

Original Research Article

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Degradation of Nicotine by the Resident Flora of the Oral Cavity in Tobacco Consumers

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ABSTRACT

Nicotine degradation by micro-organisms has received increasing attention in the past 50 years because microorganisms have the potential to reduce nicotine levels in tobacco. As an environment-friendly treatment, microbial degradation of nicotine has been considered as a promising method due to its low cost and high efficiency. However, the current understanding of nicotine metabolism in micro-organisms is poor. Therefore aim of the present investigation was to isolate nicotine degrading bacteria from oral cavity in tobacco chewers and to study its effect on nicotine degradation. Oral swab samples were collected from tobacco consumers. In the present study the bacteria identified was found to be *Lactobacillus* sp. The inhibitory concentration of nicotine for the nicotine degrading bacteria was studied. It was observed that the increased concentration of nicotine in the nicotine liquid medium was inhibitory to the growth of bacteria in the medium. It was concluded that *Lactobacillus* sp. present in mouth can utilize and breakdown the nicotine. But it should also be taken into consideration that increased amount of nicotine has been found to be inhibitory for these nicotine utilizing bacteria as well.

Keywords

Nicotine
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Oral Bacteria,
Lactobacillus sp.

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Introduction

Tobacco is a product prepared from the leaves of tobacco plants. Chewing tobacco is a serious health risk that can cause severe oral diseases and introduce pathogenic bacteria into the oral cavity. Tobacco is an addictive drug that is harmful to one's health and its use could possibly lead to death (Antolin, 2006). Consumption of tobacco leads to halitosis, stained teeth, dental restorations and serious diseases such as oral cancer (Patel *et al.*, 2004), gingivitis, periodontitis (Scott and Singer, 2004),

melanosis (increased pigmentation on the cheeks and gums), and leukoplakia (white patches or plaques on the lining of the oral mucosa) (Srinivasan and Jewel, 2001).

Nicotine, a principal pyridine alkaline in tobacco plants, is notorious for its significant contribution to tobacco addiction. However, nicotine is very toxic to humans because it is easily absorbed in the body; its hydrophilic nature contributes to the environmental contamination (Holmstedt,

1988; Liu *et al.*, 2014). Nicotine can penetrate biological membranes and the blood-brain barrier easily to exert its effects (Schievelbein, 1982). Nicotine is not a direct cause of most tobacco smoking-related diseases, but it is highly addictive (Benowitz *et al.*, 1999). Currently, regulatory strategies to control the tobacco induced disease epidemic are very much focused on nicotine.

Nicotine degradation by micro-organisms has received increasing attention in the past 50 years because microorganisms have the potential to reduce nicotine levels in tobacco (Civilini *et al.*, 1997; Wang *et al.*, 2004). Many bacteria capable of utilizing nicotine have been isolated and characterized (Brandsch, 2006). As an environment-friendly treatment, microbial degradation of nicotine has been considered as a promising method due to its low cost and high efficiency. However, the current understanding of nicotine metabolism in micro-organisms is poor. Therefore aim of the present investigation was to isolate nicotine degrading bacteria from oral cavity in tobacco chewers and to study its effect on nicotine degradation.

Materials and Methods

Isolation of bacteria from oral cavity of tobacco chewers

Oral samples (mouth swabs) were collected from tobacco chewers, using sterilized cotton buds. The collected swab samples were inoculated in liquid medium containing 1 gm/litre nicotine at 30°C with shaking at 120 rpm in an incubator. The liquid culture medium was a minimal medium containing (per litre) 13.3 g K₂HPO₄·3H₂O, 4 g KH₂PO₄, 0.2 g MgSO₄·7H₂O and 0.5 ml trace elements solution. The trace elements solution contained (per litre of 0.1 M HCl) 0.05 g CaCl₂·2H₂O, 0.05 g CuCl₂·2H₂O,

0.008 g MnSO₄·H₂O, 0.004 g FeSO₄·7H₂O, 0.1 g ZnSO₄, 0.1 g Na₂MoO₄·2H₂O and 0.05 g Na₂WO₄·2H₂O. Nicotine was added to the medium after filter-sterilization. After bacterial growth was observed, the culture was used as an inoculum and transferred twice. The final culture (0.1 ml) was serially diluted and spread onto agar plates containing nicotine. After 2 days, colonies began to appear on plates incubated at 30°C. The colonies were picked and streaked to purity on nicotine agar plates. The isolated bacteria were identified on the basis of morphological, cultural and biochemical characteristics (Wang *et al.*, 2004).

Inhibitory Effect of Different Nicotine Concentration on Isolated Bacteria

Two sets of test tubes containing liquid medium with increasing nicotine concentration were used with 8 test tubes in each set. One set was inoculated with the isolated bacteria and other was kept uninoculated. In each set one tube containing the standard concentration of nicotine was considered as Blank. Both the set of test tubes were incubated for 2 days at 30°C. After 2 days, the tubes were observed for turbidity and compared to the uninoculated tube of the respective concentrations. Optical density was taken at 620 nm. The optical densities of the inoculated tubes were compared with optical densities of the uninoculated tubes to observe the effect of increasing concentration of nicotine on the bacteria (Kumari and Thangavel, 2016).

Results and Discussion

A nicotine-degrading bacterium was isolated from oral cavity samples obtained from tobacco chewers in Nagpur. On the basis of identification *Lactobacillus* spp. was isolated. Saliva is the first biological fluid

that is exposed to tobacco which is responsible for the changes in salivary pH. The bacteria could use nicotine as the sole carbon, nitrogen and energy source (Mujahid *et al.*, 2014). It was reported that

Lactobacillus sp. were utilizing nicotine at maximum of pH 7.0 and sudden increase in pH indicates nicotine degradation (Chaudhary *et al.*, 2007).

Table.1 Inhibitory Effect of Nicotine on Nicotine Degrading Bacteria

Nicotine Concentration (g/litre)	Optical Density (nm) of Uninoculated tubes	Optical Density (nm) of Inoculated tubes
Blank	0.0	0.0
0.5	0.27	0.65
1.0	0.46	0.80
1.5	0.69	1.19
2.0	0.93	1.26
2.5	1.23	1.57
3.0	1.44	1.69
3.5	1.72	1.88
4.0	1.89	1.92

Fig.1 Inhibitory Effect of Nicotine on Nicotine Degrading Bacteria



X-axis: 0.5g/litre (Concentration of Nicotine)

The liquid nicotine medium that was prepared and suspended into test tubes with increasing amount of nicotine was inoculated with the nicotine degrading bacteria that had been isolated from the mouth of tobacco chewers. The objective was to find the inhibitory concentration of nicotine for these nicotine degrading bacteria. Consequently, it was seen that the increased concentration of nicotine in the nicotine liquid medium, that is, concentration of more than 4g/litre was toxic to the growth of bacteria in the medium (Table 1) (Figure 1). It was reported that *Pseudomonas* sp. and *Arthrobacter* carries the ability to breakdown nicotine into carbon, nitrogen and energy (Brandsch *et al.*, 1982). Nicotine utilizing organisms were also studied for nicotine degradation by some researchers (Haileiwei *et al.*, 2008). In some studies normal oral flora organisms were isolated such as *Staphylococcus* sp., *Streptococcus* sp., *Proteus* sp., *Pseudomonas* sp. and *Klebsiella* sp. and reported that *Pseudomonas* sp. has the ability to degrade nicotine (Kumari and Thangavel, 2016).

In conclusion, it can be suggested from the present study that the microorganisms isolated from the oral swab of the tobacco consumers can utilize nicotine present in tobacco and also decrease the hazardous effect that nicotine has on human health after continuous consumption. The bacterium isolated was found to be the *Lactobacillus* sp.

It can thus be said that the presence of *Lactobacillus* sp. in the mouth is an advantage in itself. The utilization of nicotine by this *Lactobacillus* sp. is beneficial to the tobacco chewers, because a part of the nicotine in the tobacco is degraded in the mouth which might help in decreasing the risk associated with it. Thus it

can be concluded that the species of *Lactobacillus* sp. present in mouth can utilize and breakdown the nicotine. But it should also be taken into consideration that increased amount of nicotine has been found to be inhibitory for these nicotine utilizing bacteria as well.

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