

Inhibition of Keratinolytic Bacteria Acting on Nails by Lemon-Salt-Alcohol Sanitizer

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Received: 📅 August 23, 2019

Published: 📅 August 29, 2019

Abstract

Keratin found in human nails is a eukeratin [1]. It is degraded by keratinase produced by bacteria like *Bacillus* sp. Degradation of human nails was brought about by soil inhabiting bacteria isolated from soil samples of Melmaruvathur, Tamil Nadu, India. The growth curve of the bacteria was determined. Inhibition was studied using various mixtures of lime juice, salt and ginger extract in alcohol. The growth of the bacteria producing keratinase isolated by us is found to be inhibited by a mixture of lime juice, salt and alcohol. It was also found that ethanol was more effective than methanol in its inhibitory action [2].

Keywords: Keratin; Inhibition; Sanitizer

Introduction

Keratin is a protein naturally found in skin, hair, feather, wool and nails. Keratin is insoluble in water and not degraded by enzymes such as trypsin, pepsin and papain. Keratin can be present either as α -keratin(α -helix) [3] or β -keratin(β -sheets) in highly coiled structures. This structure actually lends keratin its character to resist breakdown. But it is still digested by enzymes such as keratinase produced by bacteria like some species of *Bacillus*, a few fungi and actinomycetes. Keratinase acts on keratin to liberate various amino acids like Histidine, Lysine and Arginine [4] which are commercially exploited. Keratin can be classified into eukeratin and pseudo keratin depending on the ratio of the amino acids present. Human nails contain eukeratin.

The treatments available currently to degrade keratin are not as efficient as keratinase since they require large amounts of energy [5]. Microbial degradation using keratinolytic bacteria is a viable alternative. The amino acids produced by degradation of keratin are destroyed by the action of the chemical and physical factors used for industrial degradation. If the process is optimized further, it will be possible to extract amounts of amino acids enough for commercial production [6]. Keratinolytic enzymes are used in various industries like cosmetics, textiles, and leather production. Lime, ginger and garlic have been used traditionally in cooking in our country. It is well known that they possess antibacterial activity. It has been found that citric acid found in lime juice is responsible for its higher efficiency in controlling growth of bacteria. The low

pH produced by citric acid causes cytotoxicity which ultimately kills the microbes [7] (Figure 1). project we hope to isolate keratinase producing organism from the area of Melmaruvathur, check the production of keratinase by these microorganisms, study the effect of these microbes on nails and also check the inhibitory action of the lemon-salt-alcohol sanitizer which has been prepared by us.



Figure 1:

Materials and Methods

Isolation of Microorganism

Soil samples were collected from places adjoining the head tansuring areas of Melmaruvathur Adiparasakthi Amman Temple. Serial dilution of this sample was prepared by dissolving 1 g of soil in 9 ml of distilled water. Dilution was performed till a

concentration of 10⁻⁹ was obtained. Then this dilution was poured on Nutrient Agar and baited using nails since our primary aim is to isolate organisms which can degrade the keratin present in nails. The plates were incubated at 37°C for 24 hours. The colonies obtained thus around the baited nails were sub-cultured further to obtain pure cultures of the required microorganism.

Growth Curve Determination

The growth curve of the organism was determined by inoculating one pure colony obtained into 100 ml of nutrient broth containing nails. The readings were taken using a control that contained only nutrient broth and nails. Nails were added to the broth to check the effect of their presence on the growth of the bacteria (Table 1) (Figure 2).

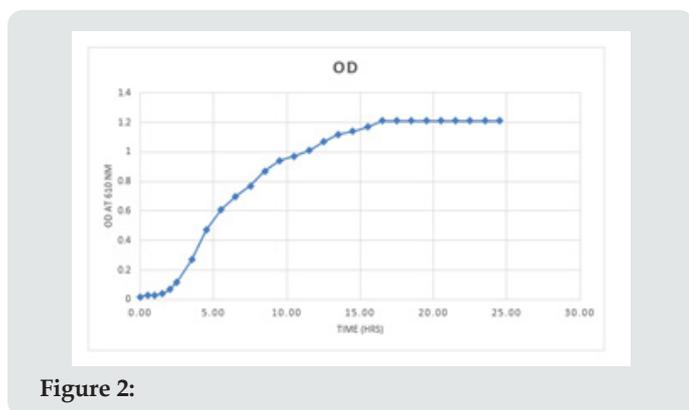


Figure 2:

Table 1.

Time	OD at 610nm	Time	OD at 610nm
0 hrs	0.02	11.5 hrs	1.01
0.5 hrs	0.03	12.5 hrs	1.07
1 hr	0.03	13.5 hrs	1.12
1.5 hrs	0.04	14.5 hrs	1.14
2 hrs	0.07	15.5 hrs	1.17
2.5 hrs	0.12	16.5 hrs	1.21
3.5 hrs	0.27	17.5 hrs	1.21
4.5 hrs	0.47	18.5 hrs	1.21
5.5 hrs	0.61	19.5 hrs	1.21
6.5 hrs	0.7	20.5 hrs	1.21
7.5 hrs	0.77	21.5 hrs	1.21
8.5 hrs	0.87	22.5 hrs	1.21
9.5 hrs	0.94	23.5 hrs	1.21
10.5 hrs	0.97	24.5 hrs	1.21

Preparation of Sanitizer

Two sanitizers were prepared by mixing 0.5 ml of alcohol (methanol/ethanol) [2] with 0.5 ml of a solution mixture containing equal proportions of lemon, table salt and ginger. A spread plate culture of our organism was made, and holes were made into it to add the sanitizer to it. Two Petri plates were taken: one each for ethanol and methanol. They were filled with nutrient agar and

allowed to solidify. 5 holes were made into the agar and filled with the given solutions: - Hole 1: Control (alcohol only); Hole 2: Alcohol+ Ginger extract; Hole 3: Alcohol+ Lemon; Hole 4: Alcohol+ Salt; Hole 5: Sanitizer. The holes were made to avoid the overlapping of zones of inhibition by mixing of solutions. After incubation at 37°C for 24 hrs the radius of zone of inhibition for each sanitizer used was noted in a table (Figure 3) (Table 2).



Figure 3:

Table 2.

Antibacterial Used	Radius of Zone of Inhibition (mm)	
	Ethanol	Methanol
Lemon	6	5.5
Salt (NaCl)	5	4
Ginger	0	0
Mixture	12.5	7.5

Results and Discussion

The mixture of crude extracts of lime, ginger and salt in alcohol is much more effective than the crude extracts separately in alcohol. It was also found that the mixture in ethanol worked better than the mixture dissolved in methanol. This is because methanol is not as effective as ethanol in causing protein denaturation and has a much-reduced bactericidal action. Also, 70% ethanol is better than absolute alcohol in this regard. Fresh ginger extract could not be used due to its unavailability. Ginger extract did not show antibacterial activity when used either in ethanolic or methanolic extract [8] (Figure 4).

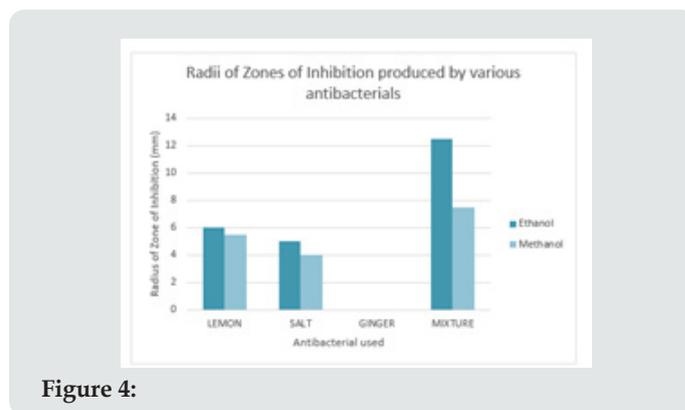


Figure 4:

Conclusion

It was found that our sanitizer which was a mix of lemon, NaCl, ginger and ethanol is more effective than the one containing methanol in inhibiting the growth of Keratinolytic bacteria. This sanitizer can be made easily as it requires very less ingredients and is cost effective.

Acknowledgements

We wish to thank School of Biosciences and Technology, VIT University and our Chancellor, Dr. G. Viswanathan for lending us the support needed to complete this project.

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DOI: [10.32474/OAJESS.2019.03.000168](https://doi.org/10.32474/OAJESS.2019.03.000168)



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