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Scrutiny of extra-cellular productions among the *Bacillus subtilis* cultures: Rotten potato used as fermentation substrate

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The agro-waste of potatoes is rich with valuable carbon sources. In present study, *Bacillus subtilis* 168 was grown on various sub-merged fermentation cultures based extracts of potato wastes like as LB_1 (TY- medium), LB_2 ($^1/_8{}^{th}$ strength TY-medium), LB_3 (5% extract of potato-peels in LB_2), LB_4 (5% extracts of peeled off potatoes in LB_2) at 37°C for 18 hrs and 40 hrs of incubation. Among the cultures, cell growth was higher in LB_1 and LB_4 harvested at 18 hrs, while it decreased significantly in all cultures harvested at 40 hrs of incubation except LB_3. Maximum reducing sugars observed in LB_3 (3.404±0.147mg ml^{-1} at 18 hours) cultures ($p \geq 0.05$), while total proteins in LB_3 cultures harvested at 40 hrs. Among the enzyme activities, amylases showed maximum activities (3.584±0.155 U. ml^{-1} min^{-1}) in LB_1 and LB_4 (3.498±0.548 U. ml^{-1} min^{-1}) cultures harvested at 40 hrs of incubation, while xylanases in LB_1 and LB_4, lipases in LB_3 and LB_4, invertases in LB_1 and LB_3 and proteases in LB_3 and LB_4 cultures (harvested at 40 hrs of incubation). In conclusion, the selection of optimal substrate is necessary to obtain maximum desired products. This experiment may be helpful in selection and utilization of low cost freely available agriculture ligno-cellulosic rich wastes for production of industrial enzymes.

Keywords: *B. subtilis*, potato wastes, cell growth, reducing sugars, proteases, total proteins.

INTRODUCTION

The agriculture plants wastes are rich with low cost carbon based energy sources. Almost 12-20 % waste is produced by the potatoes. It could be major fermentable industrial raw material with microorganisms (Mahmood, Greenman, & Scragg, 1998; Pandey et al. 2000; Pandey & Soccol, 1998). The *Bacillus subtilis* is one among the members of *Bacillus* genus has been considered very important for production of various industrial compounds. This organism is a non-pathogenic and also known as SAFE gram positive bacterium (GRAS), which is able to secrete a large many volatile heterologous extra-cellular proteins including the organic acids (WHO, 2002; Kalogeraki and Winans, 1997; Ding et al. 2014 & 2015). In the growth cultures, *B. subtilis* remains or performs effectively on population of other micro-flora and biological-compounds of food-nutrient flavors due to its biotransformation capabilities (Nakamura, 1987; Yan et al. 2013). To obtain these products on pure

commercial carbohydrates sources increases the costs of products while many alternate switches are obtainable to develop a low cost single cell based fermentation system (Dhillon et al. 2011).

The enzyme acts as the biological catalysts, which have been mostly produced by microbial fermentation. There are many commercially biochemical products obtained through fermentation like citric acid, lactic acid, ethanol, acetic acid including enzymes. The adaptive enzymes are produced by microbial cells required for usable amount of degradation or alteration in specific substrate within the growth medium (Prakasham and Sarma, 2006; Poorna and Prema, 2007). Whereas, the constitutive enzymes are only produced constantly, while irrespective either in presence or absence of the particular substrate within growth medium. The exo-enzymes perform the hydrolysis in the adjacent fermentation medium to degrade polymeric materials that are too big and hard to transport through membrane to the cell. The majority of enzymes generated by microbial fermentation are exo-cellular enzymes which are being useful for commercial purpose (Casida and Quistad, 2005; Rai and Mukherjee, 2009). The precise specifications of substrate and fermentation organism are prime requirement for the production of targets (Doran, 1995). Like as each plant organ have specific nutrient composition as well as specific extra-cellular enzymes are produced by selected fermentation organism (Kampen, 2014; Mok et al. 2019).

The *B. subtilis* is industrially important organism for the manufacture of protein based products including enzymes (proteases, amylases, lipases) to potential peptide (antibiotics) (Demain et al. 1985; Schallmey and Ward, 2004; Enan et al. 2020). This organism has great capacity for potato-raw-digestion as it has high levels of hydrolytic enzymes production in the cultures (Hayashida et al. 1988). It also depends on the availability of types of substrate in the culture (Vallander and Eriksson, 1990). For this study, *B. subtilis* is selected as fermentation organism as it is able to grow happily on the carbon rich agriculture wastes. The potato is used as a fermentation carbon source or substrate. Various parameters including the analysis of the products of sacchrifcation and extracellular hydrolytic in culture supernatant of selected fermentation organism. The present study could be helpful in near future for the selection of substrate for efficient productions through fermentation.

MATERIALS AND METHODS

Fermentation organism and cell culture

The already isolated pure culture of *Bacillus subtilis* 168 was taken from its preserved glycerol stock stored at -20°C in the institute. For the activation of *B. subtilis* 168, sterile wire-loop was dipped in the culture and streaked on the LB plates. These cultures were incubated at 37°C for overnight. The single colony was selected and transferred in the test tube which contains 2 ml LB liquid medium (Sambrook and Maniatis, 1989). Test tube was incubated (37°C) for overnight. Its 0.5 ml was transferred in fresh 50 ml LB liquid medium and incubated again at 37°C for 1 hour. Now this culture was used as a master culture.

Preparation of standard control cell culture

The standard medium for cell culture for the *B. subtilis* 168 was prepared by mixing the 1 % Tryptone, 0.5 % Yeast Extract and 0.5 % NaCl. It was used as the TY/LB (*Luria-Bertani*) liquid medium, while for LB solid or plate medium was prepared by just mixing with 1.2 % agar-agar. The pH of each medium was adjusted to 7.0 before its sterilization.

Collection and preparation of fermentation substrate

For the preparation of the fermentation substrate, the rotten potatoes were taken from the vegetable-market of the city. The collected potatoes were 50 % rotten in outer appearance. They were washed with tap-water. The peels of potatoes were excised with fine knife. Both peels and peeled potato were washed with running tap water in the laboratory. The stuff was dried with toilet tissue-papers and weighed exactly 50 g.

Table1: Composition of growth medium of *Bacillus subtilis* supplemented with extracts of rotten potatoes as fermentation substrate.

#s.	Medium	Composition of medium
01.	LB_1	LB/TY Medium: 10 gL^{-1}Tryptone, 5 gL^{-1}, yeast extract, 5 gL^{-1} NaCl
02.	LB_2	$^1/_8{}^{th}$ strength TY medium + ¾ dH_20
03.	LB_3	LB_2 + 5 % extract of peels of rotten potatoes
04.	LB_4	LB_2 + 5 % extract of peeled rotten potatoes
Note: *Each culture with 25 ml volume maintained in conical volumetric flask (200 ml). Eachculture maintained on 4-replicates.*		

They were grinded in equal volume of sterilized distilled (dH_2O), filtered with muslin-cloth and used as fermentation substrate. The both

filtrates were mixed in $^1/_{8^{th}}$ strength TY/LB medium. The designed experimental set-up is given in table 1.

Culture incubation and its growth rate

All fermentation cultures were inoculated with the master culture. The initial OD600 of each culture was adjusted to 0.02 with master culture and cultures after inoculation were incubated with 250 rpm shaking conditions at 37°C. The cultures were harvested after incubation exactly 18 and 40 hours. The OD600 of each culture was taken at the time of harvest and then centrifuged at 5,000 pm for 7 minutes. The cell pellets were transferred to fresh Eppendorf tubes and frozen at -20°C, while culture-supernatant was preserved at 4°C for further analysis.

Biochemical analysis of culture supernatant

For the measurement of total sugars contents in the supernatant, the procedure developed by Bennett (1992) followed. Exact 0.5 ml supernatant mixed with 2.5 ml conc., H_2SO_4 and then 50 µl 80% phenol was dropped into the reaction glass test-tube. After waiting 10-minutes at room temperature for its absorbance OD485 was read and finally quantified from standard curve. The reducing sugars also measured by mixing 0.5 ml sample-culture with 2 ml 2,6-dinitrosalicylicacid (DNS). For 5-minutes, the reaction mixture was heated in water-bath at H_2O-boiling point. When cool down at room temperature, its absorbance read at OD540 (Miller, 1959). The total protein contents were quantified with Lowery's method (Lowery, 1951). Briefly, 0.5 ml culture-sample mixed with 2.5 ml alkaline-copper-reagent. Reaction was kept at room-temperature for 10-minutes than 0.25 ml follin-reagent was added and after 30-minutes its OD750 was read.

The extracellular enzyme activities analysis

A number of enzyme activities were analyzed in the supernatant of *B. subtilis* 168 culture supplemented with rotten potato as carbon source. *Alpha-amylases assay*: it is based on reduction in intensity of blue colour due to starch hydrolysis by enzyme (Bajpai and Bajpai, 1989). For reaction, 1 ml crude-enzyme (culture-supernatant) mixed with 10 ml 1% starch solution and incubated at 50°C. After 10-minutes, reaction was stopped with 10 ml 0.1 N HCl. Take 1 ml of above reaction and mixed with 10 ml 0.1N HCl than its 1 ml mixed with 10 ml 0.05% iodine (0.5 % KI) solution. The absorbance of blue-coloured solution was read at OD660. *Proteases activities*: It was performed by following the reported method (Gençkal and Tari, 2006). Exact 1.0 ml supernatant was taken in glass test-tube and mixed with equal volume of freshly prepared casein (as ssubstrate). At 37°C for 60 minutes, reaction mixture was incubated. The 2.0 ml TCA (Trichloroacetic acid; 15%) added and it was centrifuged for 10-minutes at 5000 rpm. The 1.0 ml supernatant transferred to another fresh test-tube and 4.0 ml 0.5 N NaOH and 1.0 ml folin's reagents [1:1 diluted; *Folin–Ciocalteu reagent* (FCR)] added. With dH_2O, its volume was raised to 10 ml and absorbance was read at OD625 against blank on spectrophotometer. The proteases activities were also analyzed under various physio-chemical stressed conditions. Like as for metal stress, enzyme crude activity was determined under standard assay supplemented with 5 mM cadmium chloride ($CdCl_2$) as metal ionic stress.

Quantification of xylanases activity: It is a calorimetric method (Kandeler et al., 1999) and performed by taking 1.0 ml culture in glass test tube. Equal volume of fresh prepared substrate (xylose) was mixed thoroughly and incubated at 60°C (wait for 15-minutes). After that 2.0 ml of DNS reagent was added for the purpose to stop the reaction and its OD540 was read on spectrophotometer. The *lipases activities*: This assay was conducted calorimetrically by using culture supernatant as crude-enzyme (Willerding et al., 2011). Exactly, 0.05 ml culture-supernatant mixed with 2.50 ml *p*-nitrophenyl-palmitate (used as substrate) solution. This reaction mixture was kept for 10 min., at 37°C. Now the reaction is ready take absorbance at OD410 on spectrophotometer. *For invertases activities*: The 1.0 ml of culture supernatant was pipetted in the glass test tube than 0.5 ml sucrose (0.1 % in dH_2O, g/v) as its substrate. This reaction mixture kept or incubated for 15 minutes at pre-maintained 35°C than 2.0 ml DNS was added slowly. After boiling for 5 minutes, its absorbance OD540 was noted (Dustmann and Katharina, 1985).

Statistical Analysis

Each culture was maintained on 04 replicates per substrate/treatment. Collected measurements of present study were subjected for significance analysis. It was computed CoStat (version 3.03) program (CoHort, Berkeley, USA). The significant mean values from the control to treated cultures were subjected for DMR (Duncan Multiple Range) test at the level of 5 % significance (Steel and

Torrie, 1986; Behrens, 1997; Queen and Keough, 2002).

RESULTS AND DISCUSSION

Agro-wastes and *Bacillus* species

Today, many countries have been facing economic difficulties including to other factors spending inflate millions to import commercial enzymes by overseas. Cost reduction depends on different aspects like as to search out the primary available natural cheapest sources of bio-synthesizers and carbon sources. For this purpose, the agriculture-wastes are most effective for enzyme production from the microbial cells. Previously, differential output of various strains on different agro-industrial wastes has been observed for extra-cellular production including enzymes, antibiotics, amino acids and vitamins (Eppinger et al., 2011; Alina and Petruta, 2015; John and John, 2015). Many members of microbes such as bacteria, yeast and fungus have been utilized to synthesis secure prebiotics to support human health naturally (Aachary and Prapulla, 2011; Cutting, 2011; Agersø et al. 2019). A population of *Bacillus* species are growing in human gut to intestine and they are producing large numbers proteins to maintain other intestine micro-flora population (Schäfer et al. 1996; Srinivasiah et al. 2008; Lee and Siragusa, 2010). Same has also been performing various valuable bio-tasks to prepare several fermented foods (Wang & Fung, 1996; Ikeda et al. 2006; Ravasi et al. 2015).

Cell cultures and growth rate of *Bacillus subtilis*

The present study was aimed to produce extracellular enzymes with *Bacillus subtilis* 168 on carbon rich agro-waste sources under sub-merged fermentation conditions. The rotten potatoes used as agro based substrate for growth of selected fermentation organism (Table 1). These alternative phyto-based cultures have shown differential growth pattern than the standard nutrient growth cultures (Fig 1). Rate of cell multiplications observed in both harvests taken at 18 hrs and 40 hrs incubation. Maximum cell multiplication was observed in LB_1 (TY Medium) and LB_4 (¼ TY medium+ 5% Rotten potato extract) after at 18 hrs of incubation. The cell growth of these cultures was observed in a reduction trend at 40 hrs of incubation except LB_3 (¼ TY medium+ 5% Rotten potato peels) with increasing number of cells (Fig 1). Minimum growth was observed in LB_2 (¼ TY medium+ ¾ dH_20) nutrient deficit cultures in both cultures harvested at 18 hrs and 40 hours of incubation (Fig 1).

Comparatively, the *B. subtilis* cultures based on potato peels used as carbon source showed good and stable growth rates than cultures based on peeled-potatoes and even it remains good than the standard TY-medium significantly. These differential (lowest to highest) growth rates could be due to available free energy in the supplied substrates as the nutrition in the cultures (Schallmey et al., 2004). Meanwhile, overall cell growth rate was decrease in all the cultures at the harvest of 40 hrs of incubation, because of consumption of nutrition by the growing organism. Among the cultures, even after 40 hrs of incubation the stable growth was observed in LB_3 than other cultures, which was supplemented with potato peels wastes (PPW).These results about the cell growth on rotten potatoes could be the useful economic and easily accessible agro-substrate for the biosynthesis of many important industrial biocatalysts or enzymes including the other many useful secondary metabolites.

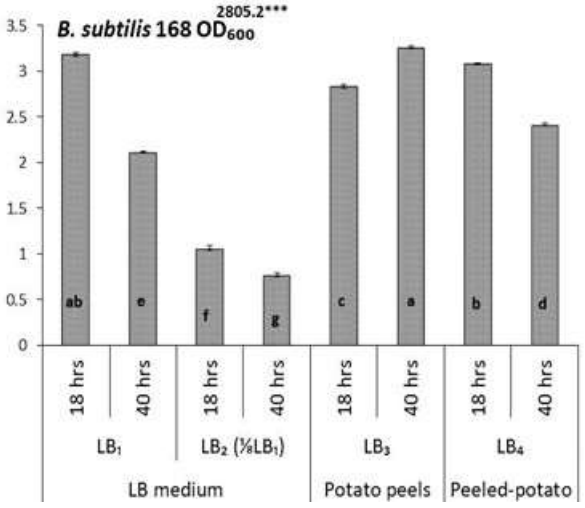

Figure 1: The rate of cell growth among the cultures of the *Bacillus subtilis* supplemented with extracts of peels and peeled rotten potatoes at two harvests with time interval of 18 and 40 hours of incubation.

Measurements of extracellular bio-chemicals

The total sugar contents observed in both culture supernatants harvested after 18 hrs and 40 hrs of incubation. Maximum sugars were observed in LB_3 cultures than others to the standard *Bacillus* growth medium LB_1 (Fig 2). The

total sugars among the cultures were increased significantly up to the time of harvest at the 18 hrs of incubation significantly, while it decreased in overall cultures at the time of second harvest after 40 hrs of incubation. The decrease or increase in the soluble sugars at the 18 and 40 hrs of culture harvests depends on the rate of cell growth and duration of culture incubation (Travert et al. 1997; Nichols et al. 2005; Qurashi and Sabri, 2012). According to this parameter has shown that the peels or skin of potatoes displayed stability in cell growth. The peels and peeled potatoes have been involved to trigger the secretion of specific enzyme in the medium initially at 18 hrs of incubation, while it is decreased due to surplus saccharification and accessibility of developed organic solutes (Volesky et al. 1984).

From the results (Fig 2), variant levels of reducing sugar productions were observed among the *Bacillus* culture supernatant. Highest reducing sugars were observed in LB_3 medium (potato peels extract) and then in LB_1 cultures harvested at 18 hrs of incubation. Lowest reducing sugars noted in both harvests taken at 18 and 40 hrs of incubation in nutrient deficit LB_2 cultures.

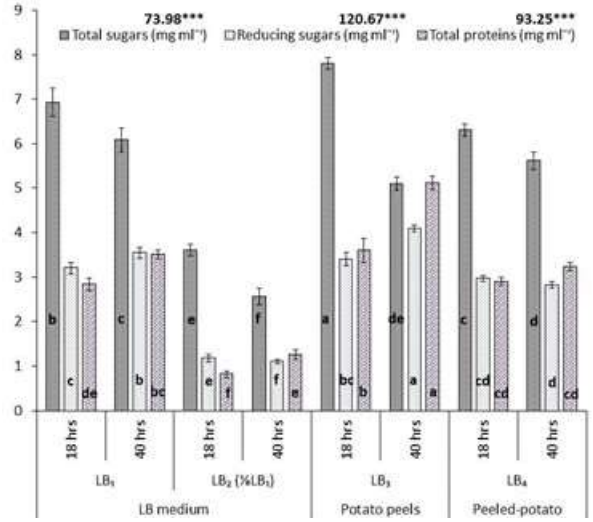

Figure 2: Analysis of the rate of saccharification and total proteins production among the cultures of the *Bacillus subtilis* supplemented with extracts of peels and peeled rotten potatoes at two harvests with time interval of 18 and 40 hours of incubation.

These increasing or decreasing trends in fermentation cultures depends on the availability of carbohydrates in the cultures to the organism and rate of their consumptions including to the other physio-chemical conditions of the cultures (Rezic et al. 2013; Yücel and Aksu, 2015; Borkowski et al. 2016).

The *Bacillus subtilis* is horse secretor of several heterologous proteins in its culture (van Wely et al. 2001). In this experiment, differential protein production rates observed among the cultures from time to time, which is also dependent on the culture growth stage of normal cell growth. The maximum proteins in the supernatant were observed in the 2nd harvest taken at 40 hrs of culture incubation, while it was less in all the cultures harvested at 18 hrs of incubation (Fig 2). As the *Bacillus* cultures harvested two time after 18 and 40 hrs growth, which has remained the major time-factor for level of cell development as well as extracellular productions levels in liquid medium. It could be due to the change in liquid nutrient medium composition in corresponding to the usable components of the nutrient in medium. At the later stages of the cultures, the nutritional stressed response may be apparently causes the specific changes in the metabolic activity of the cultured cells concerned the industrial interest in spite contaminated chemical products (den Besten et al. 2013; Święciło and Zych-Wężyk, 2013).

Analysis of enzyme activities

In this study, the amylases activities observed significantly higher in the supernatant of LB_1 (3.584±0.155 U. ml^{-1}) and LB_4 (3.498±0.548 U. ml^{-1}) cultures of *B. subtilis,* which were harvested after 40 hrs of incubation (Table 1 and 2). The *B. subtilis* showed lower amylase activities in the supernatants harvested after 18 hrs of incubation in LB_2 (1.161±0.023 U. ml^{-1}) cultures. The amylases are mostly applied for the liquefaction of starch into the mixture of simpler oligosaccharides like as maltose and fructose sugars or their syrups (Schaechter, 2009; Sailas et al. 2013).

Among the cultures, higher activities of xylanases in the supernatant of LB_1 (3.192±0.008 U. ml^{-1}) and LB_4 (3.361±0.007 U. ml^{-1}) at 40 hours were observed ($p \leq 0.05$). Despite of that, it showed lower xylanase activities in the harvest of LB_2 (0.758±0.006 U. ml^{-1}) medium at the harvest after 18 hrs of incubation (Table 2). The measurements of the xylanases hydrolytic bio-reduction of xylan showed that it was simplified into different xylo-oligosaccharides instead the significant xylose accumulation (Tan et al. 1987). There xylobi- and xylo-trioses are the hydrolysis products from the cultures of xylanase produced with *B. stearothermophilus* supplemented with oat spelt

xylan (Nanmori et al. 1990) Meanwhile, over-productions of the catabolites may be the base of xylanase suppression in the cultures(Cihan et al. 2011; Shulami et al. 2014).

Table 1: Analysis of different enzyme activities (U. ml^{-1} min^{-1}) produced among the cultures of the *Bacillus subtilis* supplemented with extracts of peels and peeled rotten potatoes at two harvests with time interval of 18 and 40 hours of incubation.

#s.	Enzymes	LB medium		Potato peels	Peeled potatoes	p-significance
		LB_1	LB_2 (¼LB_1)	LB_3	LB_4	
		Culture-supernatant harvested at 18th hrs of incubation at 37°C				
01.	*Amylases*	6.347±0.111^{d}	1.749±0.022^{f}	7.839±0.059^{b}	6.271±0.034^{d}	792.12***
02.	*Xylanases*	2.903±0.029^{d}	0.758±0.056^{h}	2.209±0.016^{f}	3.087±0.005^{c}	5918.7***
03.	*Lipases*	0.363±0.007^{d}	0.156±0.004^{e}	0.404±0.009^{c}	0.395±0.004^{c}	520.12***
04.	*Invertases*	0.439±0.005^{b}	0.106±0.004^{f}	0.368±0.006^{d}	0.340±0.003^{de}	367.86***
05.	*Proteases*	0.543±0.003^{g}	0.743±0.003^{f}	1.455±0.003^{b}	1.242±0.003^{d}	20286.3***
	Culture-supernatant harvested at 40th hrs of incubation at 37°C					
01.	*Amylases*	6.722±0.058^{d}	2.133±0.019^{e}	8.584±0.192^{a}	7.224±0.081^{c}	*As in upper rows*
02.	*Xylanases*	3.192±0.007^{b}	0.986±0.006^{g}	2.364±0.007^{e}	3.361±0.007^{a}	
03.	*Lipases*	0.388±0.002^{c}	0.161±0.004^{e}	0.492±0.006^{a}	0.471±0.006^{b}	
04.	*Invertases*	0.456±0.004^{a}	0.341±0.008^{ef}	0.399±0.007^{c}	0.378±0.005^{d}	
05.	*Proteases*	0.548±0.003^{fg}	0.764±0.003^{e}	1.548±0.003^{a}	1.427±0.003^{c}	

Note: *The data-values of different analysis are the means of 4-replicates with standard error, while letters i.e. a,b,c,d ... for DMR test and *** for representing the data significant at 0.05 level (5%).*

The lipases are enzymes, which catalyses the hydrolysis fats (lipids). In our results, the lipase activity in the *Bacillus* cultures was observed higher in the LB_3 (0.492±0.006 U. ml^{-1}) and LB_4 (0.471±0.006 U. ml^{-1}) cultures harvested at 40 hrs of incubation. The production of lipases increased with bacterial growth as reported in *Acinectobacteri* and pseudomonas species (Minden et al. 1995; Nadal et al. 2002). The lowest lipase activity noted in LB_2 (0.156±0.004 U. ml^{-1}) cultures at 18 hrs of incubation (Hasan et al., 2006). The invertases activities among the *B. subtilis* cultures were noted variant among the cultures supplemented with different parts of the same rotten potato tubers. The rate of saccharification of the fermentation organisms depends on their respective levels of enzymes production as well as on the available type of substrate in culture medium (Dipasquale et al. 2009; Schallmey et al. 2004; Awad et al. 2013). The maximum activities of the invertases were noted in LB_3 (0400±0.005 U. ml^{-1}) and LB_1 (0.456±0.004 U. ml^{-1}) cultures harvested at 40 hrs of incubation, while lower invertases activities observed in LB_2 (0.106±0.004 U. ml^{-1}) cultures, which were harvested after 18 hrs of incubation. The rate of hydrolytic enzyme production is restricted with growth phase just as activities of invertases slightly decreased after log phase (Glissmann and Conrad, 2002).

The proteases activities in the supernatant of *Bacillus* cultures were observed maximum in LB_3 (1.549±0.003 U. ml^{-1}) and LB_4 (1.408±0.021 U. ml^{-1}) cultures harvested after 40 hours of incubation, while it was minimum in LB_1 (0.140±0.003U. ml^{-1}) standard cultures than others when harvested after 18hrs of incubation. The higher activities could be the sign in particular of the steady-state or decline in culture growth phase. Probably, it may be the breakdown of proteins by protease to fulfil the national requirement of the cultured cells under deficit conditions at decline growth phase (Sternberg, 1976; Berg and Pettersson, 1977). The proteases perform big role in cleaning of dead cells as well as the secreted proteins which are not need of the growing cultures are degraded well with proteases (Davies et al. 1987; Kaminskyy and Zhivotovsky, 2012).

Effect of heavy metal on proteases activities

The monovalent and divalent metals have significant effects on the protease activities. In present experiment, the effect of divalent metal cadmium (Cd^{2+}) on the proteases was assessed in culture supernatant (crude enzyme) of *Bacillus* cultures. The enzyme activity enhanced in presence of metal ion as cadmium (Cd^{2+}) activity (Fig 3). Maximum activities of proteases under

Cd^{2+} observed in the LB_3 (potato-peels) and LB_4 (peeled-potato) in the supernatant of the 2nd harvest taken at 40 hrs of incubation and minimum in LB_1 cultures (without metals stress) when harvested after 18hrs of incubation (Table 1, Fig 3). It means that cadmium may be acting as the cofactor of proteases or prevent enzyme denaturation (Donaghy and McKay, 1993; Adinarayana et al. 2003; Beg et al. 2003). Reduction in enzyme inhibition and retention of tertiary protein structure has also been reported due to their hydrophobic interactions with metals. In addition, inhibition of relative activity by metal ions is a common phenomenon because inhibitors minimize the enzyme efficiency for industrial purposes (Kar et al. 2003).

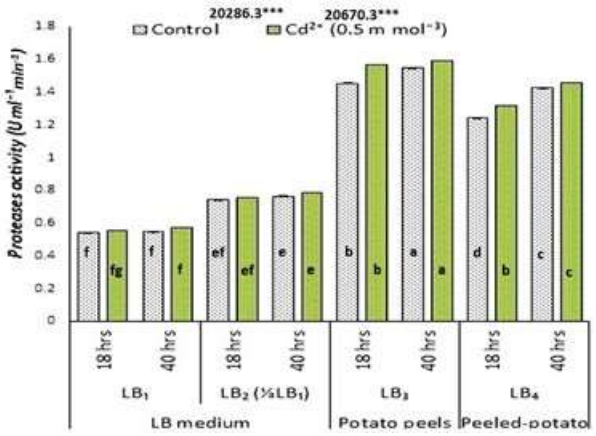

Figure 3: Measurements of the effects of cadmium metal (Cd^{2+}) on the activities of proteases produced among the cultures of the *Bacillus subtilis* supplemented with extracts of peels and peeled rotten potatoes at two harvests with time interval of 18 and 40 hours of incubation.

CONCLUSION

The industrial production of biocatalysts is needed with exponential production. These productions require excess energy, while it could be fulfilled with the utilization of low cost freely available agro-carbohydrate wastes. Potato-waste as a substrate is the cheapest carbon and nitrogen source. *Bacillus subtilis* has performed significant role in the production of various extracellular metabolites including the enzymes. Maximum cell growth other secondary metabolites production and secretion of many important stable industrial enzymes are produced in the culture supplemented with potato-peels (LB_3 cultures). This work indicates the remarkable innovative visions to increase awareness about the extra-cellular productions. This work could be helpful to improve the extracellular productions with the selection of efficient fermentation strains and its subsequent agro-waste as substrate in future.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Author 1 has carried out the present research work. Author 2 designed the objectives and guided throughout the study. Author 3 tabulated the data and statistical analysis. Author 4 has studied the manuscript critically.

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