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REVIEW ARTICLE

Microbial Avicelase: an Overview

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ABSTRACT

Cellulose constitutes the most abundant agricultural wastes and is the most dominant renewable bio resources. Bioconversion of cellulosic biomass is accomplished by an enzyme complex consists of three major type of enzymes acting synergistically in the saccharification of cellulose. Among these enzymes, exoglucanase (Avicelase, EC 3.2.1.91) cleaves cellobiosyl units from the ends of cellulose chains. Avicelase releases either cellobiose or glucose generally from non reducing end of microcrystalline cellulose. Avicelases are found to have potential applications in the bioconversion of agricultural waste materials to useful products, such as single cell protein, fuels and chemical feed stocks. It is found to be produced by few microbes and in comparison to that of endoglucanase and beta glucosidase the report of exoglucanase or avicelase is remarkably scanty. It consists of a C terminal catalytic domain, one central domain of a yet-unknown function, and an N-terminal substrate-binding domain. Existence of synergistically acting Avicelase I and Avicelase II and a third hydrolase Avicelase III is reported. A number of bacterial strains are found to produce extracellular Avicelase of which Geobacillus stearothermophilus, various strains of Bacillus spp, Clostridium spp, Acidothermus cellulolyticus Cellulomonas flavigena and actinomycetes like Streptomyces reticuli, Streptomyces flavogriseus, Streptomyces omiyaensis, Streptomyces endus, Thermoactinomyces are noteworthy. On the other hand, fungal strains, Trichoderma spp, Aspergillus spp, Anaeromyces sp., Orpinomyces sp. Rhizopus sp are reported to be potent Avicelase producing strains. The microbial Avicelases were extracted, purified and characterized and it was found that majority of microbial Avicelases showed a pH optima of 4-6 and temperature optima of 40°C-70°C. The genomic structures of microbial Avicelases are being elucidated.

Keywords: Avicelase, Exoglucanase**,** Exocellulase, cellobiohydrolase. Cellulase

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INTRODUCTION

Cellulose is a water insoluble un-branched homopolysaccharide consisting of glucose subunits joined together via β-1-4 glycosidic linkages [1]. Enzymatic hydrolysis of cellulose is of major importance from both natural and engineering aspects [2]. Cellulase (s) are important enzymes not only for their potent applications in different industries, like industries of food processing, animal feed production, pulp and paper production , and in detergent and textile industry, but also for the significant role in bioconversion of agriculture wastes in to sugar and bioalcohols [3].

Cellulase is an enzyme complex which breaks down cellulose to glucose. Generally, three major type of enzymes;endo-1,4- β -glucanase (carboxymethyl-cellulase, or Cx cellulase), cellobiohydrolase (exoglucanases, CBH,Avicelase, C1 cellulase), and β-glucosidase (cellobiase) act synergistically in the saccharification of cellulose. Endocellulase breaks internal bonds to disrupt the crystalline structure of cellulose and expose individual cellulose polysaccharide chains. Exocellulase cleaves 2-4 units from the ends of the exposed chains produced by endocellulase, resulting in the disaccharide such as cellobiose. Cellobiase or beta-glucosidase hydrolyses the exocellulase product into individual monosaccharides. Degradation of cellulose is started by the random attack of endoglucanases at regions of low crystallinity, which creates free ends for the action of cellobio-hydrolases that release cellobiose units from the ends of the chain and finally β-glucosidases cleave cellobiose to glucose [4, 5, 6] .The term "Avicelase" is therefore commonly regarded as synonymous with exoglucanase or cellobiohydrolase (CBH). This enzyme should not be confused with exo-1,3-β-D-glucan glycohydrolase, EC 3.2.1.56, also commonly called exoglucanase [7]. The latter displayed high activity against laminarins, curdlan, and 1,3-beta-oligoglucosides.

Avicelases are found to have potential applications in the bioconversion of agricultural waste materials to useful products, such as single cell protein, fuels and chemical feed stocks [8, 9, 10]. Among cellulases, the exoglucanases appear to catalyze most of the bond-cleavages in the saccharification of crystalline cellulose and is one of the major components of cellulase preparations, especially in the case of fungus-derived commercial enzymes [11].

Although a number of microorganisms were reported to produce cellulases, in comparison to that of endoglucanase and beta glucosidase the report of exoglucanase or avicelase production is remarkably scanty. Hence extensive research and in detail discussion is warranted to isolate new avicelase producing microbes and explore more applications of avicelase for manufacturing of value added products as well as for waste utilization.

The present review deals with the production and characterization of avicelases produced by various microorganisms.

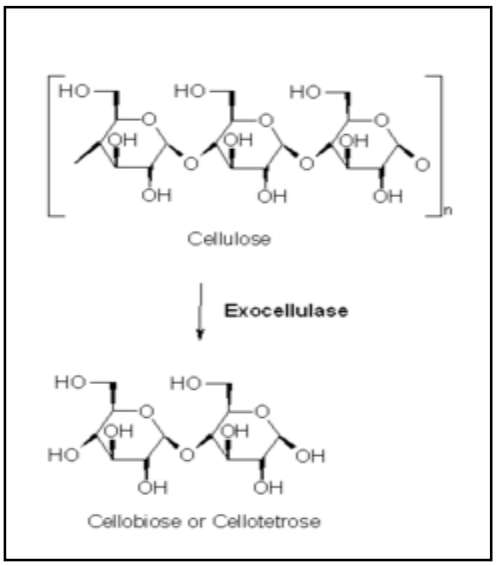

Figure 1. Hydrolysis of microcrystalline cellulose by Avicelase into smaller sugars .

MODE OF ACTION

Avicelase or exoglucanases (1,4-β-D-glucan cellobio-hydrolase, EC 3.2.1.91) are usually active on crystalline cellulose and are lacking from incomplete cellulase systems. It is also called β-1,4-glucan cellobiohydrolase; β-1,4-glucan cellobiosylhydrolase; 1,4-β-glucan cellobiosidase; exoglucanase; avicelase; CBH 1; C_1 cellulase; cellobiohydrolase I; cellobiohydrolase; exo-β-1,4-glucan cellobiohydrolase; 1,4-β-D-glucan cellobiohydrolase; cellobiosidase or exocellulase (EC 3.2.1.91). It cleaves disaccharide (cellobiose) units (Figure 1) either from non-reducing or reducing ends, whereas endoglucanases hydrolyse the cellulose chain internally [12]. More precisely, it cleaves two to four units from the ends of the exposed chains produced by endocellulase, resulting in disaccharides like cellobiose. Exocellulases are further classified into type I that works progressively from the reducing end of the cellulose chain and type II that works progressively from the non reducing end. The exoglucanases (Avicelases) are also known as cellodextrinases and act on the terminals of oligosaccharide chains generated by endocellulases, releasing glucose or cellobiose [13] Among the three major component enzymes of cellulase, exoglucanase shows rapid deactivation and contributes the most to cellulase deactivation. [14]

STRUCTURE OF AVICELASE

Avicelase consists of a C terminal catalytic domain belonging to the family E [15, 16], one central domain of an as-yet-unknown function, and an N-terminal substrate-binding domain. In *Streptomyces* [17], an extracellular 36-kDa protease processes Avicelase (with an apparent molecular weight of 82 kDa) to a truncated, catalytically active 42-kDa enzyme lacking the cellulose-binding domain and the central part of the progenitor enzyme [18] and gene libraries of *S. reticuli* DNA in the *Escherichia coli* phage vectors lambda gt11 and Charon 35, the Avicelase gene (cel1) was identified [16], which revealed that the truncated enzyme (42 kDa) corresponds to the C-terminal region whereas the inactive proteolytically derived protein (40 kDa) represents the N-terminal part of the 82 kDa Avicelase. In *Clostridium stercorarium,* cellulose-binding region was located in the C-terminal half of Avicelase I consisting of a reiterated domain of 88 amino acids flanked by a repeated sequence about 140 amino acids in length and

the enhanced cellulolytic activity of Avicelase I is imparted by the presence of multiple cellulose-binding sites. [19].

TYPES OF AVICELASE

Avicelase I and Avicelase II purified from the cellulolytic thermophile *Clostridium stercorarium* acted in synergism to hydrolyze microcrystalline cellulose showing "exo-exo" type cooperativity and presented a mechanistic model explaining the synergistic interaction between Avicelase I and Avicelase II [20]. Avi III, a novel member of the glycoside hydrolase (GH) family of enzymes, produced by *Acidothermus cellulolyticus*, was a thermal tolerant glycoside hydrolase useful in the degradation of cellulose [21]. Barr *et al*, 1996 [22] opined that one class (containing CBH I, E4, and E6) preferentially cleaves cellooligosaccharides from the reducing end, while the other (containing E3 and CBH II) preferentially cleaves from the nonreducing end and the existence of two exocellulase classes with different specificities could provide an explanation for exo/exo synergism. Similar synergism between the endo/exocellulase, Cel9A, and β-glucosidase (βgl) of *Thermobifida fusca* was noted by Chir *et al*, 2011[23]. Exoglucanase Cex from *Cellulomonas fimi* is a glycoprotein [24]. Cex produced by *Streptomyces lividans* from the cloned cex gene was also glycosylated and the extent and nature of glycosylation are similar for Cex from both organisms. [25] *Xanthomonas oryzae* secreted CbsA, was over expressed, purified and crystallized by Kumar *et al*, 2012 [26], who found that the crystal diffracted to a resolution of 1.86 Å and belonged to space group $P2_12_12_1$.

Exocellulases are known to play a major role in the hydrolysis of crystalline cellulose and two classes of exocellulases were reported: one class, found in families GH-7 and GH-48, attacks the reducing ends of cellulose molecules, processively releasing cellobiose, whereas the other class, found in family GH-6, attacks the nonreducing ends in the same way [27]. The glycoside hydrolase family 9 cellulase (Cel9) from *Clostridium phytofermentans* has a multi-modular structure and is essential for cellulose hydrolysis [28] and was dependent on a single, key hydrolase, Cphy3367 [29], which in turn was found to encode the sole family of 9 glycoside hydrolase (GH9). But, GH9 proteins in the subfamily with Cphy3367, such as *Clostridium stercorarium* CelZ, were found unusual as they acted both as endoglucanases and as exoglucanases [19].

On the other hand, *Clostridium phytofermentans* ISDg encodes a putative family 48 glycoside hydrolase (CpCel48) with a family 3 cellulose-binding module, which had high avicelase activities with cellobiose as a main product [30]. Similar multienzyme cellulosome complex was found to e present in *Clostridium thermocellum* [31] , of which Cel48S had some promising role in Avicel digestion .

Induction of avicelase production

The only carbon source that causes Avicelase synthesis is crystalline cellulose. Although pure microcrystalline cellulose supplemented culture medium induces the production of Avicelase, a number of bacterial and fungal strains are reported to synthesize extracellular Avicelase (Table 1) utilizing indigenous cellulose or cellulosic wastes like wheat straw or rice bran. In many cases glucose is found to act as the potent repressor, although the cascade of reaction following signal transduction by the presence of inducer or repressor is yet to be elucidated.

PRODUCTION OF AVICELASE BY BACTERIA AND FUNGI

Bacterial sources of avicelase:

A number of exoglucanase or avicelase producing bacterial strains were reported. The parameters for production of avicelase from a strain of *Geobacillus stearothermophilus* were statistically optimized [32] using the Box-Behnken design, which showed that Avicelase production was highest in presence of 42g/L Avicel. Treated sugarcane bagasse was found to be an effective inducer for the production of Avicelase by a thermophilic strain of *Geobacillus stearothermophilus*. [33]. Bintari, 2011 [34] studied the optimization of production and characterization of Avicelase from *Bacillus subtilis* using Avicel as inducer. Avicelase activity profiles obtained from *Bacillus subtilis* strains namely, SL9-9, C5-16, and S52-2, after 7 days with 10 g/L of carboxy methyl cellulose as a carbon source showed that all the strains produced considerable Avicelase activity after 3-4 days of cultivation [35]. A thermophilic strain of *Bacillus* sp SMIA-2 showed best Avicelase activity in a culture medium supplemented with 0.5% (w v^{-1}) pure Avicel [36]. A fairly common observation has been that Bacilli lack the complete cellulase system and does not hydrolyze crystalline cellulose [37]. However, two distinct Avicelase activities in *B. circulans* were observed [38]. A simple microbial production method was developed for production of Avicelase enzyme from sawdust by a strain of *Bacillus sp.* when grown at 37°C for 48h. [39] *Bacillus licheniformis* SVD1 displayed Avicelase activity although multi-enzyme complex (2000 kDa) lacked Avicelase activity [40] *Caldibacillus cellulovorans*, a thermophilic aerobic bacterium, was also reported to produce Avicelase [41].

Table 1. Microbial Avicelases.

Name of the microbe	Characteristic features							References
	Production parameters				Catalytic parameters			
	pH	Temp (°C)	Time (hr)	Inducer	pH	Temp (°C)	Mol.wt (K)	
Avicelase from bacteria and actinomycetes:								
Geobacillus stearothermophilus	7	50	48	SCB	7	50	-	32,33
Bacillus subtilis	6-7	50	120	Avicel	-	-	-	34
Bacillus subtilis SL9-9, C5-16, and S52-2	7	28	48	-	5	50	-	35
Bacillus sp SMIA-2	7.5-8.0	50	120	Avicel, CSL	8.5	70	-	36
B. circulans	-	-	-	Avicel	4.5	50	75-82	38
Bacillus sp.	7	37	48	Sawdust	6.6	40	-	39
Caldibacillus cellulovorans	6.8	70		Cellobiose		70	-	41
Clostridium thermocellum NKP2 and Thermoanaerobacterium thermosaccharolyticum NOI -1	7	60		Corn hulls			-	42
Clostridium A11	-	-	-	Avicel	-	-	-	43
Clostridium stercorarium	-	-	-	-	6	68	100	20
Cellulomonas flavigena	-	-	72	Avicel	6.5	50	-	46
Streptomyces reticuli	7	-	120	Avicel	-	-	42	16, 18
Streptomyces reticuli	7	50	-	Avicel	-	-	-	53
Streptomyces reticuli	-	30-50	-	Avicel	7.0	45-50	300	86
Streptomyces flavogriseus,	-	-	-	CMC	-	-	45	49
Streptomyces omiyaensis	6.5	40	120	Avicel	6.5	45	-	50
Streptomyces endus	6.5	50		Dry leaf	-	-	-	51
Alphaproteobacteria, Firmicutes, Gammaproteobacteria, Actinobacteria Bacteroidetes		28	72	Avicel	-	-	-	13
Avicelase from fungi :								
Rhizopus oryzae	5-8	37	-	Avicel	5	40	-	3
Neurospora crassa FGSC 2489	-	-	-	Stem and leaf	5	40	-	56
Strain Y-94	-	-	-	-	5.3	62	68	58
Neocallimastix hurleyensis		39	168	Wheat straw	6	50	-	59
Anaeromyces sp, *Orpinomyces* sp, *Neocallimastix* sp	-	-	72–96	-	-	-	-	60
Pleurotus ostreatus	5.5	27	240		4.8	50	45	61
Stropharia rugosoannulata	5	40	-	Maltose	-	-	-	62
Porodaedalea pini	-	25	480	-	5.5	37	-	63
Trichoderma reesei QM9414	-	-	96	Rice bran	-	-	-	64
Lecythophora sp. YP363	-	30	96	-	5.2	50	-	65
Trichoderma viride and *Ganoderma lucidum*	6	35	120	Corn stover	-	-	-	67
Pleurotus sajor-caju	-	30	56-70	Jute stick	-	-	-	68
Humicola grisea var. *thermoidea*	-	42	120	Oat flour	-	-	-	69
Aspergillus niger UC	-	-	-	-	4.5	40-50	80	72
Aspergillus niger AS 101	-	28±2	144	Corn cobs	5.5.	50	52	88
Aspergillus MAM-F35 and MAM-F40	-	28	168	Wheat straw	6.6	40	-	73
Aspergillus fumigatus	5.5	55	72	Wheat straw	4.8	55	-	74
Aspergillus flavus	5	30	48	CMC	5	50	-	75
Arachniotus sp	-	30±2	24	Maize stover	-	-	-	76
Bjerkandera adusta , *Pycnoporus sanguineus*,	4.5	28	-	Avicel	5	50	-	94
Sordariahumana DEF1	-	28	-	CMC	-	-	-	95
Trichoderma spp	4.8	48	72	Pistachio hull	4.8	50		96

Clostridium sp, as anaerobic bacterium was found to be a potent producer of Avicelase. Clostridium thermocellum NKP2 [42] produced avicelase (0.24 U) when grown on corn hull. The extracellular cellulase enzyme system of *Clostridium* A11 was fractionated by affinity chromatography on Avicel. Only Avicel-adhered fraction could degrade Avicel. Avicelase activities were studied with the extracellular enzyme system and cloned cellulases. Synergism between "avicel adhered enzyme system" and cloned endoglucanases was observed on Avicel degradation. (Benoit et al, 1995) [43]. On the other hand Avicelase I and Avicelase II purified from thermophilic *Clostridium stercorarium* was found to act synergistically on microcrystalline cellulose [20].

Avicelase II has been isolated and characterized from *Acidothermus cellulolyticus* and was patented [44] and a patent was taken on the method of thermal tolerant avicelase from it. *Cellulomonas flavigena*, a gram positive bacteria, when cultivated in the presence of 0.5% Avicel as carbon source, produced higher levels of avicelase after 72 h of fermentation but a large proportion of avicelase activity remained bound to be insoluble cellulosic substrate throughout the fermentation periods studied [45]. Rajoka 2004 [46] found α-Cellulose was the most effective inducer followed by grass straw for the production of exoglucanase in *Cellulomonas flavigena. Among Actinomycete strains studied, Streptomyces reticuli* was found to grow efficiently with crystalline cellulose (Avicel) as the sole carbon source and the enzyme could be released efficiently by nonionic detergents [16] It was found to have an apparent molecular weight of 82 kDa [18] and could efficiently hydrolyze crystalline cellulose (Avicel) to cellobiose [47] . *Streptomyces reticuli* produced a 35-kDa cellulose (Avicel)-binding protein (AbpS) which was found to interact strongly with crystalline cellulose but not with soluble types of cellulose.[48]. *Streptomyces flavogriseus*, a mesophilic actinomycete, produced high levels of extracellular exoglucanase or Avicelase [49] A strain of *Streptomyces omiyaensis* was found to produce maximum avicelase at a temperature of 45°C and pH 6.5 [50]. Another isolate, *Streptomyces endus* was found to produce Avicelase using dry leaf as the inducer [51]. Two *Streptomyces* strains, M7a and M23, from a Brazilian forest soil , were found to grow in a microcrystalline cellulose medium by Semêdo *et al*, 2000 [52] .Another strain of *Streptomyces reticuli* was found to hydrolyze crystalline cellulose (Avicel) to cellobiose by Avicelase, Cel1[53]

Extracellular Avicelase was found to be produced by *Thermoactinomyces* sp which could be separated from carboxymethyl cellulase by preparative isoelectric focusing in the pH range 3-5 [54]. Soares Júnior *et al*. 2013 demonstrated that the most abundant groups of bacteria, isolated from mangrove sediment of Brazil were Alphaproteobacteria and Firmicutes, followed by Gammaproteobacteria, Actinobacteria and Bacteroidetes had both endo and exoglucanase (avicelase) activity [13].

FUNGAL SOURCES OF AVICELASE

Rhizopus oryzae PR7 was found to was found to produce extra cellular exoglucanase or avicelase when grown on avicel or various cellulosic agro wastes in submerged fermentation [3]. Avicelase was obtained from a wood-rotting fungus *Irpex lacteus* (*Polyporus tulipiferae*) which showed a strong synergistic action with an endocellulase of higher randomness in the hydrolysis of Avicel and produced exclusively cellobiose from CMC as well as from water-insoluble celluloses such as Avicel or cotton at earlier stages of hydrolysis [55]. Avicelase activity of filamentous fungus *Neurospora crassa* FGSC 2489 was found after 7 days of cultivation. [56] Enhanced exoglucanase was found to be produced by brown rot fungus *Fomitopsis* sp. RCK2010 [57]. The microcrystalline cellulose hydrolyzing enzyme, tentatively called Avicelase II from a fungal Strain Y-94 was purified [58]. Significant increases in the Avicelase activities of *Neocallimastix hurleyensis,* a rumen anaerobic fungus was observed after 31 subcultures on Avicel [59].Three fungal strains, namely *Anaeromyces* sp., *Orpinomyces* sp. *and Neocallimastix* sp., isolated from rumen liquor and faecal samples of Murrah buffaloes showed Avicelase activity [60]. In *Pleurotus ostreatus* (Jacq.) P. Kumm. (Type NRRL-0366) mushroom from Egypt, after 12 days of cultivation, the maximum enzyme production was yielded on basal medium supplemented with 6% avicel cultured at 27°C and at initial pH value of 5.5. [61]. Avicelase production by *Stropharia rugosoannulata* was found to achieve its maximum level at 40°C, pH 5 and 0.8% maltose [62]. Although Avicelase production by a white-rot fungus *Porodaedalea pini* in fermentor culture showed the maximum production (16.8 nkat/mg) on 20th day, but in flask-agitating culture, it did not show any common and constant trends in the activity [63]. *Trichoderma reesei* QM9414 from the solid state fermentation of rice bran medium showed highest Avicelase activity [64]. It was found that the thermostable avicelase produced by *Lecythophora* sp. YP363 [65], was more stable to heat than that of *Trichoderma reesei* Rut C-30 [66]. The hyper production of exoglucanase from the co-culture of *Trichoderma viride* and *Ganoderma lucidum* in solid state fermentation of corn stover substrate after 5 days of incubation at pH 6 and 35˚C was reported [67]. From solid state fermentation (SSF) of pre treated jute stick and green jute, *Pleurotus sajor-caju* produced Avicelase during eight weeks of fermentation [68]. Studies on the enzymes of the cellulase complex of the thermophilic fungus *Humicola grisea* var. *thermoidea* were described [69] and successful cloning and expression of *Avicelase Gene* of *Humicola insolens* in *Pichia pastoris was done* [70]. One yeast

strain out of a total a total of 245 yeast isolates from Gunung Halimun National Park was reported to produce Avicelase [71]. *Aspergillus* was well known for production of extracellular cellulases including exoglucanase or Avicelase. *Aspergillus niger* UC grown on wheat bran solid medium produced Avicelase [72]. In case of *Aspergillus* MAM-F35 and MAM-F40, the highest production of Avicelase on Wheat Straw was 36 U/ml and 32 U/ml, respectively [73]. *Aspergillus fumigates* [74] produced highly active exoglucanase from the solid-state fermentation of wheat straw at 55 °C temperature, pH 5.5 after 72 h. whereas a strain of *Aspergillus flavus* [75] was found to produce exoglucanase both in submerged and solid state fermentation. *Arachniotus* sp was found to produce exoglucanase from shake culture medium supplemented with maize stover only after 24 hours of incubation [76]. In submerged fermentation of *Aspergillus niger* , addition of 2-mercaptoethanol could enhance avicelase production [77].

ASSAY OF AVICELASE

Avicelase activity was measured by incubating 0.5mL of enzyme solution with 1 g of Avicel, microcrystalline cellulose, as substrate and 1.5ml of 0.1M sodium acetate buffer (pH 5.0) for 1 h at 50℃. After incubation, the reaction mixture was centrifuged at 10,000Xg for 5min, and then 1ml of the supernatant was taken to determine reducing sugars by the DNS method. One unit of Avicelase activity was defined as the amount of enzyme that released 1 μmol of reducing sugars as glucose equivalents from Avicel per ml per min. [35]. Some authors [46, 78] considered Avicelase and FPase (cellobiohydrolase) synonymous as they called either of them as exoglucanase (E.C. 3.2.1. 91) and according to them it measures the complete cellulase complex [79].The reducing sugar release was determined by the 3, 5-dinitrosalicylic acid method [80]. The enzymatically liberated reducing sugar was calculated from a previous established standard curve using glucose as a standard. One unit of filter paper assay enzyme (FPU) was defined as the amount of enzyme releasing 1µ mole of reducing sugar from filter paper per ml per min.

Avicel is the substrate for exoglucanase activity assay because it has a low degree of polymerization (DP) and relatively low accessibility. Therefore, some researchers [81] feel that "avicelase" activity is equivalent to exo-glucanase activity but it was found that some endoglucanases can release considerable reducing sugars from avicel [82] and amorphous cellulose and soluble cellodextrins can act as substrates for both purified exo-glucanases and endoglucanases. [83]. This forces some researchers to draw the conclusion that, there is no substrate specific for exoglucanases within the cellulase mixture [81, 84]. Silveira *et al*, 2012 [85] proposed a fast method for the determination of endo- and exo-cellulase activity in cellulase preparations using filter paper.

CHARACTERIZATION OF AVICELASE

Avicelase from *Bacillus circulans* F-2 had optimal pH and temperature at 4.5 and 50° C respectively and showed high-level activity towards carboxymethyl cellulose (CMC) as well as p-nitrophenyl-beta-D-cellobioside, 4-methyl umbelliferyl cellobioside, xylan, Avicel, filter paper, and some cello-oligosaccharides [38] Characterization of Avicelase produced by thermophilic *Bacillus* sp. strain SMIA-2 revealed that the optimum pH of the enzyme was 8.5 and the enzyme retained more than 80% of its activity after incubation at room temperature for 2h at pH 6.5-8.5 and the optimum temperature of this enzyme was 70°C and the enzyme retained 67% of the original activity after 20 min. of heat treatment at 70°C. Avicelase was stimulated by Mn^{2+} and Co^{2+}, whereas Hg^{2+} greatly inhibited the enzyme activity [36]. A high-molecular-weight Avicelase (>300 kilodaltons) was purified from the cellulose components of *Streptomyces reticuli* [86] growing in presence of Avicel showed optimal activity at around pH 7.0 and temperatures of between 45-50°C. Avicelase (85KDa) from *Streptomyces omiyansis* [50] showed optimal activity at around pH 6.5 and temperatures of between 45°C. Maximal activity of avicelase I purified from *Clostridium stercorarium* was observed between pH 5 and 6. In the presence of Ca^{2+}, the enzyme is highly thermostable, exhibiting a temperature optimum around 75°C. [87]. Partially purified exoglucanase or Avicelase from *Aspergillus niger* was stable at room temperature in the pH range of 4.0-6.0 for 24h, showing optimum activity at pH 5.5and temperature of 50°C. The Km and Vmax values were found to be 55.5 mg/ml and 0.9uM/min [88]. The activity of purified Avicelase (80,000 Dalton) from *Aspergillus niger* UC [72] was optimum at pH 4.5 and 40-50°C. Purified exoglucanase from *Aspergillus fumigatus* [74] showed maximum activity at 55 °C and pH 4.8 using 1 % Avicel aqueous solution as substrate. The K(m) and V(max) were 4.34 mM and 7.29 µM/min, respectively. The purified exoglucanase of *Streptomyces flavogriseus* with molecular weight of approximately 45 000 and an isoelectric point of 4.15 showed negligible affinity towards carboxymethylcellulose and the main product of enzyme action was cellobiose [49]. The enzyme from *Rhizopus oryzae* PR7 MTCC 9642 showed optimum activity at pH 5.0 and 40°C and stability at pH range of 5-9 and about 90% activity was retained even after an exposure of 10 minutes at 80° C [3]. Avicelase was stable in the pH range 5.0 to 6.5 at temperatures below 50°C. Avicelase II from

aerobic bacterium identified as *Cellulomonas flavigena* was found most active at pH 6.5 and 50°C and stable in the pH range of 3-11 and 100% activities were lost when incubated at 70°C for 30 minutes [37….45]. The optimum pH and temperature for Avicelase of a fungal strain Y-94 was 5.3 and 62°C, respectively, The enzyme was stable between pH 4.1 and 6.0 at 4°C for 24 hr, and up to 61°C for l0min. [58] .Avicelase from thermophilic *Geobacillus stearothermophilus*had [33] an optimum pH and temperature 7.0 and 50ºC and exhibited good pH stability between 4-9 and good temperature stability between (30-80ºC).

GENETIC ANALYSIS OF AVICELASE PROTEIN

The nucleotide sequence of the *celZ* gene coding for a thermostable endo-β-1,4-glucanase (Avicelase I) of *Clostridium stercorarium* was determined by Jauris et al, 1990 [89] who proposed that the enhanced cellulolytic activity of Avicelase I is due to the presence of multiple cellulose-binding sites. It was found that CelS is the most abundant subunit [90] and an exoglucanase component of the *Clostridium thermocellum* cellulosome, multicomponent cellulase complex and the scientists studied the product inhibition pattern of CelS using purified recombinant CelS (rCelS) produced in *Escherichia coli.* Subcloning and DNA-sequencing revealed a G+C rich (72%) reading frame of 2238 bp encoding a protein of 746 amino acids. A signal sequence of 29 amino acids was identified by aligning the deduced amino acids with the characterized N-terminus of the 82 kDa Avicelase [16]. The nucleotide sequence of the *celZ* gene coding for a thermostable endo-β-1,4-glucanase (Avicelase I) of *Clostridium stercorarium* .The structural gene consists of an open reading frame of 2958 by which encodes a preprotein of 986 amino acids with an M_r of 109000. The recombinant protein expressed in *Escherichia coli* is proteolytically cleaved into catalytic and cellulose-binding fragments of about 50 kDa each. Sequence comparison revealed that the N-terminal half of Avicelase I is closely related to avocado (*Persea americana*) cellulase. Homology is also observed with *Clostridium thermocellum* endoglucanase D and *Pseudomonas fuorescens* cellulase. The cellulose-binding region was located in the C-terminal half of Avicelase I. It is proposed that the enhanced cellulolytic activity of Avicelase I is due to the presence of multiple cellulose-binding sites [19]. The combined activity of Avicelase I and Avicelase II purified from Clostridium stercorarium towards Avicel was about double the sum of the individual activities and there exists an exo-exo type cooperativity which presents a mechanistic model explaining the synergistic interaction between Avicelase I and Avicelase II [20]. Avicelase III has been isolated and characterized from *Acidothermus cellulolyticus* [44] and a patent was taken on the method of thermal tolerant avicelase from it.

The corresponding gene for Avicel-binding protein (*abpS*) was identified and sequenced in *Streptomyces reticuli [47].* By analyzing the secondary structure of the deduced AbpS sequence, a large centrally located α-helical structure exhibiting low levels of homology with the tropomyosin protein family and the streptococcal M-proteins was detected. Two exoglucanases (Cel6B and Cel48A, formerly E3 and E6) are isolated from *Thermobifida fusca* [90] .The Cel48A exoglucanase and the Cel5A endoglucanase from T. fusca were combined to produce diverse geometrical arrangements, and the resulting chimeras were tested for their activity on microcrystalline cellulose and it was found that the bifunctional enzyme with the endoglucanase on the N-terminus (CBM-5-48) exhibits enhanced cellulolytic activity compared to the N-terminally positioned exoglucanase (CBM-48-5) [91, 92]. Studies on the enzymes of the cellulase complex of the thermophilic fungus *Humicola grisea* var. *thermoidea* are described [70]. A genomic library was constructed in the phage vector EMBL 4, and from this library two clones were isolated using as a probe the cloned *cbh-1* (exoglucanase, EC 3.2.1.91) gene of *Phrochaete chrysosprium*. Sequence comparison with the equivalent genes from *P. chrysosporium* and *Trichoderma reesei* in terms of primary sequence indicated that there was about 60% homology between them [69].

CONCLUSION

From comparative data analysis, Liao *et al,* 2011 [93] opined that the fungal cellulase exhibited much faster hydrolysis rates than bacterial cellulase at the same temperature and protein mass concentration over a long period. But irrespective of the source, it was found that there was a higher occurrence of isolates with endoglycolytic activity than exoglycolytic activity [13] and even in a microorganism with both endo and exo glucanase in multienzyme complex, avicelase or exoglucanase activity is weaker than that of endoglucanase activity.

It was concluded that the combinations of endocellulase and exocellulase can lead to a rise in the cellulose degradation activity by synergy, working on both internal and external cleavage of the cellulose molecules. On the other hand, amongst a number of cellulolytic microorganisms, in comparison to that of endoglucanase and beta glucosidase the report of exoglucanase or avicelase production is remarkably scanty [3]. The catalytic module of Avicelase, a cellulase, having different types of substrate affinity and variable characteristic features are required to be analyzed with multi scale modeling, using

computational approaches as proposed by Beckham *et al* [97] .To understand the role of scaffoldin subunit of the cellulosome and cellulose binding module (CBM) [98] at atomic scale, free energy perturbation methods may be adopted which inturn may provide some insights for increased Avicelase–cellulose interactions .

Hence extensive research is warranted to explore the avicelase producing microorganisms with a detailed study of its proteomics and genomics to employ it for cellolose hydrolysis at an industrial level.

COMPETING INTERESTS

The author declares that there exists no competing interest.

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