ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Biotechnology

ISSN 1682-296X
DOI: 10.3923/biotech.2018.95.103

Research Article
Oxalic Acid as the Main Molecule Produced by *Trichoderma asperellum* MG323528 Fermented on Corn Stover Based Medium

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Abstract

Background and Objective: Organic acids have several pharmaceutical, food, agricultural and medical applications. Corn stover represents a serious environmental problem. The present study investigated the bio processing of such readily available low-cost biomass with microorganism into valuable organic acids that expected to neutralize the negative impact on the environment and minimize the production costs. **Materials and Methods:** A novel cellulolytic *Trichoderma asperellum* MG323528 was selected as a new corn stover decomposer that could transform it into various bio-products. The fungus was incorporated in corn stover-based medium for the production of organic acids. The Box-Behnken experimental design was applied to maximize the total organic acids production especially oxalic acid. **Results:** The optimum composition of solid-state fermentation medium was found to contain17.83 mg P from rock phosphate, 5.61 mg N from $(NH_4)_2SO_4$ and 9.84 mg $MgSO_4 \cdot 7H_2O$ per 1 g of corn stover, yielding a total of 209.11±1.20 mmol organic acids. According to the HPLC screening, the main organic acids detected in the fermented corn stover was oxalic acid, representing about 78% of the total organic acids, in addition to minor amounts of citric, formic, salicylic and ascorbic acids. **Conclusion:** This kind of homo-fermentation could be considered for large-scale production of oxalic acid on an economic medium of CS using the promising *T. asperellum* MG323528 strain.

Key words: Organic acids, box-Behnken design, *Trichoderma asperellum*, HPLC, bio processing

Citation: Abdulaziz Abdulrahman Al-Askar, WesamEldin Ismail Ali Saber, Khalid Mohamed Ghoneem and Younes Mohamed Rashad, 2018. Oxalic acid as the main molecule produced by *Trichoderma asperellum* MG323528 fermented on corn stover based medium. Biotechnology, 17: 95-103.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Organic acids (OA) are weak acids compounds, constituting a key group among the building-block chemicals, because of their functional groups, organic acids are extremely useful as starting materials in a variety of fields such as agricultural, food and pharmaceutical industries. For instance, lactate is typically used as a buffer solution, citric and acetic acids are used in the pharmaceuticals, food industries and antimicrobial agents, oxalic acid is utilized in cleaning, bleaching and removal of rust, itaconic acid is exclusively used in the polymer industry, coatings, adhesives, thickeners and binders and ascorbic acid has wide applications in medicine as one of the most efficient antioxidants[1-3].

In order to economize the production process, it is better to found alternatives to the conventional substrates (glucose and/or sucrose) for OA production, lignocellulosic materials such as corn stover (CS) are abundant, attractive and usually low-priced[4-5].

The lignocellulosic residue of CS represents about the half of the overall *Zea mays* L. biomass with the chemical compassion of 42% glucan, 20% xylan, 15.8% lignin, 3.6% protein, 4.2% silica and ash and 13.8% of other constituents, this relative proportions of the stover fractions makes it ideal fermentation medium, so the proper bioprocessing of CS is very promising approach and potentially offer a low-cost resource to various valuable bioproducts[4]. Besides, this process introduces environmentally safe management of CS residual.

Most of OA are final products or at least natural intermediates in major metabolic pathways[2]. Many phosphorous solubilizing microbes have the ability to produce organic acids, compared to bacteria, phosphate solubilizing fungi (PSF) have ten times higher in their ability to secrete organic acids. Therefore, PSF are considered as primary candidates in this respect[6-7].

Several fungi could be involved in the biodegradation of lignocellulose, of them, the filamentous fungi have been extensively used as cell factories for different biotechnological products, including OA synthesis, of them several species of *Aspergillus* and *Penicillium* were reported[2,7]. However, all these studies confirmed the importance of cellulolytic activity of the used fungus, since the first step in the bio conversion of lignocellulosic residuals is its degradation to simple monomers by the proper enzymes. The applied strains in the bio-production of OA are particularly suitable for food or drug production, as they have been certified as generally regarded as safe by the FDA of the USA and the WHO of the UN.

Bio conversion procedure based on solid-state fermentation (SSF) is preferable because of its high productivity, cheap fermentation media, sufficient oxygen utilization, simple preparation and low energy consumption[8]. Importantly SSF simulates the natural growth condition of the microbe, especially, when using a proper experimental design such as response surface methodology, which provides statistical modeling for studying the interactions among the fermentation parameters and calculates the optimal level of each medium components that maximize production of a given target[9]. However, little information are available regarding the bio processing of CS residual into organic acids in the presence of cellulolytic and phosphate solubilizing fungi, using proper experimental design.

In this paper, conditions favorable for the production of OA on the raw cheap CS-substrate were optimized using anew isolate of *T. asperellum*, oxalic acid could release as the main molecule in the fermented hydrolysate of CS, so OAs were further specified using high-performance liquid chromatography (HPLC).

MATERIALS AND METHODS

Corn stover substrate: Samples of CS residual (maize variety; Odeon) was collected during the summer season of 2017 from Riyadh and Al-Kharj regions in the KSA, dried at 50°C overnight, homogenized and blended in an electric grinder to obtain fine powder (maximum 0.1 cm long) to serve as a solid support and substrate for organic acid production. CS did not subject to any pretreatment to simulate the natural growth conditions of the microorganisms.

Fungal isolates: Eleven fungal isolates in the present study were previously isolated from the decayed residues of CS of maize, they were chosen based on the ability to hydrolyze carboxymethyl cellulose on agar plates by the formation of a clear zone around the grown fungus. They were; *Aspergillus* sp. (CS13 and CS69), *Chaetomium* sp. CS31, *Mucor* sp. (CS29 and CS37), *Neurospora* sp. (CS6 and CS31), *Nigrospora* sp. CS38, *Penicillium* sp. CS7, *Trichoderma* sp. (CS1 and CS5). The potent isolate number CS5 was molecularly identified as *Trichoderma asperellum* CS5, the DNA sequence was deposited in the GenBank database under the accession number of MG323528.

Cellulolytic activity of the fungal isolates: The culturing conditions were performed based on the solid-state fermentation (SSF) technique for screening the obtained fungi

for cellulolytic activity, total organic acid production as well as during optimization study. CS-based medium (1.0 g) was used to simulate the natural growth conditions. The contents were mixed thoroughly with 5 mL salt solution composed of $MgSO_4$; 1 g L^{-1}, $(NH_4)_2SO_4$; 1.6 g L^{-1} and KH_2PO_4; 4 g L^{-1} with pH 5 in 250 mL Erlenmeyer flasks and autoclaved for 15 min at 121°C.

Inoculation was carried out using three 0.5 cm PDA disks of 5 days old culture from the tested fungus. During 7 days incubation period at 28°C, the moisture content was kept at 65% by the addition of sterilized water when needed,then, 10 mL of 0.01% Tween 80 was mixed with the fermented components of each flask and kept on a rotary shaker at 150 rpm for 30 min, the culture was then filtered through filter paper, followed by centrifugation at 5000 rpm for 20 min. The post-culture filtrate was used for examination of both cellulolytic and xylanolytic enzymes.

Enzymatic assay: Cellulolytic activity on carboxymethyl cellulose (CM case) and xylanolytic activity on oat spelt xylan (xylanase) were determined according to Ghose[10] and Bailey *et al.*[11], respectively. The released reducing sugars by both enzymes were determined according to Miller[12]. The amount of enzyme required to release 1 μmol min^{-1} glucose or xylose under the test conditions was defined as the unit of CM case or xylanase, respectively.

Box-Behnken optimization design: For maximization of OA production by the selected fungus, the response surface optimization was performed using the Box-Behnken design matrix. SSF procedure was employed using 1 g of CS in Erlenmeyer flasks (250 mL) and 5 mL of the previous salt solution, replacing KH_2PO_4 withrock phosphate (RP) that contains 8.0% phosphorus, obtained from Soils, Water and Environment Research Institute, Agricultural Research Center, Giza, Egypt. The salts of the solution as the independent variables were studied each at three levels. Accordingly, the independent variables were; rock phosphate (RP), $(NH_4)_2SO_4$ and $MgSO_4 \cdot 7H_2O$. The levels of each independent variable and the design of the experiment were indicated in Table 1. The behavior of the three-factor system was explained by the following second order polynomial Eq.:

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ij} X_i X_j + \beta_{ii} X_i^2$$

Where:

Y = Predicted response (organic acids)
β_0 = Intercept term
β_i = Linear effect
β_{ij} = Interaction effect
β_{ii} = Squared effect
X_i = Independent variable (RP, $(NH_4)_2SO_4$
X_j = Independent variable $MgSO_4 \cdot 7H_2O$)

The regression equation was generated using Design-Expert version 7 software by setting the optimum conditions that yield the maximum value of OA. The total OA in the filtrate was spectrophotometrically determined at 500 nm[13]. The statistical model was experimentally validated with respect to OA production under the conditions predicted by the preceding model under SSF conditions.

Table 1: Organic acids biosynthesis on 1 g CS-based medium, depending on Box-Behnken design with the three tested independent variables after 7 days of incubation

	Tested independent variable			
Run	RP, mg P (X_1)	$(NH_4)_2SO_4$, mg N (X_2)	$MgSO_4 \cdot 7H_2O$, mg (X_3)	Response (Total OA, mmol g^{-1} CS)±SD[b]
1	5	3	10	135.62±8.17
2	35	3	10	116.01±9.80
3	5	9	10	129.08±6.57
4	35	9	10	70.26±3.27
5	5	6	5	132.35±4.90
6	35	6	5	81.70±8.17
7	5	6	15	122.55±6.54
8	35	6	15	93.14±3.21
9	20	3	5	130.72±6.54
10	20	9	5	58.82±6.54
11	20	3	15	88.24±6.53
12	20	9	15	111.11±3.26
13[a]	20	6	10	205.29±8.16
14[a]	20	6	10	203.66±11.44
15[a]	20	6	10	203.66±4.90

[a]Center point, [b]Standard deviation

Specification of OA using HPLC: Based on the optimum conditions obtained from the preceding optimization trials of Box-Behnken matrix. Sample of post-culture filtrate was extracted by mixing 1.0 g sample with methanol (20 mL), at 40°C, then filtered and concentrated under reduced pressure to dryness at 40°C and re-dissolved in acidified water (pH 2) with HCl, followed by evaporation under reduced pressure to dryness (40°C) and re-dissolved in 1 mL of 0.01 N H_2SO_4, of which 20 µL was examined by HPLCunit (Agilent 1200 Infinity Series, United Kingdom), using a C18 column at 30°C. Elution was carried out isocratically with 0.01 N H_2SO_4, as the mobile phase, with a flow rate of 0.1 mL min^{-1}, for 120 min. UV detection was performed at 214 nm.The concentration of OA was determined using the obtained peak areas and retention times of the OA standards[14].

Experimental design and statistical analysis: The statistical analysis software; CoStat version 6.4 (CoHort Software) was used for one-way analysis of variance (ANOVA) of the data. Compare among means of coenzymes activity was performed using Duncan's new multiple range test. The statistical software package; Design-Expert version 7 (Stat-Ease, Minneapolis, MN) was used for generating the Box-Behnken matrix and performing ANOVA. The software was also used for the generation of thesecond order polynomial model, estimation of the optimum level of each independent variable that maximizes OA production. The value of probability (P) <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Quantitative screening of fungi for cellulolytic activity: To initiate the present study, the eleven fungal isolates were screened on the base of the ability of the fungal candidates to degrade cellulosic biomass, to select the efficient fungus that has the complementary enzymatic system (CM case and xylanase) that able to ferment CS-based medium into various organic molecules.

As presented in Table 2, the eleven selected fungal isolates showed a wide range of variation in the CM case and xylanase activities. In this connection, *T. asperellum* CS5 was the most active isolate, recording 6.914 and 14.626 U g^{-1} CS, respectively. The other isolates had a low to moderate activity range. Additionally, all the tested isolates showed various reduction degrees in the pH of their filtrates, indicating the presence of molecules with acidic nature such as OAs in the filtrate.

It is already known that an assortment of cellulolytic fungi inhabit diverse sorts of agrarian biomass and play a critical role in their disruption. For instant, 21 cellulose degrading fungi were isolated from rice straw, of them two *Aspergillus* spp., two *Penicillium* spp. and one *Trichoderma* sp. showed high cellulase activity, in any case, fungal efficiency to degrade the crystalline cellulosic materials depends upon the presence of complete cellulase activities in adequate quantity, i.e., endo-glucanase, exo-glucanase and β-glucosidase activities[8].

Table 2: Screening of fungal isolates for the secretion of cellulose degrading enzyme and organic acids

	Enzymatic activity (µmol g^{-1} CS min^{-1})	
Fungus	CM case	Xylanase
Aspergillus sp. CS13	1.534^{f}	1.463^{cd}
Aspergillus sp. CS69	5.872^{c}	6.777^{b}
Chaetomium sp. CS31	0.511^{h}	2.584^{c}
Mucor sp. CS29	6.554^{b}	0.488^{d}
Mucor sp. CS37	4.887^{d}	5.510^{b}
Neurospora sp. CS31	0.189^{i}	0.000^{d}
Neurospora sp. CS6	1.250^{g}	0.000^{d}
Nigrospora sp. CS38	1.591^{f}	6.826^{b}
Penicillium sp. CS7	1.193^{g}	0.000^{d}
Trichoderma sp. CS1	2.046^{e}	0.000^{d}
Trichoderma asperellum CS5	6.914^{a}	14.626^{a}

Means within the column followed by the same letter(s) are not significantly differed at $p \leq 0.05$

The medium composition was based on CS that composed mainly of cellulose, hemicellulose and lignin, this complex structure, reduces vigorously its biodegradation into simpler units and so requires the aid of several enzymes during the degradation to fermentable monomers. Cellulose is the main targeted polysaccharide because it is composed only of D-glucose units, which is a fermentable sugar for most microorganisms[15]. Cellulases catalyze the hydrolysis of cellulose into glucose, of them, the randomly acting endoglucanases leave the β-1,4-glucosidic bonds in the inner part of cellulose, representing the intermediate step of hydrolysis, combined catalytic action with other cellulases leading to liberation of glucose monomers[16]. The hydrolysis of xylan requires the combined action of endo-1,4-β-xylanase and β-D-xylosidases, releasing fermentable D-xylose monomer from xylan[17].

The obvious activity of CM case and xylanase could be considered indicators for the complementary enzymatic system of CS degradation by the tested fungi in general and *T. asperellum* CS5 in certain, leading to the liberation of hexoses and pentoses as the major product. These monosaccharides represent the building stone of various organic molecules, including Oas. Accordingly, *T. asperellum* CS5 was chosen for use throughout the course of further studies as the most active cellulolytic fungus.

Table 3: ANOVA of OA production obtained from Box-Behnken experiment by *T. asperellum* CS5

Source of variance	Sum of squares	Degree of freedom	Mean square	F-value	p-value
Model	30920.0	9	3435.56	1456.7	<0.0001*
X_1	3140.2	1	3140.15	1331.5	<0.0001*
X_2	1282.9	1	1282.89	544.0	<0.0001*
X_3	16.4	1	16.35	6.9	0.0464*
X_1X_2	384.5	1	384.47	163.0	<0.0001*
X_1X_3	112.8	1	112.80	47.8	0.0010*
X_2X_3	2245.4	1	2245.40	952.1	<0.0001*
X_{12}	6093.3	1	6093.32	2583.6	<0.0001*
X_{22}	9542.0	1	9542.02	4045.9	<0.0001*
X_{32}	11639.7	1	11639.72	4935.4	<0.0001*
Residual	11.8	5	2.36		
Lack-of-fit	10.0	3	3.34	3.8	0.2176^{NS}

*Significant value, NS: Non-significant value

Optimization of OA production: To examine the interaction effect of various concentrations of the independent factors (RP, $(NH_4)_2SO_4$ and $MgSO_4 \cdot 7H_2O$) on total OA production by *T. asperellum* CS5, the Box-Behnken design was employed at three levels each. The obtained results (Table 1) were fitted to the preceding second order polynomial equation to explain the dependence of OA production on the selected medium components, which was found to be:

$$OA = -164.273+6.5\ X_1+52.122X_2+34.31\ X_3-0.218\ X_1X_2+0.071X_1X_3+1.580X_2X_3-0.181\ X_{12}-5.648X_{22}-2.246X_{32}$$

The ANOVA of the quadratic regression model (Table 3) suggested that the model is significant aswas cleared from the model F-value (1456.7). Another, the p-values denoted the significance of the coefficients and also important in understanding the pattern of the mutual interactions between the variables. Values of p less than 0.05 implied the model terms were significant. In the present case, all model terms including X_1, X_2 and X_3, as well as their interaction and quadratic terms were significant. If there were many insignificant model terms, terms reduction may improve the model, however, this reduction procedure is not applied in the present model, since p-values of all terms are significant and important, being lower than 0.05.

The ANOVA implied that the lack-of-fit was not significant and there was a 21.76% chance for the model to be not fitted, the non-significant lack of fit is a good indicator for the model to fit. The model's goodness of fit was also checked by determination coefficient (R^2) being 0.9996, the closer to 1 the better correlation between the observed and predicted responses. The predicted R^2 of 0.9947 is in reasonable agreement with the adjusted R^2 of 0.9989. The higher the adjusted R^2 the more accuracy of the relationships between the three variables (X_1, X_2 and X_3) and OA production response. Similar to adjusted R^2, predicted R^2 indicates how well the model predicts response for new observations, whereas adjusted R^2 indicates how well the model fits your data. Predicted R^2 can prevent over-fitting the model and is more useful than adjusted R^2 for comparing models. Larger values of predicted R^2 suggest models of greater predictive ability. However, the higher the three kinds of R^2 the more accurate of the model design.

Finally, the predicted residual sum of squares value (164.20) was relatively low, indicating the lower possibility of error during the experimental work. The value of the adequate precision ratio (signal to noise ratio) recorded 115.454. The value of ratio more than 4 is an adequate indicator for the overall model to be suitable with in the design space. The coefficient of variation (CV) recorded 1.22, reflecting the high degree of reliability of the experiments that is usually indicated by the low value of CV.

To highlight the roles played by the three factors affecting OA production, the three-dimensional surface response plots were generated in pairwise combination by varying two independent variables, of the three tested factors, within the experimental range and holding the third factor constant at the central point. Figure 1 showed the relationship among the three variables, generally, the maximum production of OA was achieved around the center points of the tested variables. This, in turn, reflecting the accuracy of both variables selection and their tested concentration ranges.

Solving response optimization problem led to obtaining the levels at 17.83 mg P from RP, 5.61 mg N from $(NH_4)_2SO_4$ and 9.84 mg $MgSO_4 \cdot 7H_2O$ per 1 g CS. The theoretical value of the response OA is 206.84 mmol. These calculated levels of the tested variables were experimentally validated; the obtained response recorded 209.11±.20 mmol OA. The calculated value of OA was inline with these experimentally validated, reflecting the suitability and precision of the model to be applied within the experimental range, thus proving the validity of the model.

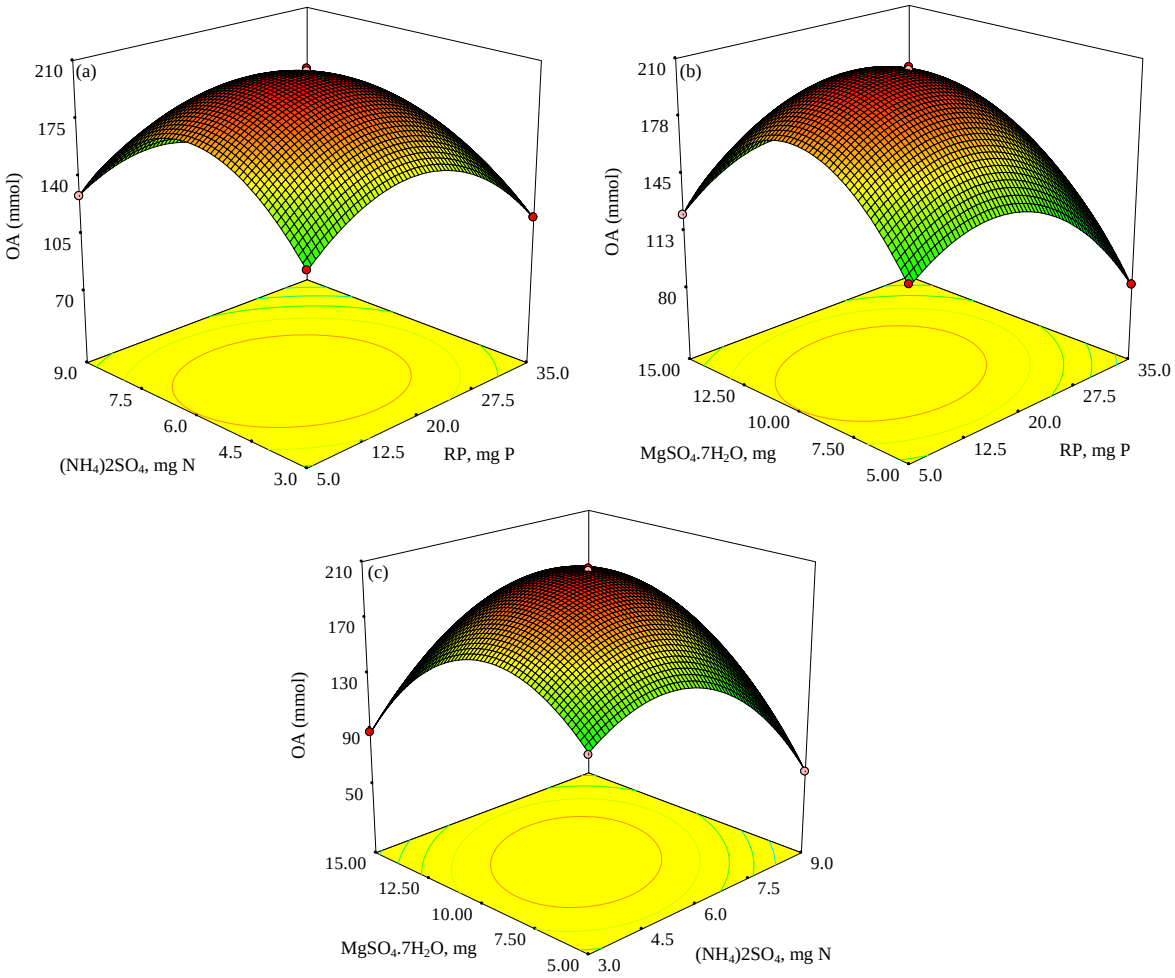

Fig. 1(a-c): Three-dimensional response surface plot for the effect of every pair wise combination on OA production by *T. asperellum*, keeping the third factor at the center point

The fermentation medium was supplemented with RP as a provoker for OA production by the tested fungi, the presence of RP as a sole complex source of P in CS medium induced the fungi towards the solubilization, assimilation and utilization of RP-phosphorus, one possible explanation of solubilization of complex phosphate is the secretion of OA, that is why RP was applied in the OA production medium, another proposed mechanism for the formation of OA is the production of phytase and/or acid phosphatase[5,8].

Indeed, *Aspergillus oryzae* and *A. niger* were characterized by the production of large amount of organic acids in the presence of RP, such organic acids have been also, recognized for phosphate solubilization by several species of fungi, also, the nature of organic acids produced has a considerable effect on the solubilization of insoluble phosphates, the position and type of functional group within each acid seems to be a dominant factor that influences the amount of released P[5,18]. As far as we know, non or scanty information is available about the types and production of OA by the genus *Trichoderma*.

The combinations of inorganic salts of $(NH_4)_2SO_4$ and $MgSO_4 \cdot 7H_2O$, were investigated to obtain ahigher yield of OA. In this connection, Saber *et al.*[8] reported that $(NH_4)_2SO_4$ was favorable nitrogen sources for malic acid production, together with lower concentration $MgSO_4 \cdot 7H_2O$, resulted in the highest yields of malic acid, through the promotion of the mycelial growth and increased the amount of soluble minerals.

HPLC screening of OA in the hydrolysate of CS: Based on the above trial, the scale-up production of OA was carried out using medium under optimized conditions. The hydrolysate of SSF of CS by the *T. asperellum* was quantified for various expected organic acids. The HPLC technique was used for specification of OA because of simplicity, accuracy and speed

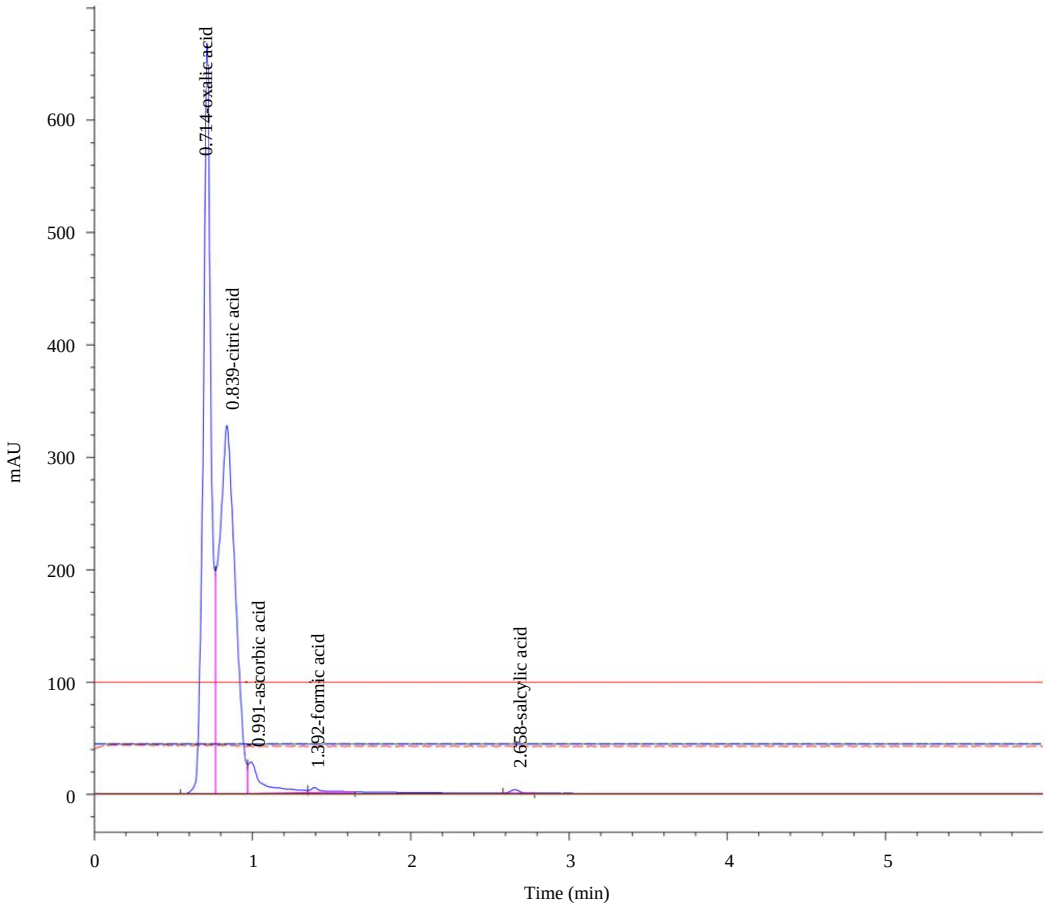

Fig. 2: Diagram of the specification of organic acids analyzed by HPLC, biosyn the sized on the optimized fermented CS-based medium by *T. asperellum* CS5

of analysis, the HPLC technique is an attractive method. The results of HPLC are introduced in Fig. 2. Noticeably, there were only five peaks in the diagram of HPLC analysis, representing all OAs detected in the post-culture filtrate of the 7 days old fermented CS. More illustration was depicted in Fig. 3, as could be seen the mount (mg g^{-1} CS) of the detected OA in descending order were oxalic (27.55), citric (4.96), formic (1.57), salicylic (0.66) and ascorbic (0.47) acids. Oxalic acid was produced in the highest quantity, representing about 78% of the five detected acids. The other four acids occupied about 22%. Therefore, this kind of fermentation could be considered homofermentative, which wholly or principally the single end-product; oxalic acid was produced.

Scanty information is available on types and quantity of organic acids produced during the bioprocessing of various plant biomass residues. This study is spotting some light in this area. Microbial hydrolysis is the initial step in this process followed by biochemical metabolism, as aninstance, citric acid and oxalic acids were reported as the major acidic metabolites during fungal fermentation, each one at different conditions, citric acid is promoted under nitrogen-limited conditions, while oxalic acid under carbon-limited conditions[2].

Most of the low-molecular-weight OA, such as oxalic acid, the predominant acid in the present study, is presumably derived from the tricarboxylic acid cycle, the prominent oxalate production in fungi occurs by a process called glyoxylate oxidation and oxalic acid is biosynthesized from glucose catalyzed by cytosolic oxaloacetase, with hydrolysis of oxaloacetate to oxalate and acetate[2,7,19].

Generally, each acid has unique biosynthesis pathway and some acids share similar pathways. For example, each of citrate and oxalate fermentations is no more than one enzymatic step from the primary pathway of D-glucose and D-fructose metabolism; likewise, the malate production mechanism is abbreviated by one-step of the pathway of fumarate formation. Other acids have more than biosynthesis mechanism such as ascorbic acid, which could be formed by either fermentation, followed by chemical synthesis steps or by fermentation to produce 2-keto-Lgulonic acid, followed by a chemical conversion to ascorbic acid. Another

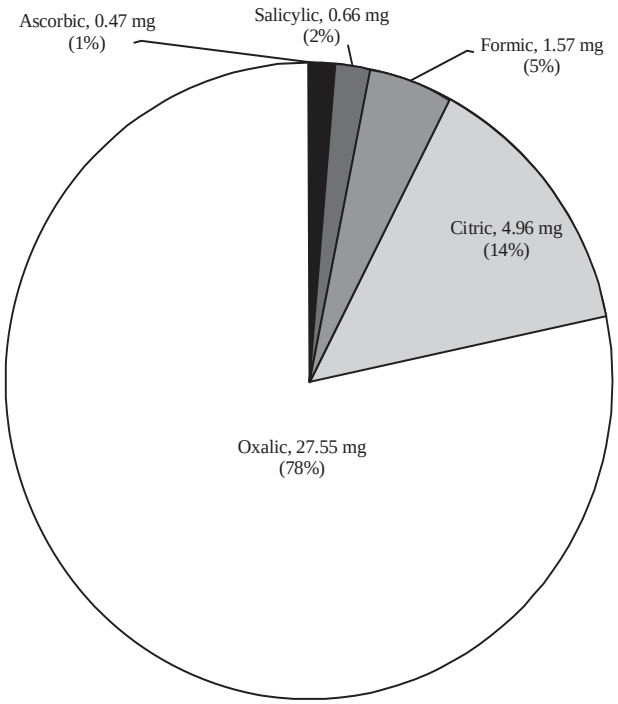

Fig. 3: Concentration of the individual organic acid per one gram of CS detected in the filtrate of CS fermented medium by *T. asperellum* CS5. Number between brackets represents the percentage of every organic acid in relation to the total amount of the 5 organic acids detected by HPLC

one-step fermentation process has been reported using *Chlorella pyrenoidosa*. Likewise, salicylic acid is synthesized from cinnamic acid by either decarboxylation of cinnamic acid to benzoic acid followed by 2-hydroxylation to salicylic acid or cinnamic acid could be first 2-hydroxylated to o-coumaric acid and then decarboxylated to salicylic acid[1,2,20].

Regarding the fermentation conditions, the ability of OA secretion is controlled by the fermentation conditions, basically, carbon to nitrogen ratio that could a ect the types of organic acids, it was proposed that OA could be formed under carbon and nitrogen limiting conditions as well as high carbon to phosphorus ratio[7,18] such as the present CS fermentation medium. However, variability encountered in the composition of CS with seasonal changes must be taken into consideration, although, thesechanges varies in a narrow range.

CONCLUSION

This investigation spotted some light on the basic scientific information for the bio-production of OA on CS using *T. asperellum* MG323528, which is newly reported here as CS decomposer. The SSF technique and the Box-Behnkenmatrix were applied to maximize the yield. HPLC screening showed that oxalic acid was the main OA, represented by 78% of the total OA detected. The resulted oxalic acid has several applications in various fields such as food and pharmaceutical industries. This work could positively share in several ecological, economical and healthy aspects, by the bioconversion of CS as an environmental problem into valuable compounds such as oxalic acid.

SIGNIFICANCE STATEMENT

This study discovers new *T. asperellum* isolate that can ferment corn stover into oxalic acid; this strain can degrade cellulose and, in the same time, solubilize complex phosphate the proposed fermentation technique can be beneficial for reducing the production cost as well as updating the biotechnological procedures into higher level of economic productivity of organic acids.

ACKNOWLEDGMENTS

The authors would like to express their thanks and gratitude to the General Directorate of Research Grants (GDRG) at King Abdulaziz City for Science and Technology (KACST) for funding this project (Grant No. LGP-37-171).

REFERENCES

1. Running, J.A., R.P. Burlingame and A. Berry, 2003. The pathway of L-ascorbic acid biosynthesis in the colourless microalga *Prototheca moriformis*. J. Exp. Bot., 54: 1841-1849.
2. Sauer, M., D. Porro, D. Mattanovich and P. Branduardi, 2008. Microbial production of organic acids: expanding the markets Trends. Biotechnol., 26: 100-108.
3. Dhama, K., R. Tiwari, R.U. Khan, S. Chakraborty and M. Gopi *et al*., 2014. Growth promoters and novel feed additives improving poultry production and health, bioactive principles and beneficial applications: The trends and advances-a review. Int. J. Pharmacol., 10: 129-159.
4. Pordesimo, L.O., B.R. Hames, S. Sokhansanj and W.C. Edens, 2005. Variation in corn stover composition and energy content with crop maturity. Biomass Bioenergy, 28: 366-374.
5. Kumari, A., K.K. Kapoor, B.S. Kundu and R.K. Mehta, 2008. Identification of organic acids produced during rice straw decomposition and their role in rock phosphate solubilization. Plant Soil Environ., 54: 72-77.

6. Son, H.J., G.T. Park, M.S. Cha and M.S. Heo, 2006. Solubilization of insoluble inorganic phosphates by a novel salt- and pH-tolerant *Pantoea agglomerans* R-42 isolated from soybean rhizosphere. Bioresour. Technol., 97: 204-210.
7. Li, Z., T. Bai, L. Dai, F. Wang and J. Tao *et al*., 2016. A study of organic acid production in contrasts between two phosphate solubilizing fungi: *Penicillium oxalicum* and *Aspergillus niger*. Scient. Rep., Vol. 6. 10.1038/srep25313.
8. Saber, W.I.A., N.E. El-Naggar, M.S. El-Hersh and A.Y. El-Khateeb, 2015. An innovative synergism between *Aspergillus oryzae* and *Azotobacter chroococcum* for bioconversion of cellulosic biomass into organic acids under restricted nutritional conditions using multi-response surface optimization. Biotechnology, 14: 47-57.
9. Zhang, Y.J., Q. Li, Y.X. Zhang, D. Wang and J.M. Xing, 2012. Optimization of succinic acid fermentation with *Actinobacillus succinogenes* by Response Surface Methodology (RSM). J. Zhejiang Univ. Sci. B, 13: 103-110.
10. Ghose, T.K., 1987. Measurement of cellulase activities. Pure Applied Chem., 59: 257-268.
11. Bailey, M.J., P. Biely and K. Poutanen, 1992. Interlaboratory testing of methods for assay of xylanase activity. J. Biotechnol., 23: 257-270.
12. Miller, G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem., 31: 426-428.
13. Montgomery, H.A.C., J.F. Dymock and N.S. Thom, 1962. The rapid colorimetric determination of organic acids and their salts in sewage-sludge liquor. Analyst, 87: 949-955.
14. Silva, B.M., P.B. Andrade, G.C. Mendes, R.M. Seabra and M.A. Ferreira, 2002. Study of the organic acids composition of quince (*Cydonia oblonga* Miller) fruit and jam. J. Agric. Food. Chem., 50: 2313-2317.
15. Karp, S.G., A.L. Woiciechowski, V.T. Soccol and C.R. Soccol, 2013. Pretreatment strategies for delignification of sugarcane bagasse: A review. Braz. Arch. Biol. Technol., 56: 679-689.
16. Badhan, A.K., B.S. Chadha, J. Kaur, H.S. Saini and M.K. Bhat, 2007. Production of multiple xylanolytic and cellulolytic enzymes by thermophilic fungus *Myceliophthora* sp. IMI 387099. Bioresour. Technol., 98: 504-510.
17. Zanoelo, F.F., M.D.L.T. de Moraes, H.F. Terenzi and J.A. Jorge, 2004. Purification and biochemical properties of a thermostable xylose-tolerant β-D-xylosidase from *Scytalidium thermophilum*. J. Ind. Microbiol. Biotechnol., 31: 170-176.
18. Narsian, V. and H.H. Patel, 2000. *Aspergillus aculeatus* as a rock phosphate solubilizer. Soil Biol. Biochem., 32: 559-565.
19. Makela, M., S. Galkin, A. Hatakka and T. Lundell, 2002. Production of organic acids and oxalate decarboxylase in lignin-degrading white rot fungi. Enzyme Microbial Technol., 30: 542-549.
20. Running, J.A., D.K. Severson and K.J. Schneider, 2002. Extracellular production of L-ascorbic acid by *Chlorella prototheco*ides, *Prototheca species* and mutants of *P. moriformis* during aerobic culturing at low pH. J. Ind. Microbiol. Biotechnol., 29: 93-98.