Technical Report

Significance of *cellulase* in CanXida Restore (Formula RST)

"In CanXida Restore (Formula RST), ***cellulase*** *aids digestion, reduces Candida overgrowth, and enhances nutrient absorption for improved digestive health.*

Contents

Executive Summary

Cellulases, vital enzymes with broad applications, play a key role in breaking down cellulose for various industries. Produced by microorganisms, they employ catalytic mechanisms like retaining and inverting to efficiently break glycosidic bonds in cellulose microfibrils, releasing essential products. Functional properties of cellulase, such as hydrolytic activity, synergistic action, thermostability, substrate specificity, and involvement in biomass bioconversion, dietary fiber digestion, and health-related processes, highlight their versatility.

Cellulase's health benefits include improved digestion, increased nutrient absorption, and potential roles in treating metabolic disorders. Its biosafety profile is generally favorable, though caution is advised. Effective targets of cellulase include cellulolytic microorganisms, biofilms, and microbial ecosystems in cellulose-rich environments. In formulations like CanXida Restore, cellulase offers benefits like aiding fiber digestion, reducing Candida overgrowth, enhancing nutrient absorption, and influencing the gut microbiome*.

1. Introduction

Cellulases are very significant enzymes, with crucial roles in both industrial and natural environments. They have a substantial impact on the global carbon cycle by breaking down insoluble **cellulose** into soluble sugars (Wilson, 2009).

Cellulose, a vital plant cell wall component, is a linear chain of glucose units linked by β-1, 4 glycosidic linkages.

Breaking the glycosidic bonds of cellulose microfibrils involves the enzymatic action of cellulases. These enzymes specifically target the β-1,4 glucosidic linkages between glucose units in cellulose. The hydrolysis process results in the release of various products, including oligosaccharides, cellobiose, and individual glucose molecules (Rodrigues, 2016).

Cellulases, the third-largest industrial enzyme, find widespread applications in food, textiles, pharmaceuticals, detergents, biofuels, and paper industries. Their role in catalyzing cellulose hydrolysis contributes to improved flavors, textures, fabric finishing, drug formulation, cleaning efficiency, biofuel production, and enhanced paper quality. This versatility positions cellulases as crucial agents in advancing sustainable practices and innovation across diverse sectors (Rodrigues, 2016).

2. Cellulases sources & mechanism

Cellulases have been commercially available for over 30 years and are widely used in various industries. These are produced by a variety of microorganisms, such as fungus and bacteria, when they grow on cellulose materials. They are inducible enzymes. As a result, cellulose undergoes a conversion process into simple sugar (Ejaz et al., 2021a).

Cellulases utilize two different catalytic mechanisms, to break down glycosidic bonds using acid-based catalysis, namely (Jayasekara et al., 2019)

- Retaining
- Inverting

Retaining Mechanism:

The retaining mechanism ensures that the anomeric configuration of the target glucosidic bond remains unchanged following a double-displacement hydrolysis.

The process consists of two essential steps:

- Glycosylation
- De-glycosylation.

Inverting Mechanism:

The inverting mechanism involves the inversion of the anomeric carbon's configuration through a single nucleophilic displacement hydrolysis.

The original anomeric configuration of the glycosidic bond is not preserved.

3. Functional Properties of *cellulase*:

The functional properties of cellulase include:

3.1. Hydrolytic activity:

Cellulase catalyzes the hydrolysis of cellulose, breaking it down into smaller sugar units called cellobiose and eventually into glucose (Jayasekara et al., 2019). This process is essential for the release of energy stored in plant cell walls.

3.2. Synergistic action:

Cellulase is composed of three enzymes:

- Endo-1,4-β-D-glucanase (endoglucanase)
- B-glucosidase
- Exo-1,4-β-D-glucanase (exoglucanase)

The three enzymes play a crucial role in breaking down cellulose through a synergistic process, ensuring efficient and successful hydrolysis (Jayasekara et al., 2019).

Similarly, cellulases often work in synergy with other enzymes, to efficiently break down complex plant cell wall structures, such as hemicelluloses and pectinases(Behera et al., 2017)

3.3. Thermostability:

Some cellulases are thermostable, meaning they can maintain their activity at high temperatures(Patel et al., 2019). This property is particularly valuable in industrial processes, where elevated temperatures may be employed.

3.4. Substrate specificity:

Cellulases can exhibit different degrees of specificity for cellulose substrates. Substrate specificity pertains to an enzyme's capacity to meticulously choose the exact substrate from a range of chemical compounds (Sulyman et al., 2020).

For instance, some are highly specific to cellulose, while others may also act on related polysaccharides like hemicellulose*.

3.5. Bioconversion of biomass:

Cellulase enzymes play a crucial role in the bioconversion of biomass into valuable products such as biofuels. This is achieved by breaking down cellulose into fermentable sugars that can be further converted into biofuels through microbial fermentation (Ejaz et al., 2021).

3.6. Dietary fiber digestion:

Cellulases assist in breaking down cellulose, a major component of dietary fiber. While humans lack endogenous cellulases, gut bacteria produce these enzymes, contributing to the digestion of dietary fiber in the colon (Guan et al., 2021).

3.7. Production of short-chain fatty acids (SCFAs):

The fermentation of cellulose-derived products by gut bacteria results in the production of short-chain fatty acids (SCFAs) (Den Besten et al., 2013), such as acetate, propionate, and butyrate. SCFAs play a vital role in maintaining gut health.

3.8. Prebiotic effects:

The breakdown of cellulose and other fibers can produce oligosaccharides and other soluble components that act as prebiotics (Davani-Davari et al., 2019). Prebiotics promote the growth and activity of beneficial gut bacteria, contributing to a healthy microbial balance in the gut.

4. Health Benefits of *cellulase:*

While cellulase itself is not typically consumed directly as a supplement, the health benefits associated with cellulase are often attributed to its role in aiding digestion and supporting overall gut health. Here are some potential health benefits:

4.1. Improved Digestion:

Cellulase, a key component in enzyme blends, is pivotal for promoting digestive health, especially in individuals with decreased gastric motility and impaired digestion (Fujimori, 2021; Vachher et al., 2021). This enzyme facilitates the breakdown of cellulose in plant cell walls, aiding in the digestion of dietary fiber and enhancing nutrient absorption*.

4.2. Increased Nutrient Absorption:

By breaking down cellulose into simpler sugars, cellulase may enhance the absorption of essential nutrients, such as vitamins, minerals, and antioxidants, from plant-based foods*.

4.3. Promotion of gut microbiota diversity:

Cellulose degradation and the subsequent fermentation processes support a diverse microbial community in the gut. A rich and diverse gut microbiota is associated with improved gut health and overall well-being (Hubkova, 2017).

4.4. Treating Metabolic Disorders

Digestive aids such as “Digestin” and “Polyenyne Plus”, enriched with cellulase, are emerging as promising tools in the treatment of metabolic disorders. Cellulase, a key enzyme in these formulations, contributes to the efficient breakdown of complex carbohydrates, aiding digestion and potentially addressing metabolic issues (Ejaz et al., 2021; Jayasekara et al., 2019).

4.5. Improving the nutritional value

Cellulases play a crucial role in enhancing nutritional value by breaking down anti-nutritional components such as oligosaccharides, β-glucan, pectins, lignin, inulin, dextrins, cellulose, and arabinoxylans. Through their enzymatic action, cellulases effectively degrade these compounds, ultimately improving the nutritional composition of the substrate. This process facilitates the release and absorption of essential nutrients, contributing to an overall increase in the nutritional quality of the material (Ejaz et al., 2021).

4.6. Biofilm Removal

Pathogenic microorganisms form biofilms, and cellulases can be studied for effectively removing these biofilms (Ejaz et al., 2021; Jayasekara et al., 2019).

4.7. Phytobezoar Treatment:

Phytobezoars are masses of indigestible plant material that can accumulate in the digestive tract, causing blockages.

The use of cellulase in phytobezoar treatment is based on its ability to break down cellulose, aiding in the digestion and dissolution of plant material causing the obstruction (Ejaz et al., 2021; Jayasekara et al., 2019).

4.8. Acanthamoeba Keratitis Control:

Acanthamoeba keratitis is an eye infection caused by the amoeba Acanthamoeba.

Some research suggests that cellulase enzymes may have potential in controlling Acanthamoeba infections, possibly by breaking down the cyst walls of the amoeba (Ejaz et al., 2021; Jayasekara et al., 2019).

Table 1: *Clinical trials that have used Lactobacillus rhamnosus for either treatment or prevention are detailed here. Source: clinicaltrials.gov*

Clinical trial ID	Health Condition	Status
NCT05520411	• Bloating • Stomach Distended	*Completed*
NCT00881322	• Abdominal Cramps • Abdominal Pain • Flatulence	*Completed*

5. Biosafety Profile of *cellulase*

Cellulase, an enzyme pivotal in cellulose degradation, is predominantly derived from microorganisms like bacteria and fungi (Li et al., 2023).

The biosafety profile of cellulase in drug supplementation is generally considered favorable (Silano et al., 2021; Ullah et al., 2023). However, caution is advised to prevent potential allergenic reactions.

The production of cellulase enzymes can generally be considered safe. Cellulases are enzymes naturally produced by various microorganisms, including bacteria and fungi. When produced for industrial or commercial purposes, such as in the food and beverage industry or for biofuel production, stringent regulations and quality control measures are in place to ensure the safety of the process*.

* *These statements have not been evaluated by Food and Drug Administration. This product is not intended to diagnose, treat, cure or prevent any disease.*

The use of genetically modified organisms (GMOs) in cellulase production may be subject to specific regulations depending on the country or region. It is essential for companies involved in cellulase production to adhere to regulatory guidelines and conduct thorough safety assessments to ensure that the final product is safe for its intended applications*.

6. Effective Targets of *cellulase*

Here are some ways in which cellulase activity can affect microbial targets*:

6.1. Cellulolytic bacteria and fungi:

Cellulase acts on cellulose, breaking it down into glucose and other simple sugars. This process is particularly important for cellulolytic microorganisms, including certain bacteria and fungi. By targeting cellulose, cellulase indirectly affects the growth and survival of these microorganisms.

6.2. Biofilm disruption:

Some bacteria, including pathogenic ones, can form biofilms on surfaces. Biofilms are communities of microorganisms encased in a matrix of extracellular polymeric substances (EPS), which often include cellulose. Cellulase can potentially disrupt biofilms by breaking down the cellulose component, making it more challenging for the bacteria to adhere to surfaces and form a stable biofilm.

Some of the examples of microbial targets are:

Bacteria:

Staphylococcus: Opportunistic pathogens often associated with skin infections.

Escherichia: Commonly found in the intestines; some strains can cause gastrointestinal infections.

Pseudomonas: Versatile genus with species involved in various ecological niches; some are opportunistic pathogens.

Bacillus: Diverse group; some used in industry, while others can cause infections.

Enterococcus: Present in the intestines; can be opportunistic pathogens, especially in healthcare settings.

Klebsiella: Found in the respiratory, intestinal, and urinary tracts; some strains can cause infections.

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Fungi:

Aspergillus: Common mold genus; some species are used industrially, while others can be pathogenic, especially in immunocompromised individuals.

Candida: Opportunistic yeast genus; includes species like Candida albicans, often associated with mucosal infections in humans.

7. Significance of *cellulase* in CanXida Restore Formula

If cellulase is included in the CanXida Restore Formula, its potential benefits may be attributed to several factors. Firstly, cellulase is known for its role in breaking down cellulose, a complex carbohydrate found in plant cell walls. While humans lack sufficient native cellulase in the gut, supplementing with cellulase could assist in the digestion of dietary fiber. This may contribute to alleviating digestive discomfort such as bloating or constipation, providing a potential avenue for enhancing overall digestive well-being*.

Moreover, it can also reduce Candida overgrowth. Candida, being a fungus, possesses cell walls containing cellulose. The inclusion of cellulase might be suggested to help break down these cell walls, potentially aiding in the reduction of Candida*.

Additionally, cellulase in the formula could be considered for its potential role in enhancing nutrient absorption from plant-based foods. By breaking down plant cell walls, cellulase might facilitate the release of nutrients, contributing to their absorption in the digestive tract*. Furthermore, it can also influence the gut microbiome by modulating bacterial growth*.

In conclusion, it has the potential to offer benefits related to digestive health, including the breakdown of dietary fiber and potential implications for Candida overgrowth*.

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