SUMMARY

Lignocellulosic polymers resulted from agro-wastes are considered as the cheapest and most available biopolymers worldwide. Lignocellulose is the main component of plant cell wall. Production of lignocellulosic wastes is estimated as 150-170 X 10^9 tons annually all over the world. So, removal of these lignocellulosic wastes is a critical matter otherwise, they will be accumulated causing many types of pollution. In the past and currently in some developing countries, people habituated to get rid of lignocellulosic waste materials (LWM) simply by burning in the open air or even treat them chemically. Certainly in Egypt, burning of rice straw in open fields is the most famous example for removal of LWM forming the "black cloud" which affects the human health harmfully.

Thus, biodegradation of LWM is the best, cheapest and eco-friendly method for safe removal of LWM. Biodegradation of LWM relies on using cellulolytic microorganisms or cellulolytic enzymes for hydrolysis of agro-wastes. Fungi, actinomycetes and bacteria are mainly responsible for biodegradation of LWM.

The current study aimed to isolate different samples of lignocellulolytic actinomycetes. Actually, we can collect 25 samples from cow manure in Beni-Suef Governorate, Egypt. By screening the cellulolytic activity of all the isolated samples using Congo red method, a total of two samples (8%) were found to be cellulolytic producing large clearance zones around colonies spotted on CMC agar plates. The halo zones formed due to degradation of CMC, the sole carbon source, as role of cellulases secreted by cellulolytic isolates. The cellulolytic activity of the two isolates were assayed

using 3,5-dinitrosalisylic acid (DNS) method that measures the total reducing sugars released from hydrolysis of cellulosic polymers.

Then, the cellulolytic activities of the isolated actinomycetes (0.051 and 0.061 U/ml) were compared with that of reference strain, *Thermobifida cellulosilytica* TB100^{T} ordered from German Type Culture Collection (GTCC). *Thermobifida cellulosilytica* has the highest cellulolytic activity (0.087 U/ml) relative to the other isolated strains what is motivated us to study and analyze its secretome more deeply especially that researches regarding this reference cellulolytic strain are low.

Thermobifida cellulosilytica TB100^{T} is a thermophilic cellulolytic actinomycete that has the potential of production of variable lignocellulases. Cellulases produced from *T. cellulosilytica* were partially characterized. Interestingly, *Thermobifida cellulosilytica* is a promising source of thermostable cellulases which are active over a wide range of pH and temperature. The stability of cellulases produced from *T. cellulosilytica* to high temperature and pH enabled their successful applications on large industrial scale such as biofuel production.

LC-MS/MS based proteomic analysis revealed that *T. cellulosilytica* secretome has variable cellulases which are efficient for the degradation of simple/complex lignocellulosic materials. Complex substrates such as rice straw can induce the expression of full cellulase system as well as other lignocellulolytic enzymes more than simple substrates. However, the complex substrate needs to be firstly pretreated to facilitate the enzymes accessibility to their substrates. Pretreatment process includes different types; chemical, physical, physicochemical and biological. Generally,

pretreatment is a two-sided coin having advantages and drawbacks specific to each pretreatment process.

This research focused on expression protein profiles of different cellulases secreted from *T. cellulosilytica* upon growth on different carbon sources; carboxymethylcellulose (containing 6 proteins) and rice straw (containing 31 proteins) as simple and complex substrates, respectively. Among 31 proteins produced in the rice straw secretome there is an important enzyme having A0A147KKN7 uniprot accession code which belongs to lytic polysaccharide monooxygenases (LPMOs) class. Lytic polysaccharide monooxygenases are group of oxidative enzymes recently discovered and that have great stimulatory effect on the cellulolytic activity of ordinary cellulases.