LAIQETFLRLK++
BfOOAH: 219  AAKTVKEFAPWPILLNMVENG STPIITTKEAQ EMGFRIMIFSFAALAPAY LAIQETFLRLKRD 281

HR62: 124    GVVGTPKNVTPRALFEVCGLQESIVIDTAAGGGSFADGV 162
             GVVGTPKN+TP+ ALF+VCGL++SIV+DT  AGGG+FADGV
BfOAH: 282   GVVGTPKNLTPKALFDVCGLKDSIVVDTTAGGGAFADGV 320
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B

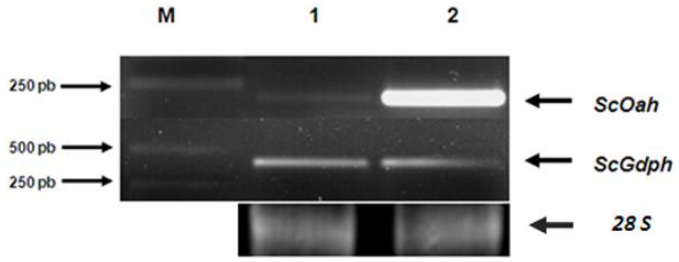

Figure 4. Homology between HR62 clone a putative *ScOah* from *S. cepivorum* Berk and *BfOah* from *Botryotinia fuckeliana* Whetz and its expression level in driver and tester DNA. (A) Alignment between putative OAH proteins from *S. cepivorum* Berk and *B. fuckeliana* Whetz using the blastx algorithm from NCBI, +, indicates similar amino acids. (B) RT-PCR detection of *ScOah* (175 bp) transcript by amplification with specific primers. Lane M, 1 kb DNA marker; lane 1, *ScOah* amplified from cDNA driver; lane 2, *ScOah* amplified from cDNA tester. *ScGpdh* (369 bp) was used as housekeeping gene.

shown to play roles in heterotypic and homotypic cell-cell adhesion processes, morphogenetic pathways and invasive/pseudohyphal growth, so both genes could be involved in *A. sativum* Linnaeus recognition by *S. cepivorum* Berk. Blastx analysis showed that clone HR62 (accession number DR774651) displays high similarity with *Botryotinia fuckeliana* Whetz Oah gene (Score, 297.00; E-value 2e-79), (Joosten et al., 1999; Guevara-Olvera and Acosta-Garcia, 2011) which encodes an oxaloacetate acetylhydrolase (OAH), Mn^{2+}-dependent enzyme, that catalyses formation of oxalic acid. OAH cleaves oxaloacetate to oxalate and acetate.

Oxalic acid plays an important role in a compatible pathogen-host interaction since, in several cases its secretion has been shown to be required for patho-genesis (Dutton and Evans, 1996). Oxalate is produced by a variety of fungi, including saprophytic and phytopathogenic species. Additionally, the role of oxalic acid secreted as a pathogenicity factor by the ubiquitous phytopathogenic Ascomycete fungus *Sclerotinia sclerotiorum* in inhibiting the oxidative burst from host plants is strongly documented (Cessna et al., 2000). The oxidative burst is the controlled release of O_2 and H_2O_2 at the site of pathogen invasion (Wojtaszek, 1997; Ebel and Mithofer, 1998), which is one of the earliest responses against microbial invasion. It have been shown that Oxalic acid (OA) secreted by the *S. sclerotiorum* is a key pathogenicity factor; moreover, transgenic oilseed rape plants constitutively expressing TaOxo from wheat (*Triticum aestivum*) display considerably increased oxalate oxidase (OXO) activity and enhanced resistance to *S. sclerotiorum* (Dong et al., 2008). Speculation regarding the mechanism or mechanisms by which oxalate secretion might enhance *Sclerotinia* virulence currently centers on three modes of action (Dutton and Evans, 1996). First, several of the fungal enzymes secreted during invasion of plant tissues such as endopolygalacturonase (EP) have maximal activities at low pH. Several researchers have postulated that oxalate might aid virulence by decreasing the apoplastic pH to a value better suited for enzymatic degradation of plant cell walls (Bateman and Beer, 1965). Second, oxalate may be directly toxic to host plants, presumably because of its acidity, the secretion of oxalate has been suggested to weaken the plant, thereby facilitating invasion (Noyes and Hancock, 1981). Finally, chelation of cell wall Ca^{2+} by the oxalate anion has been proposed both to compromise the function of Ca^{2+}-dependent defense responses and B to

weaken the plant cell wall (Bateman and Beer, 1965). Additionally, maceration of onion (*Allium cepa* Linnaeus) host tissue by *S. cepivorum* Berk correlates with fungal oxalic acid secretion and EP activity (Stone and Armentrout, 1985).On the other hand, RT-PCR analysis for ScOah transcript described in this study (Fig. 4B), demonstrate that this gene is more strongly expressed during pathogenesis. Consequently, we speculate that HR62 (ScOah) gene plays an important role during garlic (*A. sativum* Linnaeus) white rot caused by *S. cepivorum* Berk, codifying the OAH enzyme to synthesize oxalic acid avoiding the oxidative burst of garlic defense response. Only clone HR15 (Accession number DR774655) with homology (Score, 42.00; E-value, 0.008) to a hypothetical protein of *Streptococcus suis* (Guevara-Olvera et al., 2006) could represent a novel gene involved in *S. cepivorum* Berk pathogenesis toward garlic. Seven among 14 clones exhibited P values between 1.80-8.80 units (Table 1), suggesting that all of them are putative genes, these values could change obtaining 5´and 3´ cDNA by rapid amplification of cDNA ends (RACE). In summary, molecular cloning of 14 gene fragments identified in this work raises the possibility to use them as genetic targets for garlic white rot control which could give great benefit for agriculture.

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