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Antimicrobial Potential of Genes from Garlic (*Allium sativum* L.)

Hafiz Muhammad Khalid Abbas, Xi Kong, Jia Wu,
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Abstract

With the advancements in agriculture, farming community less or more started to rely on synthetic chemicals to increase the crop production and protection. But the negative impact of these chemicals on environment and cropping system urges the scientists to discover some new ways to combat with crop disease. By keeping in view, garlic is a well-known economically important vegetable throughout the world and recognized as reservoir for a number of bioactive compounds to treat various diseases; scientists have developed a strategy to identify and isolate antimicrobial genes from garlic. By using *B. subtilis* expression systems, a total of 48 antimicrobial genes, including *AsR 416*, were identified with the potential to significantly retard the growth of economically important fungal and bacterial pathogens. Furthermore, these antimicrobial genes exhibited the thermal stability along with nontoxic effects on mammalian blood cells, which indicate its potential use in the development of human medicines. These genes can revolutionize the way to treat with pathogens and also give a new wave of knowledge to explore the other organisms for the search of antimicrobial genes. This will also help to search the other cost-effective ways for the treatment of plant and human diseases.

Keywords: *Allium sativum* L., antimicrobial peptide, *Bacillus subtilis*, resistance gene

1. Introduction

Garlic (*Allium sativum* L.) is one of the most important species of the genus *Allium* and recognized as economically important vegetable throughout the world, especially around the Mediterranean basin where it is considered as main agricultural product [1, 2]. It is also of great importance because of its therapeutic properties and health-related benefits against various kinds of diseases such as aches, deafness, diarrhea, constipation, tumors, and respiratory problems. Health benefits from *Allium* species, especially garlic, have been used for centuries to treat various kinds of disorders, and still, there is need of research to explore its health-related potential [3–5]. It is a historic medicinal plant, originated from central Asia about 6000 years ago, and had been started to use as medicine in India since 5000 years ago and 3000 years ago in China [6, 7]. Volatile sulfur compounds, especially thiosulfates, responsible for pungent aroma, are the main compounds

responsible for its physiological effects [8]. Because of its health benefits, garlic is usually recommended as dietary supplement.

During the past few decades, antimicrobial resistance has become one of the most serious and challenging threat for the prevention and treatment of the infectious diseases [9, 10]. Nowadays, much of the attention has been paid to search some new and natural therapeutic agents, which can be used to treat human diseases with high efficacy and minimum adverse effects [11, 12]. Recent advances in research have revealed that there are several natural products with the potential to eliminate or alleviate several serious human diseases, especially cardiovascular, neurodegeneration, cancer, and several other important diseases [13–15]. A large number of researches have elaborated several herbs with the ability to produce antimicrobial compounds as their defense response against the number of different stresses including microbes [16, 17].

With the advancements in agriculture, farming community started to rely more on synthetic chemicals, which have been considered as an important source for crop production and protection. But, hazardous effects of these synthetic chemicals to environment and cropping system make their use questionable [18, 19]. Besides, pathogens also tended to increase their resistance against these synthetic chemicals and threaten the agriculture sustainability [20, 21]. By keeping these challenges in view, the need of identifying new strategies as an alternative source is increasing interestingly. Recently, scientists are trying to understand the chemistry of secondary metabolites from plants, as studies have revealed these secondary metabolites important in several ways, especially allelopathy, biological control, and biofertilizers, and also some compounds have been identified as biostimulants [22–24]. Consequently, understanding the mechanism of these secondary metabolites/bioactive compounds from plants can be useful for agricultural community.

2. Antimicrobial potential of *Allium* species

A number of *Allium* species have antimicrobial potential against variety of microbes including fungi, bacteria, viruses, and other parasites. Among all the *Allium* species, garlic is considered most for antimicrobial research after onion [25].

2.1 Antibacterial potential

Allium extracts containing thiosulfinates have the potential to retard the growth of Gram-positive and Gram-negative bacteria. It is, however, reported that garlic can inhibit the Gram-negative bacteria more than Gram-positive bacteria [26]. The permeability of inhibitory compounds from *Allium* might be affected by the cell wall and cell membrane structure. However, the results were quite opposite with diallyl trisulfide and dimethyl trisulfide and with garlic extracts to conclude that the Gram-positive bacteria were more sensitive than Gram-negative bacteria [27, 28].

Extracts from the garlic are reported to exhibit the effective results against saprobic and pathogenic bacteria, which are resistant to various drugs [29]. Garlic along with ciprofloxacin exhibits the pronounced inhibition of *E. coli* Z17, O2:K1:H- and *Helicobacter pylori*, but no significant evidence was found in the case of *H. pylori* infection in human [30, 31]. It has previously been proved that allicin is the main compound in garlic responsible for the antimicrobial activity, as garlic oil and extracts deficient in allicin do not exhibit any kind of antimicrobial activity [32]. It was later found that garlic oil and its constituting sulfides exhibit the more and significant inhibition of microbes and work as strong antifungal than the antibacterial agent [28].

Studies have reported that oils and sulfides from elephant (*A. ampeloprasum*) and shallot (*A. ascalonicum* L.) garlic have the potential to inhibit the food-borne pathogenic bacteria [33, 34]. Ajoene, an unsaturated disulfide, has been reported for its broad-spectrum antibacterial activities, which can be reduced by cysteine, a sulfhydryl compound [35]. Later, it was proved that disulfide in ajoene is a necessary component for the inhibition of bacteria as reduction by sulfhydryl compounds reduces the antibacterial activity. Gram-positive bacteria and yeast are more sensitive to ajoene than Gram-negative bacteria.

2.2 Antifungal potential

It is reported in different studies that oils and sulfides from the *Allium* have the more potential to inhibit the fungi than bacteria [28, 36]. Antifungal activity of sulfide molecules is directly proportional to increase in the number of sulfur atoms up to sulfur number three or four in sulfide molecules [28, 37].

Another study has also reported that sterilized/autoclaved garlic and its active compounds exhibit significant antifungal activities than that of antibacterial. Further analysis of garlic antimicrobial products revealed that these products are the heterocyclic sulfides [38], allyl alcohol [39], and 3-(allyltrisulfanyl)-2-aminopropanoic acid [40]. For bacteria and yeasts, minimum inhibitory concentrations (MICs) of heterocyclic sulfides are more than 100 and 1–6 ppm [38], respectively, while for the allyl alcohol, 4% and 55–140 ppm MICs are recorded for bacteria and yeasts [39], respectively. In the case of 3-(allyltrisulfanyl)-2-aminopropanoic acid, 100 ppm and less than 50 ppm MICs are observed for bacteria and yeasts [40], respectively. In previous studies, it was mistakenly stated that autoclaved garlic exhibit less antimicrobial activities than fresh garlic. For this statement, the only reason was that they tested autoclaved garlic against bacteria, which was already very less sensitive than yeasts against garlic [41]. Recent studies have explored the germicidal potential of sterilized/autoclaved garlic.

2.3 Antiviral activity

Diallyl polysulfides, as transformation product of allicin, and ajoene exhibit the antiviral activities. From all the reported *Allium* products, it is observed that ajoene exhibits more inhibition than other compounds like allicin and thiosulfates, but on the other hand, allicin is considered as strong antimicrobial agent [42, 43]. It is thought that antimicrobial compounds from garlic react with viral envelope and inhibit the penetration and exponentiation of influenza virus in animal kidney cells [44]. Garlic aqueous extracts have also been studied to observe the inhibition against potato virus Y under in vivo and in vitro conditions [45].

2.4 Antiparasitic potential

A number of parasites, including *Leishmania donovani* [46], *Spironucleus vortens* [47], and *Eimeria papillata* [48], are sensitive to garlic extracts. The MIC values of allicin, dithiins, and ajoene for the inhibition of *S. vortens* growth are higher than the MICs reported for the inhibition of bacteria and fungi, indicating the high tolerance of *S. vortens* for *Allium* extracts [46].

From the above discussed literature, it is clear that garlic has a certain pool of antimicrobial genes which can be isolated and studied further to explore their mechanisms. It will provide some new directions for antimicrobial research. Now we will discuss some techniques to isolate and study the antimicrobial genes from garlic.

3. Systems for the isolation of antimicrobial genes from garlic

3.1 *Bacillus subtilis* and *Escherichia coli* expression systems

An experiment was designed to study the antimicrobial genes from the garlic. For this purpose, cDNA libraries from garlic were constructed by using two different vectors, pBE-s and pET22 (b), and then transformed into expression systems, *B. subtilis* and *E. coli*, respectively. For the library quality analysis, two parameters were considered, recombination rate and library titer [49]. For the *E. coli* expression system, 96.7% and 4.6×10^6 pfu/ml, recombination rate and library titer were observed, respectively. On the other hand, recombination rate and library titer for *B. subtilis* expression system were 91.7% and 7.8×10^6 pfu/ml, respectively. Quality analysis revealed gene library in *E. coli* expression system was marginally better than that of the *B. subtilis* expression system.

For the screening of libraries, it was considered that because of the toxicity of protein products of cDNA libraries, *B. subtilis* and *E. coli* cells would be showing autolysis to indicate the antimicrobial potential of these libraries' inserts. For more confirmation, trypan blue dye was also used to indicate the viability of *E. coli* cells [50]. By using this strategy, a number of antimicrobial genes were screened from garlic to reveal its further potentials. For example, in case of *B. subtilis* expression system, a total of 48 antimicrobial genes were screened, including *AsR 416*, while *AsRE 67* was identified by using *E. coli* expression system [51].

3.2 Antimicrobial potential of genes from *A. sativum*

Antimicrobial potential of *A. sativum* genes was studied against fungi and Gram-positive and Gram-negative bacteria [50], and the results were observed as follows (Tables 1–3).

3.3 Action mechanism of antimicrobial proteins

A study was designed to explore the action mechanism of antimicrobial peptides. In this study, *B. subtilis* cells were treated with antimicrobial peptide, *AsR 416*,

Genes	<i>Fusarium</i> spp.	<i>Botrytis cinerea</i>	<i>Phytophthora capsici</i>
WB800	—	—	+
<i>AsR 379</i>	—	—	—
<i>AsR 117</i>	—	—	+
<i>AsR 412</i>	—	—	—
<i>AsR 416</i>	—	—	—
<i>AsR 453</i>	—	—	+
<i>AsR 36</i>	—	—	—
<i>AsR 174</i>	—	—	—
<i>AsR 864</i>	—	—	—
<i>AsR 498</i>	—	—	—
<i>AsR 845</i>	—	—	+
<i>AsR 853</i>	—	—	—

—, indicate no inhibition, +, indicate inhibition.

Table 1.
Antimicrobial potential of *A. sativum* genes against fungi.

Gram-negative bacteria				
Genes	<i>Xanthomonas campestris</i> pv. <i>oryzicola</i>	<i>Agrobacterium</i> <i>tumefaciens</i>	<i>E. coli</i> DE3	<i>Ralstonia</i> <i>solanacearum</i>
WB800	—	—	—	+
AsR 379	—	—	—	+
AsR 117	—	—	—	+
AsR 412	—	—	—	+
AsR 416	—	—	—	+
AsR 453	—	—	—	+
AsR 36	—	—	—	+
AsR 174	—	—	—	+
AsR 864	—	—	—	+
AsR 498	—	—	—	+
AsR 845	—	—	—	+
AsR 853	—	—	—	+
—, indicate no inhibition, +, indicate inhibition.				

Table 2.
Antimicrobial potential of *A. sativum* genes against Gram-negative bacteria.

Gram-positive bacteria							
Genes	<i>Clavibacter</i> <i>michiganensis</i> subsp.	<i>C. fangii</i>	<i>B. anthracis</i>	<i>B. subtilis</i> 330–2	<i>B. cereus</i>	<i>B. subtilis</i> 168	<i>B. subtilis</i> WB800
WB800	—	+	—	+	+	—	—
AsR 379	+	+	+	+	+	+	+
AsR 117	+	+	+	+	+	+	+
AsR 412	+	+	+	+	+	+	+
AsR 416	+	+	+	+	+	+	+
AsR 453	+	+	+	+	+	+	+
AsR 36	+	+	+	+	+	+	—
AsR 174	+	+	+	+	+	+	—
AsR 864	+	+	+	+	+	+	+
AsR 498	+	+	+	+	+	+	+
AsR 845	+	+	+	+	+	+	—
AsR 853	+	+	+	+	+	+	+
—, indicate no inhibition, +, indicate inhibition.							

Table 3.
Antimicrobial potential of *A. sativum* genes against Gram-positive bacteria.

and then PI (propidium iodide) staining was performed [51]. PI is fluorescent agent that has the ability to bind with DNA through broken cell membrane. Red fluorescence in all bacterial cells treated with antimicrobial peptide was observed under confocal laser microscope [52], while the flow cytometry analysis revealed that cell membrane damages increase with increase in the protein concentration [53].

All findings collectively support that the target of antimicrobial peptide is to destroy the cell membrane of target bacteria.

3.4 Thermal stability and safety analysis of antimicrobial proteins

Proteins from *AsR 117*, *AsR 416*, and *AsR 498* were heated at different temperatures for 15 min, and it was found that *AsR 117* and *AsR 416* proteins were thermally stable at all temperature ranges, while *AsR 498* became thermally unstable after 50°C, as it exhibited the reduced antimicrobial activity. For the safety analysis, these proteins were analyzed against sheep red blood cells [54, 55]. This analysis revealed these antimicrobial proteins as nontoxic to mammalian cells with maximum 1000 µg/ml concentration [51]. From the thermal and safety analyses, it is also obvious that antimicrobial genes from garlic can also be used in human medicines in the future, which needs further investigations.

4. Conclusion

It is an adverse need of modern agriculture to search cost-effective ways to treat the crop diseases, as the potential use of synthetic chemicals also increases the resistance in pathogens. Garlic is a famous vegetable for its potential to treat various kinds of diseases. So, it is obvious that antimicrobial genes from garlic are the best source to incorporate resistance in plants without affecting the other environmental factors. This way of introducing resistance can also help to understand the mechanisms of plant biology to further explore the new strategies.

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Conflict of interest

The authors declare that they have no conflict of interest.

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