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Original Article

Comparative growth potential of different *Bacillus* species on fruit peels

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Abstract

Fruit peels are domineering constituents of agro industrial leftover encumbered into the environment. Six bacterial isolates *(Bacillus amyloliquefaciens, B. horikoshii, B. safensis, B. nealsonii, B. megaterium* and *B. licheniformis)* were grown in 2% dried watermelon, mango, apple, sugar cane bagasse, potato and banana peels to check their comparative growth potential. Growth evaluation through OD_{600} sign posts that all the *Bacillus* species have potential to grow on all these agro- wastes, whereas, the highest growth was observed on watermelon. Based upon higher growth yields as well as the substrates' aptitudes, watermelon peels and *B. megaterium* were optimized at pH 7, 27ºC under aerated conditions. Multiple metal and antibiotic resistances were found for select *B. megaterium*. Results show that environmental burden can be reduced by the use of agricultural byproduct/waste as a substrate for scale up biomass production of commercially important bacterial species for a variety of value added products.

Key words: Agro-industrial wastes, cultivation of bacteria, *Bacillus megaterium,* metal resistant bacteria

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INTRODUCTION

Fruit peels are important components of agro-industrial wastes loaded into the environment. The peels have potential to be used as substrates for bacterial growth. Applications of agro-industrial wastes not only reduces the environmental burden but also minimizes the cost of select microbial cultivations. Microbial cultivation media are mainly comprising of plants' origin ingredients. Fortunately, it is not impossible to trace support from existing actualities in favor of economical replacement of conventional commercial media with agro-industrial wastes. Potential of agro-industrial wastes to serve as microbial growth enhancing materials lies in their composition. Anhwange (2008) analyzed banana peels, which are rich of minerals and reported concentrations of protein, crude lipid, carbohydrate and crude fibre as 0.90, 1.70, 59.00 and 31.70%, respectively. Processing of mango into various value added products, generates huge amount of waste peels, that are loaded in the environment. Comprising up to 15–20% of fresh mango, the mango peels are rich of various bioactive elements and exhibit potential antioxidant properties. Al-Sayed and Ahmed (2013) reported moisture, ash, fat, protein and carbohydrate contents of watermelon rinds. Watermelon rinds contain different types of phenolic compounds, the most abundant one is 4-hydroxybenzoic acid (958.3μg/g dw) followed by vanillin (851.8μg/g dw), while the lowest phenolic compound is coumaric acid (8.8μg/g dw). In this context, Pakistan is specially blessed with a multiplicity of climatic and topographical regimes which are apt to nurture almost all varieties of foodstuff, fiber and cash crops as well as fruits and vegetables. Consequently, there is an immense production of agricultural wastes which are highly nutritious.

The present study was aimed at cultivating economically the commercially important *Bacillus* species in agricultural wastes for recognizing economically feasible low cost growth media for scale up production of value added products.

MATERIALS AND METHODS

Collection and processing of agro-industrial wastes:

Peels of watermelon, mango, apple, banana and potato and sugar cane bagasse were collected on daily basis from Punjab University hostel fruit shops, and brought to the

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laboratory for further processing. The collected materials were thoroughly washed with distilled water to remove particulate matter/dust. These were then cut down into smaller units to increase surface area for sun drying, followed by oven drying session at 80°C for 1 to 2 weeks till the attainment of consistant weight. Dried peels were crushed into fine powder with the help of a grinding mill and stored in air tightend jars.

Bacterial cultivation

Six bacterial species *(Bacillus amyloliquefaciens, B. horikoshii, B. safensis, B. nealsonii, B. megaterium* and *B. licheniformis)* after obtaining from bacterial culture depository of microbial Biotechnology lab. Zoology dept. Punjab University, Lahore were revived on nutrient agar plates at 37°C and preserved on slants.

Dried pulverized peels of watermelon, mango, apple, banana, potato and sugar cane bagasse suspended in distilled water served as low cost medium for the bacterial cultivation. Each suspension comprising of 2% of a given substrate was autoclaved, following centrifugation, the supernatant were collected. Then this supernatant again autoclaved after adjusting neutral pH.

The fresh culture (100µl) was inoculated in agro-industrial based extracts and incubated for 24 hours at 37°C. Bacterial growth was measured spectrophotometrically at 600nm. The bacterial species as well as the substrate was selected on the basis of best growth for optimization.

Optimization of growth conditions

The select bacterial species was optimized for growth by cultivating at different temperatures (27^0 C, 37^0 C, and 45^0 C), pH (5, 7 and 9), inocula sizes (2%, 5% and 10%) and under aerated and non-aerated condition. The bacterial growth was measured after 24 hours of incubation.

Measurement of MIC for metals

Select bacterial species was cultivated under pre optimized conditions in the presence of different concentrations of metals incorporated select watermelon based medium using salts of $CuSO_4$, $Pb(C_2H_3O_2)_2$, $NiCl_2$, $ZnCl_2$, $MnSO_4$, $HgCl_2$ and K_2CrO_4. The metals concentration at which bacterial growth was not detectable after 24 hours of incubation were considered as minimum inhibitory concentrations (MIC) or maximum tolerance limit.

Antibiotic resistance

Antibiotic resistance activity was determined by disc diffusion method (Bauer *et al.,* 1966). For this purpose antibiotic discs were loaded in triplicates on freshly prepared lawn of bacterial isolates and incubated at 37°C for 24h. Then results were recorded by measuring the diameters of grow inhibition zone in mm.

RESULTS

In present study, all the *Bacillus* species showed growth in 2% aqueous extracts of the different agro-industrial wastes. However, the highest growth of the bacteria was recorded in watermelon peels (Table I).

Bacillus megaterium attained optical density values of 2.42±0.21, 2.11±0.19, 1.33±0.25, 1.23±0.03, 1.17±0.02 and 0.68±0.30 following 24 hours of incubation in aqueous extract of watermelon, apple, banana, bagasse, mango and potato, respectively.

Based upon highest growth as well as the substrate efficiencies, watermelon peels and the bacterial species *B. megaterium* were selected for optimization of growth conditions. The *B. megaterium* was cultivated in 2% aqueous extract of watermelon peels. After 24 hours of incubation, *B. megaterium* grew best at initial pH 7 with O.D value of 0.25±0.02. Whereas the strain showed maximum growth at 27^0 C with corresponding absorbance value of 0.349±0.004. The select strain yielded best growth with 10% inoculum size. Following incubations the optimum pH, temperature and inoculums size, *B. megaterium* yielded best growth with aeration (Fig. 1).

The *B. megaterium* showed potential to grow in the presence of different metals. The minimum inhibitory concentration (MIC) values for different metals ranged from 60 to 550 mg/L.The order of metal resistance was recorded as 60(Hg), 450 (Pb),500(Cu, Cr, Ni) and 550mg/l for each Zn and Mn (Table II).

Bacillus megaterium was found to be highly sensitive against cafteriaxone, cefuroxime sodium, penicillin, rifampicin and gentamycin. Whereas inhibitory trend was observed against streptomycin and vanacomycin (Table III).

Table I: Growth screening of different *Bacillus* species in 2 % aqueous extract of different agro-industrial wastes at 37 ^{0} C after 24 hours incubation.

BACTERIAL ISOLATES	Agro-industrial wastes					
	Water melon	Mango	Apple	Bagasse	Potato	Banana
B.amyloliquefaciens	3.3±0.06 ^{a}	0.59±0.15^{b}	0.13±0.01 ^{ab}	0.68±0.11 ^{ab}	0.72±0.12 ^{b}	0.50±0.01 ^{ab}
B. horikoshii	5.4±0.13 ^{a}	0.67±0.19 ^{b}	0.64±0.23 ^{ab}	0.61±0.20 ^{ab}	0.46±0.10 ^{b}	0.88±0.01 ^{ab}
B. safensis	1.25±0.04 ^{a}	0.27±0.14 ^{b}	0.63±0.03 ^{ab}	0.46±0.14 ^{ab}	0.83±0.36 ^{b}	0.50±0.11 ^{ab}
B. nealsonii	1.21±0.05 ^{a}	0.49±0.11 ^{b}	0.65±0.10 ^{ab}	0.42±0.03 ^{ab}	0.23±0.08 ^{b}	1.19±0.16 ^{ab}
B. megaterium	2.42±0.21 ^{a}	1.17±0.02 ^{b}	2.11±0.19 ^{ab}	1.23±0.03 ^{ab}	0.68±0.30 ^{b}	1.33±0.25 ^{ab}
B. licheniformis	1.41±0.01 ^{a}	0.32±0.03 ^{b}	0.62±0.04 ^{ab}	0.67±0.01 ^{ab}	0.54±0.05 ^{b}	0.62±0.18 ^{ab}

Means that do not share a letter are significantly different within the specific column following one way ANOVA.

Table II: Minimum inhibitory concentration (MIC) of different metals for growth of *B. megaterium*

Sr. No.	Metals	Conc. (mg/L)
1	Cu	500
2	Pb	450
3	Hg	60
4	Zn	550
5	Cr	500
6	Ni	500
7	Mn	550

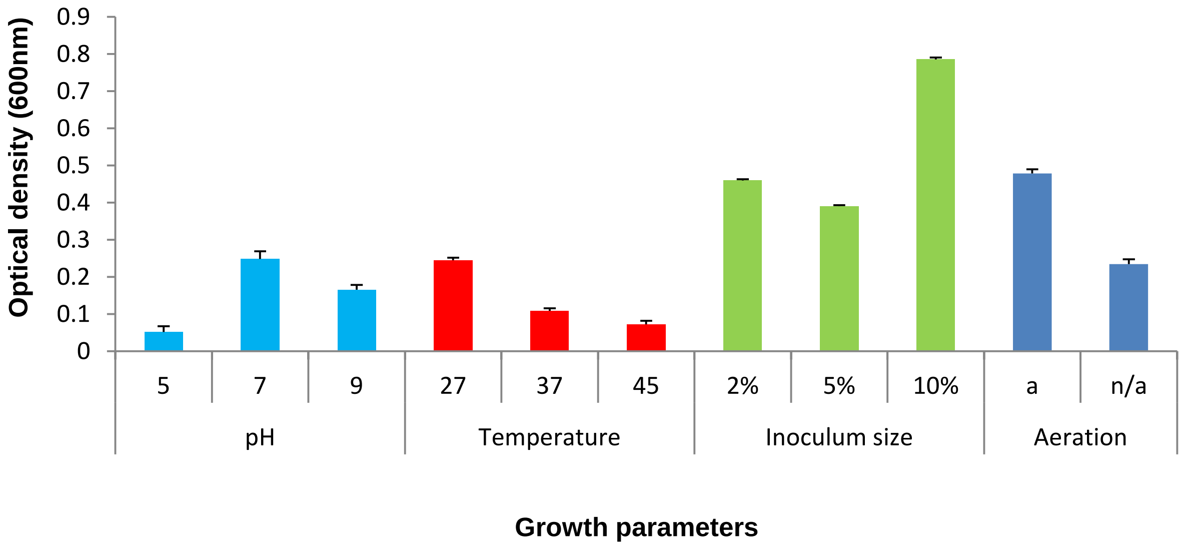

Figure 1: Growth conditions optimization (mean±SEM) of *Bacillus megaterium* of 24 hours incubated culture using 2% aqueous extract of watermelon.

Table III: Effect of antibiotics on *B. megaterium*

Sr. No.	Antibiotic	Concentration	Activity (mm)
1	Cafteriaxone (CRO)	30 µg	Nil
2	Cefuroxime sodium (CTX)	30 µg	Nil
3	Rifampicin	50mcg	20.2S
4	Streptomycin	10µg	17
5	Vanacomycin	30µg	14.5
6	Penicillin G	10units	Nil
7	Chloramphenicol	30 µg	17.6S
8	Gentamycin	10µg	18S
9	Erythromycin	15 µg	9R
10	Polymyxin B	300units	8.5R

R= the bacterium was resistant to the drug; S= sensitive

DISCUSSION

The present study was intended to apply certain agro-industrial waste substrates for cultivating economically important *Bacillus* species. The agro-industrial wastes capable of replacing the conventional and expensive laboratory media ingredients may by pass the economical constrains in scalable application of these species for their value added products. While on the other hand, consumption of such wastes will solve the issue of agricultural waste management.

The *Bacillus* species screened in the present study expressed better levels of growth in aqueous extracts of peels of watermelon, mango, apple, banana, potato and sugar cane. The trend of select microbial growth dictates useful consumption of the bio-wastes for their more or less predictable utilization as substrates for different biotechnological processes. In present study, among six *Bacillus* species, the *B. megaterium* grew well in 2% aqueous extract of watermelon peels as compared to the other substrates. The absorbance of *B. megaterium* recorded up to 2.42±0.21 in 2% aqueous extract of the watermelon peels. Watermelon crop is approximately wasted up to 20% of its annual yield in the fields. Secondly, a threshold neutraceutical value exhibited by lycopene and L-citrulline of watermelon makes it worthy of consideration for microbial growth. The findings of present study dictate that the select bacterial species *B. megaterium* grew best at neutral pH, 27°C with 10% inoculum size when aerated conditions. Neutral optimum pH and temperature optima in the mesophilic range render the microbe a good candidate of processes to be run without additional input of stresses to the environment.

Multiple metal resistances along with antibiotic resistance potential have been found in the select *B. megaterium*. *B. megaterium* has been scientifically engaged for over 50 years, due to its owns some very worthwhile rare enzymes and extraordinary aptitude for the fabrication of exoenzymes. Moreover, It is an appropriate cloning host for intact proteins production; due to absence of external alkaline proteases and can soundly sustain a range of plasmid vectors. Genetic implements for this species consist of transducing phages and a number of mutants covering the progressions of biosynthesis, division, sporulation, germination, catabolism, recombination and antibiotic resistance. The plasmids of *B. megaterium* strain have numerous rare metabolic genes that may be beneficial in bioremediation (Vary *et al.,* 2007). The information provides solution for agricultural waste management with concomitant benefit of industrial scale production of value added products. For concerned bacterial growth, nutritional cost becomes the major hurdle. Results of this attempt demonstrate application of suitable agri-wastes for cultivation of bacteria to address bioremediation process in an economically affordable way.

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