ANTIMICROBIAL ACTIVITY OF MICROBIAL TREATED WHEAT FLOUR EXTRACT AGAINST SENSITIVE AND RESISTANT MICROBIAL STRAINS

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ABSTRACT: *The antimicrobial killing activity toward pathogenic strains has been a serious emerging global issue. In a continuing search for compounds with antibacterial activity against several microorganisms including E.coli, S.aureous, Micrococcus spp., Salmonellas spp, Pseudomonas spp. and MRSA strains was found from wheat flour. The I4 isolate is isolated from tobacco farm isolate. Wheat flour is treated by using this isolate and baker's yeast and prepared wheat flour are used for antibacterial activity against E.coli, S.aureous, Micrococcus spp., Salmonellas spp, Pseudomonas spp. and MRSA strains. MIC of I4 treated wheat flour extract against sensitive organism was demonstrated in range of 1.0 to 4.5 mg/ml concentration for different strains and MIC of Baker's Yeast treated wheat flour was demonstrated in range of 2.0 to 4.5 mg/ml concentration for different strains. The comparative study of I4 and Baker's yeast treated wheat flour indicates I4 strain treated wheat flour extract, displayed higher antimicrobial activity in comparison to backer's yeast against organism E.coli, S.aureous, micrococcus, Salmonellas spp, pseudomonas spp. and MRSA strains.*

Key Words: *MRSA, Antimicrobial activity, MIC, Wheat flour*

Introduction

The growing incidence of infectious diseases due to development of increasing antibiotic resistant pathogens justify the attempts to search for new antimicrobial agents as well as compounds that are capable of inhibiting resistance bacterial mechanisms to classical drugs. The continuous use of the same class and type of antimicrobial compounds seems to contribute to a significant increase in resistant bacteria, particularly resistant Gram-positive organisms [1]. MRSA has become the most common cause of infections among many pathogenic bacteria, leading to many life-threatening diseases such as endocarditis, pneumonia and toxin shock syndrome [2], [3].

The decreasing effectiveness of conventional drugs is continuously haunting both clinicians and drug researchers, and the critical shortage of new antibiotics in development against MRSA and other multidrug-resistant bacteria is of great concern worldwide. New targets with novel strategy of therapy and mechanism of action for development of antibacterial agents against MRSA are urgently needed [4]. Plant natural resources have been demonstrated to possess great chemical and biological diversities and promising findings of antibacterial phytochemicals [5]. Wheat, (*Triticum* species) a cereal grass of the *Gramineae (Poaceae)* family. Wheatgrass is rich in chlorophyll, minerals and trace elements. Wheat is known to contain antioxidant enzymes superoxide dismutase (SOD) and cytochrome oxidase that have the potential to convert reactive oxygen species (ROS) to a hydrogen peroxide and an oxygen molecule. Chlorophyll, one of the primary components in the wheatgrass, was found to augment blood formation and strengthen the immune system through inhibition of metabolic activation of carcinogens. It also possesses the ability to inhibit oxidative DNA damage [6].

Yeast (*Saccharomyces cerevisiae*), is a proven model eukaryote widely used for investigating various molecular and cellular biological functions. Yeast is also genetically well defined: its entire genome sequence has been elucidated and the corresponding databases are generally accessible[7]. Most germs are milled as part of mill feed, and a smaller portion is separated during the milling process. Separated wheat germ can be subjected to fermentation with Saccharomyces cerevisiae (yeast) to yield the benzoquinone 2,6-dimethoxy-benzoquinone (DMBQ) and 2-methoxy-benzoquinone [8][9]. These benzoquinones are present in unfermented wheat germ as glycosides; yeast glycosidase activity present during fermentation leads to release of the benzoquinones as a glycones. The standardized manufacturing technology included wheat germ fermented with Saccharomyces cerevisiae for 18 hours at 30˚C [7].

Materials and Method

A. Soil Sample Collection

Soil sample collection was carried out from tobacco farm from Anand agriculture university, Anand (Gujarat). Total four samples were collected from different locations of that tobacco farm.

B. Enrichment of Microorganisms

1 ml of sample was inoculated into 50 ml of Glucose Yeast Extract broth in 100 ml flask. The Flask was kept at room temperature on orbital shaker having speed 120 rpm for 48 hrs.

C. Isolation of Microorganisms

After 48 hours serial dilution (10^{-1}, 10^{-2}…..10^{-6}) was prepared from each broth, Spread 0.1ml of culture from directly broth and its each dilution on GYE agar plates respectively. Incubate at room temperature for 48 hours.

D.Characterization of Isolates

Colony Characteristics were observed on GYE agar plate and Gram staining was performed to observe the Gram reaction. The isolates were inoculated in the different biochemical media for testing their biochemical reactions.

E. Collection of Plant material

Wheat flour was collected from the flour mill located in the local market of Patan.

F. Preparation of Plant material

3.5 gm cell mass was obtained by centrifuging 200ml young culture of isolate, was suspended into 100ml distilled water. 10 gm freshly grinded wheat germ collected from local grain milling company, was added into yeast suspension. The mixture was incubated for 48 hours in shaking condition at room temperature at 120 rpm. After 48 hrs the mixture was centrifuged, concentrated and dried in hot air oven at 40^0 C temperatures in Petri plates. After that powder was scraped out and stored in screw cap plastic bottle tube at 0-4^0C temperature for further use [10].

G. Antibacterial activity of microbially treated wheat flour

Antibacterial activity was carried out by streaking multiple organisms like sensitive strains and MRSA strains by using Nutrient agar plate having 0.1mg/ml, 0.5mg/ml, 1.0mg/ml,1.5mg/ml, 2.0mg/ml, 2.5mg/ml, 3mg/ml, 3.5mg/ml, 4.0mg/ml, 4.5mg/ml, 5mg/ml of microbially treated wheat flour respectively. Incubate all plates for 24 hours at room temperature. After incubation observed the growth

Results and Discussion

A. Sampling sites and Physical properties of samples

Soil samples were collated from Tobacco farm of Agriculture University, Anand. Soil samples were coded as F1A4P2K3, F1A4, F2C4, MC4 and AAU. The results of physical properties of soil samples are mentioned in the Table 3.1.

Table 3.1: Characteristics of soil

Soil sample	Humidity				PH
	E.P.W	Soil weight	Dry weight	Difference	
F1A4P2K3	35.48	1 gm	33.39	3.09	7.0
F1A4	22.32	1 gm	23.21	0.11	6.0
F2C4	32.29	1 gm	33.21	0.08	7.0
MC4	31.08	1 gm	32.01	0.07	7.0
AAU	23.09	1gm	23.92	0.17	7.0

B. Isolation and Screening of Yeast

In the present study, isolation was carried out using GYE agar plate by spread plate method. The four strains I1, I2, I3, I4 which were isolated from F1A4, AAU, MC4 and F1A4 soil sample respectively (Table 3.2).

Table 3.2: Observation of isolates growth on GYE agar plate

No	Soil sample	Code	Observation
1	F1A4	I1	Peach color colony
2	RKP	I2	Off white moist colony

3	MC4	I3	Pure white colony
4	F1A4	I4	Off white dry colony

C. Colony character of isolates

Colony characteristics of all of four strains were noted from GYE agar plate (Table 3.3).

Table 3.3: Colony characteristics of isolates on GYE Agar plate

Isolates	Size	Shape	Margin	Elevation	Texture	Opacity	Pigment
I1	Big	Round	Even	Convex	Smooth	Opaque	Pitch
I2	Big	Round	Even	Low convex	Smooth	Opaque	Colorless
I3	Medium	Round	Even	Convex	Smooth	Opaque	Colorless
I4	Big	Irregular	Undulated	Flat	Rough	Opaque	Colorless

D. Morphological characteristics of isolates

Four strains were reported as Gram Positive under microscope by performing Gram's staining method (Table 3.4).

Table 3.4: Morphologic Characteristics of Isolates

Isolates	Size	Shape	Arrangement	Gram's reaction
I1	**Big**	**Oval**	**In pair and cluster**	**Gram positive**
I2	**Big**	**Oval**	**In pair and cluster**	**Gram positive**
I3	**Big**	**Oval**	**In pair and cluster**	**Gram positive**
I4	**Big**	**Oval**	**In pair and cluster**	**Gram positive**

E. Biochemical characteristics of isolate

According to ViTech identification system, they perform 46 different tests for yeast identification. In which I4 isolate gave 18 tests positive (Table 3.5).

Table 3.5: Biochemical Characteristics of Isolates

Test no	Test Name	I4
1	L-lysine-arylavidase	-
2	L-MALATE assimilation	+
3	Leucine-ARYLAMIDASE	+
4	ARGININE GP	+
5	ERYTHRITOL assimilation	-
6	GLYCEROL assimilalation	-
7	Tyrosine ARYLAMIDASE	+
8	BETA-N-ACETYL-GLUCOSAMINIDASE	-
9	ARBUTIN assimilation	-
10	AMYGDALIN assimilation	-
11	D-GALACTOSE assimilation	+
12	GENTIOBIOSE assimilation	+

13	D-GULUCOSE assimilation	+
14	LACTOSE assimilation	+
15	METHYL-A-D-GLUCOPYRANOSIDE assimilation	+
16	D-CELLOBIOSE assimilation	+
17	GAMMA-GLUTAMYL-TRANSEFARASE	+
18	D-MALTOSE assimilation	+
19	D-RAFFINOSE assimilation	-
20	PNB-N-acetyl-BD-galactosaminidase 1	-
21	D-MANNOSE assimilation	-
22	D-MELIBIOSE assimilation	-
23	D-MELEZITOSE assimilation	-
24	L-SORBOSE assimilation	-
25	L-RHAMNOSE assimilation	-
26	XYLITOL assimilation	+
27	D-SORBITOL assimilation	+
28	SACCHAROSE/SUCROSE assimilation	+
29	URASE	-
30	ALPHA-GLUCOSIDASE	-
31	D-TURANOSE assimilation	+
32	D-TREHALOSE assimilation	+
33	NITRATE assimilation	-
34	L-ARABINOSE assimilation	+
35	D-GALACTURONATE assimilation	+
36	ESCULIN HYDROLYSIS	+
37	L-GLUTAMATE assimilation	+
38	D-XYLOSE assimilation	-
39	D- LACTATE assimilation	-
40	ACETATE assimilation	-
41	CITRATE(SODIUM)assimilation	-
42	GLUCURONATE assimilation	+
43	L-PROLINE assimilation	+
44	2-KETO-D-GLUCONATE assimilation	-
45	N-ACETYL-CLUCOSAMINE assimilation	-
46	D-GLUCONATE assimilation	-

F. Antibacterial activity of microbially treated wheat flour

Antibacterial activity of microbially treated wheat flour extract was carried out by streaking multiple sensitive strains on Nutrient agar plate having 0.1mg/ml, 0.5mg/ml, 1.0mg/ml, 1.5mg/ml, 2.0mg/ml, 2.5mg/ml, 3mg/ml, 3.5mg/ml, 4.0mg/ml, 4.5mg/ml, and 5mg/ml concentration of microbially treated wheat flour

MIC of I4 treated wheat flour extract against sensitive organism was demonstrated in range of 1.0 to 3.5 mg/ml concentration for different strains (Table 3.6).

Table 3.6 Antibacterial activity of I4 containing wheat Flour

Isolates	concentration of wheat flour											MIC
	0.1mg	0.5mg	1mg	1.5mg	2mg	2.5mg	3mg	3.5mg	4mg	4.5mg	5mg	
E.coli	+	+	+	+	+	+	+	-	-	-	-	3.5mg
S.aureous	+	+	+	+	-	-	-	-	-	-	-	2mg
Micrococcus	+	+	-	-	-	-	-	-	-	-	-	1mg
Salmonella	+	+	+	+	+	-	-	-	-	-	-	2.5mg
Psedomonas	+	+	+	+	-	-	-	-	-	-	-	2mg

MIC of Baker's Yeast treated wheat flour was demonstrated in range of 2.0 to 3.5 mg/ml concentration for different strains (Table 3.7).

Table 3.7 Antibacterial activity of BY containing wheat flour

Isolates	concentration of wheat flour											MIC
	0.1mg	0.5mg	1mg	1.5mg	2mg	2.5mg	3mg	3.5mg	4mg	4.5mg	5mg	
E.coli	+	+	+	+	+	-	-	-	-	-	-	2.5mg
S.aureous	+	+	+	+	+	+	-	-	-	-	-	3mg
Micrococus	+	+	+	+	+	+	+	-	-	-	-	3.5mg
salmonella	+	+	+	+	+	+	+	-	-	-	-	3.5mg
Psedomonas	+	+	+	+	-	-	-	-	-	-	-	2mg

G. Comparative study of Antibacterial activity between I4 and Baker's yeast treated wheat flour

Comparative study of antibacterial activity between I4 treated wheat flour extract and Baker's yeast treated wheat flour extract was carried out. Baker's yeast treated wheat flour extract showed higher inhibitory effect on *S.aureus*, *Micrococcus spp. Salmonellaspp*and *Pseudomonas aeruginosa*.as compared to I4 treated wheat flour extract. Whereas for *E.coli* I4 treated wheat flour extract showed higher inhibitory effect as compared to Baker's yeast treated wheat flour extract (Figure: 3.1).

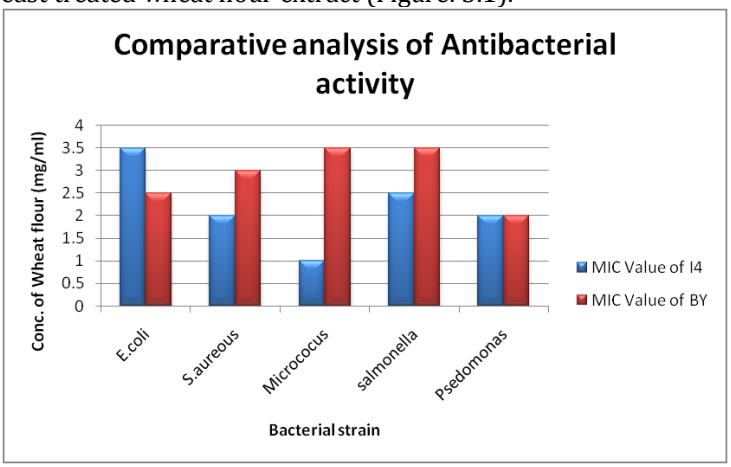

Figure 3.1: Comparative analysis of antimicrobial activity of I4 and BY containing Wheat flour

H. Anti-MRSA activity of microbially treated Wheat flour

Anti-MRSA activity of microbially treated wheat flour was carried out by streaking multiple MRSA strains on Nutrient agar plate having 0.1mg/ml, 0.5mg/ml, 1.0mg/ml, 1.5mg/ml, 2.0mg/ml, 2.5mg/ml, 3mg/ml, 3.5mg/ml, 4.0mg/ml, 4.5mg/ml, 5mg/ml concentration of I4 and Baker's Yeast treated wheat flour.

MIC of I4 treated wheat flour was demonstrated in range of 2.5 to 4.5 mg/ml concentration for different strains (Table 3.8).

Table 3.8 Anti MRSA activity of I4 containing wheat flour

Isolates	concentration of wheat flour											MIC
	0.1mg	0.5mg	1mg	1.5mg	2mg	2.5mg	3mg	3.5mg	4mg	4.5mg	5mg	
MRSA1	+	+	+	+	+	+	-	-	-	-	-	3mg
MRSA2	+	+	+	+	+	+	+	-	-	-	-	3.5mg
MRSA4	+	+	+	+	+	+	+	-	-	-	-	3.5mg
MRSA5	+	+	+	+	+	-	-	-	-	-	-	2.5mg
MRSA6	+	+	+	+	+	-	-	-	-	-	-	2.5mg
MRSA7	+	+	+	+	+	+	+	+	-	-	-	4mg

MIC of Baker's Yeast treated wheat flour was demonstrated in range of 3.0 to 4.5 mg/ml concentration for different MRSA strains (Table 3.9).

Table 3.9 Anti MRSA activity of BY containing wheat flour

Isolates	concentration of wheat flour											MIC
	0.1mg	0.5mg	1mg	1.5mg	2mg	2.5mg	3mg	3.5mg	4mg	4.5mg	5mg	
MRSA1	+	+	+	+	+	+	-	-	-	-	-	3mg
MRSA2	+	+	+	+	+	+	+	+	+	-	-	4.5mg
MRSA4	+	+	+	+	+	+	+	+	-	-	-	4mg
MRSA5	+	+	+	+	+	+	+	-	-	-	-	3.5mg
MRSA6	+	+	+	+	+	+	-	-	-	-	-	3mg
MRSA7	+	+	+	+	+	+	+	+	-	-	-	4mg

I. Comparative study of Anti-MRSA activity between I4 and Baker's yeast treated wheat flour extract

Comparative study of anti-MRSA activity between I4 treated wheat flour extract and Baker's yeast treated wheat flour extract was carried out. I4 treated wheat flour extract showed higher inhibitory effect on *MRSA*

2, MRSA 4 and MRSA 5 as compared to Baker's yeast treated wheat flour extract. Whereas for MRSA 1 and MRSA 7 both treated wheat flour extract showed similar sensitivity. (Figure: 3.2).

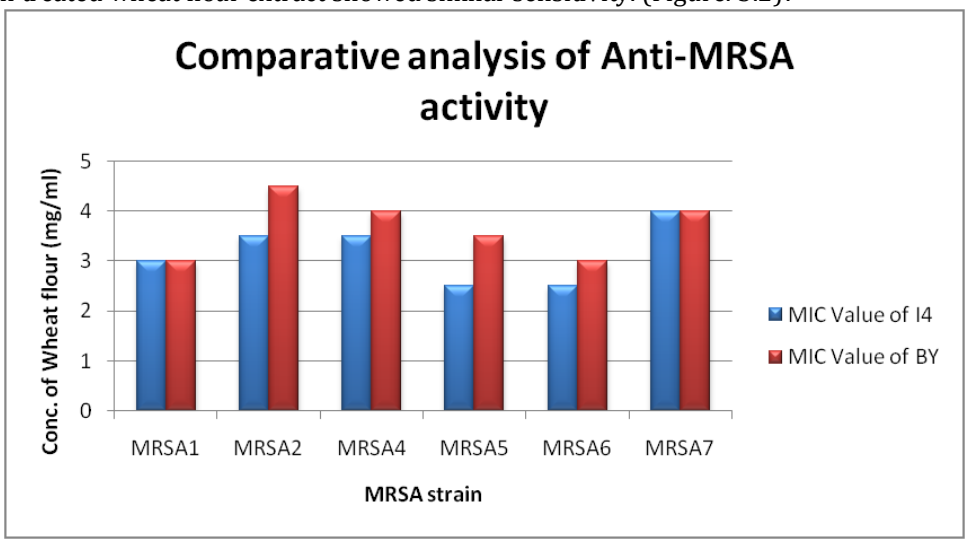

Figure3.2: Comparative analysis of anti-MRSA activity of I4 and BY containing Wheat flour

Conclusion:

In present study, total five soil samples were collected from tobacco farm which were used for preliminary screening of isolates. The tobacco farm isolates were studied for morphological, colonical and biochemical characterization. Whereas biochemical characterization was studied by Vitech based identification system in which the isolates remain unidentified, which indicate the possibility of novel strains. I4 strain treated wheat flour extract, displayed higher antimicrobial activity in comparison to backer's yeast against organism *E.coli, S.aureous, Micrococcus spp., Salmonella*s *spp, Pseudomonas spp*. and MRSA strains.

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