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The effect of radiation on The Antibacterial Activity of Allicen

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الخلاصة

من المعروف إن للثوم تأثيراً مضاداً لطيف واسع من الأحياء المجهرية الموجبة و السالبة لصيغة جرام، وإن فاعلية تأثير الثوم المضاد للأحياء المجهرية يعزى أساسا إلى مادة الاليسين, وهي المادة الفعالة الموجودة في نبات الثوم.

لا توجد دراسات تبين مدى تأثير الإشعاع المؤين على فاعلية مادة الاليسين. لقد استخلصت مادة الاليسين مائياً حسب طريقة الدليمي ثم شععت تركيز مختلفة من مستخلص الاليسين بجرع مختلفة من أشعة كاما من جهاز (gamma-cell 20) . وبينت النتائج أن الجرع المستخدمة ولحد الجرعة 3 ميكاراد ليس لها أي تأثير على فاعلية مادة الاليسين, بينما الجرع العالية الأكثر من 5 ميكاراد و لحد 15 ميكاراد المستخدمة في هذه الدراسة يثبط كلياً فاعلية الاليسين كمادة مضادة للأحياء المجهرية.

من هذه الدراسة يتضح إن الجرع العالية من الإشعاع المؤين غير مرغوب استخدامها في حفظ فصوص الثوم لما لها من تأثير مثبط لفاعلية المادة الفعالة في فصوص الثوم و هي الاليسين.

Abstract

It is known that garlic cloves had antibacterial action on a wide range of microorganisms both g (+) and g (-) bacteria. The antibacterial activity of garlic is mainly attributed to allicin, the active principle of this vegetable.

The antibacterial activity of allicin is inhibited by different chemical and physical agents.

No studies were available to elustrate the effect of ionizing radiation on the activity of this compound. Allicin was water extracted according to AL-Delamy, and different concentrations of this extract were irradiated with different doses of gamma-radiation from γ-cell 20. The results obtained indicated that doses of radiation up of 3 megarad did not affect the antibacterial activity of allicin, whereas higher doses, 5 megarad and up, will inhibit the activity completely.

We conclude from these results that higher doses of radiation should not be used for preservation of garlic cloves because of its inhibitory effect on the active principle of garlic, allicin.

Introduction

It has been shown by many investigators that Allium sativum, the common garlic has a therapeutic as well as antibacterial activity.

The main antimicrobial effects of allicin is due to its chemical reactions with thiol groups of various enzymes e.g. alcohol dehydrogenase , thioreductase and RNA polymerase which can affect essential metabolites of cystein proteinase activity involved in the virulence of enteric pathogenic species and food poisoning bacteria [1,2].

Culter and Wilson[3] were studied the antimicrobial activity of aqueous extract of allicin on 30 clinical isolates of methicillin-resistant Staphylococcus aureus, They showed that all strains were killed at conc. of 32μg/ml aqueous extract of allicin.

Garlic and its extract have been used to treat infection for thousands of years[4], allicin is considered to be the main biologically active antimicrobial phytochemical produced in garlic extract and was recognized as such in 1944[5].

There are some studies demonstrating the preservation of garlic cloves using ionizing radiation [6, 7].

Vajdi and Pereira 1973, showed by using gamma-irradiation complete sterilization from microorganisms of selected spices including garlic powder and showed that the garlic flavour did not affected by such treatment[8].

For our best of knowledge, no studies are available to elustrate the effect of ionizing radiation on the active principle, the garlic antibacterial allicin. The present work was done to demonstrate the effects of gamma radiation on the activity of allicin as antibacterial agent.

Materials and methods

Bacterial strains and growth conditions:

The following bacterial species were used, E.coli, Salmonella typhi, Bacillus megaterium , Micococcus spp, and Pasteurella hemolyticus .

Stock cultures of microorganisms were maintained on nutrient agar slant at 4ºc. For experiments bacteria were incubated with shaking at 37ºc for 18 hr. A thousand fold dilution of 18 hr bacterial culture were allowed to proliferate in the logarithmic phase for two hours at 37ºc. At the end of that time the suspension, which contain 1-2 x10^6 cfu/ml. was added to all test tubes.

Isolation of Allicin:

Garlic cloves were washed with sterilized distilled water and pealed. Fifty grams was homogenized with 100 ml of sterilized distilled water for three minutes. The mixture was then suction filtered through Watman No. 1 filter paper. This solution was considered as stock allicin extract (1:2) dilution according to the method adapted by AL- Delamy [9].

Irradiation of allicin:

Samples of 5ml (1:2) allicin extract were irradiated in polyethylene container with the following doses, 0.1, 0.25, 0.5, 0.75 ,1,2,3,5,10 and 15 megarad gamma radiation respectively and serial dilutions were made in sterilized nutrient to a final volume of 5ml .

Antibacterial test:

Five ml of sterile broth in test tubes were prepared and the required amount of non-irradiated allicin solutions was added to give the dilutions required. Then 1ml of 1:1000 dilution of an eighteen-hour broth culture of the test organisms was mixed. Growth was observed after eighteen hours of incubation.

Results and discussion

To elucidate the action of allicin on microorganisms, experiments were conducted where different concentrations of allicin were added into nutrient broth tube in amounts to give a final volume of 5 ml. Species of microorganisms in concentrations of 1-2 x 10^6 cfu/ml. were mixed and incubated for eighteen hours. The end point of inhibition was very sharp, the effective concentration showing no growth. The next tube in the series showed good growth. A concentration of 1:216 of allicin showed no growth in case of E.coli, Salmonella typhi, Bacillus megaterium and Pasteurella heamolyticus. Whereas a concentration of 1:46656 of allicin allowed no growth with respect to Micrococcus spp . (Table 1).

The concentration of allicin which allow no growth were tested for stasis by removing 0.1 ml from the test and added to 5 ml of fresh broth, all organisms grew, this will support the fact that the action of allicin is considerably more bacteriostatic than bacteriocidal and at the same time it is equally effective against g (+) and g (-) bacteria.

It is noteworthy that no studies are available dealing with the effects of ionizing radiation on the antibacterial activity of allicin. As a matter of fact numerous studies were available elustrating the preservation of garlic cloves using ionizing radiation[10]. Therefore experiment was conducted to demonstrate the effect of gamma-irradiation on the antibacterial action of allicin. Samples of 5 ml allicin extract (1:2) conc were irradiated with the different doses of gamma-rays from gamma cell-20,(0.1,0.25,0.75,1,2,3,5,10 and 15 megarad) and different concentration of irradiated allicin samples were testes for its antibacterial action on different species of bacteria. Table 2 , shows the effect of 0.1 megarad gamma-radiation on the antibacterial activity of allicin , its evident that at this dose and doses up to 3 megared (data not shown) , the antibacterial activity of allicin did not affected , where as at higher doses , 5 megarad (Table-3) and more (data not shown) the activity completely inhibited. These results are in accord with the fact that higher doses of radiation are required to destroy

or inactivate biological molecules in vitro whereas small doses are required for their inactivation in vivo [11]. These results also indicate that high doses of radiation are needed to destroy the dially1 sulfide, unstable sulfer in alky1 polysulfide, acrolin or similar unsaturated aldehyde in which the antibacterial activity of garlic are attributed.

Its noteworthy that the flavor of garlic which due to factors need to be determined was not affected or lost during the exposure of allicin to even very high doses of radiation, these results coincide with the finding of Vajdi and Pereira [8] and both might indicate that the dially1 sulfide or other unsaturated aldehyde which was presumed to be the active principle of garlic, and responsible for antibacterial activity was not responsible for the special and specified flavour of garlic.

We recommend, from these results the use of gamma radiation in low doses e.g less than 3 megarad for preservation of garlic cloves and at the same time, these doses would be save enough to affect or inhibit the active principle of garlic, the allicin.

Bacterial spp	Concentration of allicin						
	0	1:6	1:36	1:216	1:1296	1:7776	1:46656
E.coli	+	-	-	-	+	+	+
Sal . typhi	+	-	-	-	+	+	+
B. megaterium	+	-	-	-	+	+	+
Past . heamolyti cus	+	-	-	-	+	+	+
Micrococcus . spp	+	-	-	-	-	-	-

N.B (+) means growth, (-) means no growth

Table-1: Antibacterial action of allicin extract on different species of g (+) and g (-) bacteria.

Bacterial spp	Concentration of allicin							
	0	1:6	1:36	1:216	1:1296	1:7776	1:46656	
E .coli	+	-	-	-	+	+	+	Non irradiated.
	+	-	-	+	+	+	+	Irradiated.
Sal .typhi	+	-	-	-	+	+	+	Non irradiated
	+	-	-	+	+	+	+	Irradiated.
B.megater--ium	+	-	-	-	+	+	+	Non irradiated
	+	-	-	+	+	+	+	Irradiated.
Past .heam—olyticus	+	-	-	-	+	+	+	Non irradiated
	+	-	-	-	+	+	+	Irradiated.
Micrococc—us. spp	+	-	-	-	-	-	-	Non irradiated
	+	-	-	+	+	+	+	Irradiated.

N.B the same results were obtained with higher doses up to 3 megarad.

Table-2: Potential inhabitation test of irradiated allicin with 0.1 megarad dose of gamma radiation in comparison with non-irradiated allicin for different species of g (-) and g (+) bacteria.

Bacterial spp	Concentration of allicin							
	0	1:6	1:36	1:216	1:1296	1:7776	1:46656	
E .coli	+	-	-	-	+	+	+	Non irradiated
	+	+	+	+	+	+	+	Irradiated.
Sal .typhi	+	-	-	-	+	+	+	Non irradiated
	+	+	+	+	+	+	+	Irradiated.
B.megater--ium	+	-	-	-	-	+	+	Non irradiated
	+	+	+	+	+	+	+	Irradiated.
Past .heam—olyticus	+	-	-	-	-	+	+	Non irradiated
	+	+	+	+	+	+	+	Irradiated.
Micrococc—us. spp	+	-	-	-	-	-	-	Non irradiated
	+	+	+	+	+	+	+	Irradiated.

N.B the same results were obtained with higher doses up to 5 megarad.

Table -3: Potential inhabitation test of irradiated allicin with 5.0 megarad dose of gamma radiation in comparison with non-irradiated allicin for different spp of g (-) and g (+).

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