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Studies on molecular characterisation and enzymatic potentials of *Micrococcus luteus* isolated from organic kitchen waste

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ABSTRACT

Kitchen waste is a type of organic waste produced from households, restaurants, canteens, hotels. Thus, in this study an effective *Micrococcus Luteus* having enzymatic potentials of amylase, protease, lipase, gelatinase, cellulase and pectinase which will be helpful in the conversion of organic wastes into simple form was isolated, identified by molecular characterization and the enzyme production by it was quantitatively assayed. The study revealed that *Micrococcus luteus* isolated from organic kitchen waste showed greater potential for all the enzymes. Amongst all the obtained isolates from the waste after the primary screening i.e zone of hydrolysis on selective media viz starch, casein agar, tributyrin agar, carboxy methyl cellulose (CMC) agar and pectin agar positive for maximum number of enzymes, were tentatively identified. From them, the obtained *Micrococcus* was subjected for molecular characterisation and further used for enzyme production studies. It was clearly seen that this bacterium was highly potential for the production of amylase, protease, lipase, gelatinase, cellulase and pectinase in quantities as 3.6, 4, 4,3.2,3.5,4.2 EU/ml of enzyme after 5 days of incubation period

Key words: *Micrococcus luteus, Selective media, Amylase, Protease, Lipase, Cellulase*

Introduction

Waste is an unavoidable by-product of most human activity. According to Rajan *et al.* (2019) any unwanted solid, liquid, and gaseous substance is known as waste. Moderately degradable wastes include the tough textured components containing wastes and they are slowly degrading wastes. Examples of such wastes are wood and cardboards (Bhat *et al.*, 2018). The ability of organisms to degrade organic matter depends on their ability to produce enzymes that are needed to degrade the substrate's components (cellulose, hemicellulose and lignin). Kitchen waste is characterized by a high organic content containing soluble sugars, starch, lipids, proteins, cellulose, and other compounds that are readily biodegradable, and generally contain few compounds that inhibit bacteria (Wang *et al.*, 2018). Organic waste degradation requires enzymatic action of microorganisms to hydrolyse highly complex polymers into simpler degradable molecules. Hence, inoculation of biodegradable waste with various beneficial microorganisms, such as bacteria, fungi, and actinomycetes for the synthesis of extracellular enzymes, such as lipase, pectinase, protease, amylase, and cellulase enhances the organic waste degradation rate (Saha *et al.*, 2014). Organic waste components present in the environment can help to convert complex organic substrates to simple by action of active enzymes. Effective bacteria in-

clude Bacillus, Micrococcus, actinomycetes etc. are found to have tremendous potentials of producing the extracellular enzymes in maximum quantity. Thus this study focused on the studying the extracellular enzymatic potentials of a Micrococcus Luteus isolated from organic kitchen waste.

Materials and Methods

Collection of soil samples: Soil samples were collected from kitchenwaste dumping areas of hotels, canteens, households etc.

Primary screening of bacteria: All the soil samples were streak inoculated on special media viz, starch, casein, pectin, tributyrin, carboxymethyl cellulose agar (CMC), and gelatin. The colonies showing zone of hydrolysis around them were selected as positive. All these isolates were tested for their ability to produce maximum number of enzymes.

Characterization of the isolates: All the positive isolates were then identified using cultural morphological and biochemical characteristics. The identified *Micrococcus species* was characterized using 16 s ribosomal r RNA gene sequencing.

Production of enzymes using *Micrococcus Luteus*

The Micrococcus isolated from primary screening was subjected to enzyme production studies. Amylase, protease, pectinase, gelatinase, lipase and cellulase assays were performed.

Results and Discussion

Results of primary screening of the bacterial isolates obtained from organic kitchen waste

The total 23 isolates were obtained from the various sources of organic kitchen waste.

The results of primary screening of the obtained bacterial isolates are shown in Table 1

Identification of Bacterial isolates

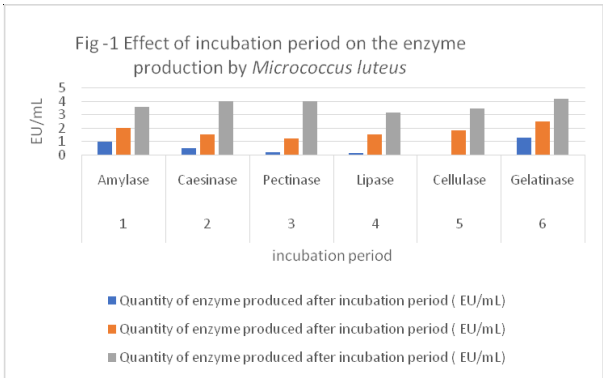
The bacterial isolates were identified by cultural,

Table 1. Isolates showing different enzymatic activities on special media

Sr. No.	Isolate no	Enzyme produced						No. of positive enzyme activities of particular isolates
		Amylase	Caesinase	Pectinase	lipase	cellulase	gelatinase	
1.	KW1	L	M	L	0	L	L	5
2.	KW2	0	L	L	0	L	0	4
3.	KW3	M	M	L	L	L	L	6
4.	KW4	M	L	0	L	M	L	5
5.	KW5	0	L	M	L	L	L	6
6.	KW6	0	0	M	M	L	L	5
7.	KW7	M	M	M	L	L	L	6
8.	KW8	E	L	E	M	L	L	7
9.	KW9	M	M	M	L	L	M	7
10.	KW10	M	L	M	M	L	0	6
11.	KW11	L	0	L	M	0	L	4
12.	KW12	M	M	L	L	L	M	7
13.	KW13	E	L	L	M	M	L	6
14.	KW14	L	0	L	L	M	0	5
15.	KW15	0	M	L	0	M	L	5
16.	KW16	0	L	M	L	L	0	6
17.	KW17	M	L	L	0	L	M	6
18.	KW18	0	L	L	M	L	L	6
19.	KW19	L	M	L	L	E	M	7
20.	KW20	L	M	M	M	L	L	7
21.	KW21	L	L	L	0	M	L	6
22.	KW22	M	L	L	L	L	L	7
23.	KW23	L	L	0	L	0	L	5

Excellent - E, Moderate – M, Low - L

Sr. No.	Enzyme produced	Quantity of enzyme produced after incubation period (EU/mL) 2 days	3 days	5 days	6 days
1	Amylase	1	2.0	3.6	3.6
2	Caesinase	0.5	1.5	4	4.1
3	Pectinase	0.2	1.2	4	4.1
4	Lipase	0.1	1.5	3.2	3.1
5	Cellulase	—	1.8	3.5	3.45
6	Gelatinase	1.3	2.5	4.2	4.2



morphological and biochemical characterization. The bacterial isolate no 8 was tentatively identified as *Micrococcus luteus* and characterized by 16S ribosomal rRNA gene sequencing method.

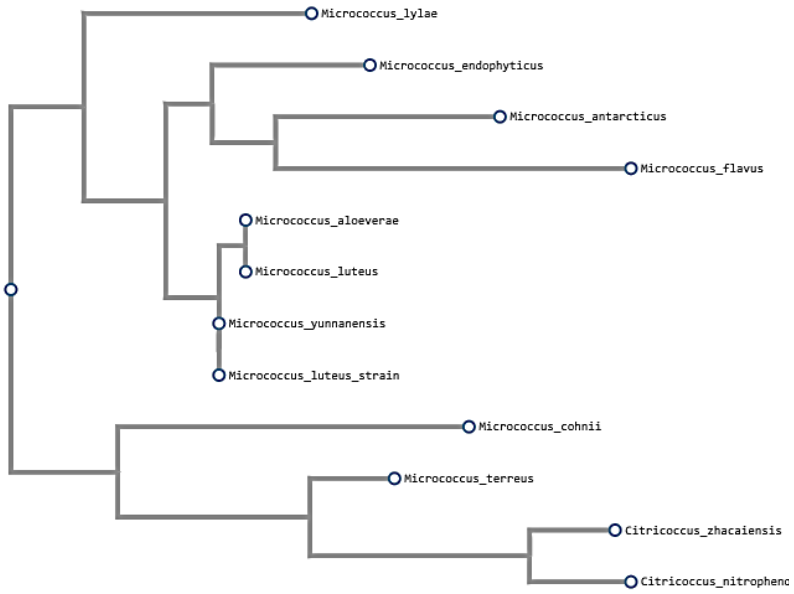
Molecular characterization of isolates KW8

The results revealed that isolate KW8 shared 99.88% sequence similarity with *Micrococcus luteus* NCTC2665(T) (CP00628). The obtained 16S ribosomal rRNA gene sequence of isolates KW8 was submitted to the DDBJ (DNA Data Bank of Japan) and identified as *Micrococcus luteus* with accession number OP 482489.

Results on effect of enzyme production by *Micrococcus luteus* The production of enzymes by *Micrococcus* was quantified after 2, 3 and 5 days. From the Fig. 1 and Table 2 it was clearly indicative that the *Micrococcus luteus* was a potential strain capable of producing all six enzymes concomitantly after 5 days at significant quantities. In 6-days it is almost constant and hence 5-days incubation is optimal. From this it can be concluded that it can be a potential isolate which can be used in organic kitchen waste degradation.

Conclusion

The above results showed a clear indication that the *Micrococcus luteus* isolated from organic kitchen waste is apromising potential bacterium that be further used for its enzyme production studies and their applications in Bioremediation studies in organic waste pollution removal.



Conflict of interest: The authors declare that there is no conflict of interest in the Research work published.

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