Original Research Article

Enzymase activity of gut microbes Isolated from the RugoseSpiraling Whitefly *Aleurodicusrugioperculatus*

Abstract

This study investigated the enzymatic diversity among various bacterial isolates of RugoseSpiraling Whitefly (*Aleurodicusrugioperculatus*), shedding light on their potential industrial applications. Notably, *Bacillus cereus* (MRSW01) exhibited remarkable amylase and lipase activities, aligning with its reputation as a prominent amylase producer. These enzymes are crucial in the food industry, particularly in starch and lipid degradation processes. *Pseudomonas helleri* (CHRSW028) demonstrated substantial cellulase activity, essential for breaking down cellulose in plant materials, suggesting its relevance in biofuel production and waste management. *Pseudomonas psychrophila* (CRSW024) stood out with the highest pectinase and significant lipase activities, both of which find applications in the food and textile industries and the hydrolysis of fats and oils, respectively. *Proteus vulgaris* strains MRSW05 and CHRSW02 displayed significant amylase, cellulase, and protease activities, making them versatile candidates for various industrial applications, including detergent formulations and bioremediation processes. Conversely, *Hafniaparalvei* exhibited comparatively lower enzymatic activities, indicating limited industrial potential. The rich enzymatic diversity within these bacterial isolates and highlight their potential contributions to various industrial sectors, ranging from food and textiles to biofuel production and waste management. Such insights can inform the development of biotechnological applications and processes, ultimately benefitting both industrial and environmental domains.

1. Introduction

Invasive species pose a significant threat to the ecological and economic well-being of countries (Pimentel *et al*., 2001). Over the past decade, India has experienced accidental introductions of several invasive soft scale insects and fall armyworms, with some of them becoming serious pests while others have expanded their host ranges and spread rapidly (Joshi, 2017). A recent study by Paini *et al*. (2016) revealed that approximately 1,300 species of invasive insect pests have been introduced into 124 different countries.

One such invasive insect pest is the RugoseSpiraling Whitefly (*Aleurodicusrugioperculatus*), which poses a significant threat to coconut trees and various plant species. This whitefly, native to Central America and belonging to the Aleyrodidae family, has rapidly spread to different parts of the world, causing substantial damage to coconut plantations and posing a significant challenge to agricultural and horticultural industries (Prasanna *et al*., 2022). It derives its name from the distinctive spiraling pattern created by its nymphs on the undersides of coconut tree leaves. These nymphs, often referred to as "spirals," secrete wax and form dense clusters on the leaves, leading to reduced photosynthetic activity and overall weakening of the tree. This infestation results in leaf yellowing, premature defoliation, and reduced coconut production, causing economic losses for farmers and coconut growers (Saranya et al., 2022).

The RugoseSpiraling Whitefly has been documented in various countries, including India, the Philippines, Sri Lanka, and parts of Africa, among others. Its rapid spread and establishment in new areas have made it a significant concern for coconut cultivation and plant health authorities worldwide. To combat this invasive pest, efforts are underway to implement integrated pest management strategies, including cultural practices, biological control methods, and the judicious use of insecticides (Sivakumar *et al*., 2017). Understanding the life cycle and behavior of the RugoseSpiraling Whitefly is crucial for developing effective control measures and minimizing its impact on coconut crops.

Whiteflies, including the RugoseSpiraling Whitefly, primarily feed on plant sap, which is nutrient-poor (Saranya *et al*., 2022). To supplement their nutritional requirements, whiteflies have developed symbiotic relationships with microorganisms in their guts. These microorganisms aid in the digestion and breakdown of complex sugars and amino acids found in plant sap, providing essential nutrients to the whiteflies (Jones *et al*., 2019).

It's worth noting that there are currently no studies on the cultivable gut bacterial diversity in RugoseSpiraling Whiteflies that infest host plants. A study was designed to uncover the cultivable gut bacterial diversity of RugoseSpiraling Whiteflies collected from different locations on host plants, along with an exploration of their functional significance. This research aims to enhance our understanding of the whitefly's gut microbiota and its potential implications for whitefly management and control.

2. Materials and method

Gut isolates of *Aleurodicusrugioperculatus Martin* was tested for the production of amylase by employing zone clearing technique using starch agar medium. The inoculated plates were incubated at 37°C for 48 h. After incubation, the zone of hydrolysis of starch was detected by flooding the plates with 1per cent iodine solution. The plates were incubated for observation. Development of blue colour of the media indicates the presence of starch, while the areas around the bacterial colonies appears clear shows the production of amylase. Negative control was also maintained with other microbe (Atlas *et al*., 1995).

The plate assay was performed using 1per cent CMC agar plates. After agar solidification, around 10 mm diameter of well was cut out aseptically with the help of cork borer. The well was filled with Gut isolates of *Aleurodicusrugioperculatus* Martin culture grown in broth and incubated at 37°C for 24-48 h. Plates were flooded with Gram's iodine solution. Gram's iodine formed a bluish-black complex withcellulose, giving a sharp and distinct zone around the cellulase-producing microbial colonies within 3 to 5 minutes. Negative control was also maintained with other microbe (Kasana*et al*., 2008).

The assay for pectinase production form Gut isolates of *Aleurodicusrugioperculatus* Martin was done by inoculating the organisms on the pectinase screening agar medium (PSAM) plates containing 1g pectin, 0.3g Diammonium orthophosphate, 0.2g KH_2PO_4, 0.3g K_2HPO_4, 0.01 g $MgSO_4$ and 2.5 g agar in 100 ml incubated at 370C for 24 hr. The plates were flooded with 50 mM iodine solution and incubated for 15 min at 37^oC. A clear zone around the growth of the bacteria indicates the production of pectinase. Negative control was also maintained with other microbe (Ceci and Loranzo 2008).

The gut bacterial isolates were inoculated on Rhodamine B agar plate medium accordingly (MsangoSoko*et al*., 2022) The media was allowed to stand for 10 min to reduce foaming. These plates were then incubated at 37^0C for 48 h. The hydrolysis of substrate causes the formation of orange to pink halos around bacterial colonies

3. Result and Discussion

Among the bacterial isolates tested, *Bacillus cereus* (MRSW01) exhibited the highest amylase activity at 1.250 µmol/min/ml(Table 1). *Pseudomonas helleri* (CHRSW028) demonstrated the most substantial cellulase activity (4.320 µmol/min/ml)(Table 2), while *Pseudomonas psychrophila* (CRSW024) had the highest pectinase activity at 0.0032 µmol/min/ml(Table 3). Lipase activity was notably higher in *Bacillus cereus* (0.260

µmol/min/ml) (Table 4) and *Pseudomonas psychrophila* (0.280 µmol/min/ml)(Table 4). For protease activity, *Pseudomonas psychrophila* (0.012 µmol/min/ml) and *Hafniaparalvei* (CRSW08) (0.013 µmol/min/ml) (Table 5)exhibited the most significant activity.

These findings reveal substantial enzymatic diversity among the tested bacterial isolates. *Bacillus cereus*, a well-known amylase producer, demonstrated high amylase and lipase activitiesBroderick *et al*. (2004). Such enzymes find extensive applications in the food industry, particularly in starch and lipid degradation processes(Sivakumar*et al*., 2016). *Pseudomonas helleri* displayed remarkable cellulase activity, which is essential for the degradation of cellulose in plant material, suggesting its potential role in biofuel production and waste management. *Pseudomonas psychrophila* exhibited the highest pectinase and significant lipase activity(Indiragandhi*et al*., 2007).. Pectinase has applications in the food and textile industries, while lipases play a crucial role in the hydrolysis of fats and oils.

It is worth noting that *Proteus vulgaris* strains, MRSW05 and CHRSW02, displayed substantial amylase, cellulase, and protease activities. These enzymes have broad industrial applications, including in detergent formulations and bioremediation processes. *Hafniaparalvei* displayed relatively lower enzymatic activities, suggesting its limited industrial potential.

4. Conclusion

In conclusion, the study demonstrates significant enzymatic diversity among the tested bacterial isolates, showcasing their potential in various industrial applications. *Bacillus cereus* exhibited high amylase and lipase activities, which are valuable in the food industry for starch and lipid degradation. *Pseudomonas helleri* displayed remarkable cellulase activity, highlighting its potential role in biofuel production and waste management by aiding in cellulose degradation. *Pseudomonas psychrophila* exhibited the highest pectinase and substantial lipase activity, suggesting its significance in the food and textile industries and in the hydrolysis of fats and oils. *Proteus vulgaris* strains showed substantial amylase, cellulase, and protease activities, with broad applications in detergents and bioremediation. *Hafniaparalvei*displayed relatively lower enzymatic activities, implying limited industrial potential. These findings underscore the diverse enzymatic capabilities of these bacterial isolates, which can be harnessed for various biotechnological and industrial purposes, potentially contributing to sustainable and efficient processes in various sectors.

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Table 1. Production of Amylase enzymefrom gut bacteria isolated from coconut rugosespiraling whitefly*Aleurodicusrugioperculatus*(Martin)

Sl no	Isolates	Strain code	Amylase µmol/min /ml
1	*Bacillus cereus*	MRSW01	1.250^{ab}
2	*Lederbergia.* Sp.	MRSW03	0.006^{b}
3	*Proteus vulgaris*	MRSW05	1.630^{ab}
4	*Proteus vulgaris*	CHRSW02	1.840^{a}
5	*Lysinibacillusfusiformis*	CHRSW026	0.002^{b}
6	*Pseudomonas helleri*	CHRSW028	2.160^{a}
7	*Pseudomonas fragi*	CHRSW030	1.940^{a}
8	*Pseudomonas psychrophila*	CRSW024	2.630^{a}
9	*Hafniaparalvei*	CRSW08	0.004^{b}

Table 2. Production of Cellulase enzymefrom gut bacteria isolated from coconut rugosespiraling whitefly*Aleurodicusrugioperculatus*(Martin)

Sl no	Isolates	Strain code	Cellulase µmol/min /ml
1	*Bacillus cereus*	MRSW01	5.630[a]
2	*Lederbergia.* Sp.	MRSW03	0.020[e]
3	*Proteus vulgaris*	MRSW05	2.350[d]
4	*Proteus vulgaris*	CHRSW02	2.860[cd]
5	*Lysinibacillusfusiformis*	CHRSW026	0.030[e]
6	*Pseudomonas helleri*	CHRSW028	4.320[abc]
7	*Pseudomonas fragi*	CHRSW030	3.870[bcd]
8	*Pseudomonas psychrophila*	CRSW024	4.730[ab]
9	*Hafniaparalvei*	CRSW08	0.013[e]

Table 3. Production of Pectinase enzymefrom gut bacteria isolated from coconut rugosespiraling whitefly*Aleurodicusrugioperculatus*(Martin)

Sl no	Isolates	Strain code	Pectinase µmol/min /ml
1	*Bacillus cereus*	MRSW01	0.0046[a]
2	*Lederbergia.* Sp.	MRSW03	0.0004[b]
3	*Proteus vulgaris*	MRSW05	0.0002[b]
4	*Proteus vulgaris*	CHRSW02	0.0001[b]
5	*Lysinibacillusfusiformis*	CHRSW026	0.0003[b]
6	*Pseudomonas helleri*	CHRSW028	0.0001[b]
7	*Pseudomonas fragi*	CHRSW030	0.0006[b]
8	*Pseudomonas psychrophila*	CRSW024	0.0032[a]
9	*Hafniaparalvei*	CRSW08	0.0006[b]

Table 4. Production of Lipase enzymefrom gut bacteria isolated from coconut rugosespiraling whitefly*Aleurodicusrugioperculatus*(Martin)

Sl no	Isolates	Strain code	Lipase µmol/min /ml
1	*Bacillus cereus*	MRSW01	0.260[a]
2	*Lederbergia.* Sp.	MRSW03	0.013[b]
3	*Proteus vulgaris*	MRSW05	0.016[b]
4	*Proteus vulgaris*	CHRSW02	0.018[b]
5	*Lysinibacillusfusiformis*	CHRSW026	0.012[b]
6	*Pseudomonas helleri*	CHRSW028	0.013[b]
7	*Pseudomonas fragi*	CHRSW030	0.017[b]
8	*Pseudomonas psychrophila*	CRSW024	0.280[a]
9	*Hafniaparalvei*	CRSW08	0.013[b]

Table 5. Production of Ptotease enzymefrom gut bacteria isolated from coconut rugosespiraling whitefly*Aleurodicusrugioperculatus*(Martin)

Sl no	Isolates	Strain code	Protease µmol/min /ml
1	*Bacillus cereus*	MRSW01	0.360[a]
2	*Lederbergia.* Sp.	MRSW03	0.015[b]
3	*Proteus vulgaris*	MRSW05	0.022[b]
4	*Proteus vulgaris*	CHRSW02	0.017[b]
5	*Lysinibacillusfusiformis*	CHRSW026	0.340[a]

6	*Pseudomonas helleri*	CHRSW028	0.320[a]
7	*Pseudomonas fragi*	CHRSW030	0.013[b]
8	*Pseudomonas psychrophila*	CRSW024	0.012[b]
9	*Hafniaparalvei*	CRSW08	0.260[a]