Microbial diversity of Areca nut

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Abstract

One hundred twenty-two adults were enrolled, including 21 without a history of betel nut use, 37 previous betel nut users, and 64 present betel nut users. In 10 chewers, leukoplakia and submucous fibrosis were shown to be oral premalignant lesions. Within-sample bacterial diversity was considerably lower in current chewers with oral lesions compared to non-chewers and in long-term (>10 years) chewers compared to never chewers. By chewing status and oral lesion status, between-sample bacterial diversity based on Unifrac distances considerably varied. *Streptococcus infantis* was much more prevalent among current chewers, and different *Actinomyces* and *Streptococcus taxa* were also more and less prevalent. The genera *Actinomyces* and *Streptococcus*. Chewers who chewed for a long time had lower Parascardovia and Streptococcus levels. Chewers with oral lesions had considerably higher concentrations of Actinomyces, Oribacterium, and *Streptococcus*, including *Streptococcus* anginosus. Current betel nut chewing remained the only predictor of oral premalignant lesions in multivariate models that controlled for smoking, oral HPV, S. anginosus, and S. infantis levels.

1. Introduction

Chewing areca (betel) nuts is a major contributor to mouth cancer in some regions of Asia and the Pacific. 10%–20% of people worldwide chew betel nuts, which derive from the Areca catechu palm tree; South and Southeastern Asia and the Pacific have the highest rates of consumption.{1} Melanesia, which includes Papua New Guinea and the Solomon Islands, has the highest rate of oral cavity tumours in the world because betel nut chewing is so popular there.{2} Betel nut chewing is common in Guam, a U.S. territory in the western Pacific {3}

It hasn't been thoroughly investigated how the oral bacterial microbiome may play a part in mouth cancer development linked to betel nuts. More than 300 bacteria, the majority of which are commensals, live in the oral cavity of healthy people and are crucial for maintaining homeostasis. These bacteria also protect against pathogenic species, control inflammation, including the production of proinflammatory cytokines, and convert nitrate and nitrite to nitrogen oxide and other reactive nitrogen intermediates.{4-5}.Betel nut chewers often experience poor oral hygiene{6}Leukoplakia, erythroplakia, and oral submucous fibrosis are the precursor lesions most closely associated with oral cancer in betel nut chewers, however other oral lesions can also occur in the early stages of the disease.{7,8}Alkaloids and polyphenols, including tannins, are the main chemical components of betel nut. The main alkaloid, arecoline, is a muscarinic acetylcholine receptor agonist that acts as a cholinergic agonist on the parasympathetic nervous system as well as a psychoactive substance.{9}

Chemical structure

Alkaloids, tannins, flavonoids, triterpenoids, steroids, and fatty acids are just a few of the many compounds that have so far been discovered in betel nuts.

1.1 Alkaloids

There are between 0.3% and 0.7% of alkaloids in betel nuts, according to estimates. Guvacoline, guvacine, arecatannin A1, arecatannin A2, isoguvacine, homoarecoline, nicotine, and dichroine are other alkaloids.{11}The components of the betel nut-like alkaloids are currently being thoroughly studied. Two novel alkaloids, acatechu A and acatechu B, have been extracted from betel nuts and given their chemical structures.

1.2 Tannins

Betel nuts are bitter and astringent due mostly to tannin. Catechins, arecatannin, and proanthocyanidin A and B are among the proanthocyanidins that make up the majority of the betel nut's tannin content.

1.3 flavonoids

From betel nut, flavonoids have also been extracted that have a range of biological properties, including antibacterial and anticancer effects. The primary families of betel nut flavonoids include isorhamnetin, chrysoeriol, luteolin, quercetin, glycyrrhizin, jacareubin, flavan-3-ol, flavan-3,4-diols, 4′,5-dihydroxyl-3′,5′,7-trimethoxyflavone, and 5,7,4′-trihydroxy-3′,5′-dime

1.4 Triterpenoids and Steroids

Additionally, the betel nut includes triterpenoids such as isotic acid and its derivatives, 3-acetylursolic acid, aromatic alcohol, aromatic methyl ether, fernenol, aroin, cyclic altonil, and cycloartenol.

1.5 Fatty Acids

Fatty acids are typically extracted using supercritical fluid extraction (SFE), and the volatile components of betel nuts are typically analysed using GC/MS.The research found that the peel of betel nuts contains (E,E)-2,4-decadienaldehyde, lauric acid, nutmeg acid, palmitic acid, stearic acid, oleic acid, and hexadanic acid.

Microbes found in betel nut-

Long-term chewing of betel nuts causes the oral cavity's bacteria, such *Veillonella* and *streptococcus*, to create a lot of acid. The endogenous acid-producing bacteria become prominent and can aid in the development of oral disorders like dental cavities or periodontitis if the saliva's capacity to buffer this acidity is exhausted. As a result of its poisonous qualities, eating areca nuts influences microbial diversity and causes physical damage to the oral cavity wall.While homeostasis is compromised after just five minutes of chewing, it was restored by the end of the experiment. *Streptococcus, Veillonella,* and Neisseria were shown to be the primary bacterial genera within the oral cavity. Chewing and oral bacterial numbers were enhanced. Therefore, it stands to reason that these bacteria are crucial for preserving oral homeostasis and mediating host-areca (betel) nut interactions. The processes of carcinogenesis are not well known, despite the fact that eating betel nuts has been proven to be an independent cause of mouth cancer. An analysis was done to see how eating betel nuts affected the oral microbiota and premalignant lesions in the mouth.From a Guam dental clinic, study subjects were gathered. There were oral interrogations and structured interviews. By analysing the 4 region of the 16S rRNA bacterial gene and genotyping for HPV, oral swab and saliva samples were examined. A total of 122 persons were enrolled, including 21 individuals without a history of betel nut consumption and 64 individuals who now chew betel nuts. Ten chewers were found to have oral premalignant lesions, such as

leukoplakia and submucous fibrosis. Long-term (>10 years) chewers compared to non-chewers and present chewers with oral lesions compared to non-lesioned subjects both had considerably decreased within-sample bacterial diversity. As comparison to past/never chewers, present chewers had levels of streptococcus infantis that were four times greater. Current betel nut users had either a considerably higher or lower abundance of certain genus-level *streptococcal* OTUs compared to never or past users, with each OTU likely representing another separate *Streptococcal* species. Also considerably increased or decreased among contemporary chewers were Actinomyces OTUs at the genus level. As comparison to past/never chewers, present chewers had levels of *streptococcus* infantis that were four times greater. Current betel nut users had either a considerably higher or lower abundance of certain genus-level *streptococcal* OTUs compared to never or past users, with each OTU likely representing another separate *Streptococcal* species. Also considerably increased or decreased among contemporary chewers were Actinomyces OTUs at the genus level. Betel nuts may have antibacterial characteristics, which might account for the decline in specific bacteria species, according to in vitro research. It has been demonstrated that prolonged exposure to betel nut aqueous extracts, particularly tannic acid, inhibits the development of common *Streptococcal* species cultivated from saliva.*Streptococcus* intermedius, S. anguinis, and S. mutans from saliva and supragingival plaque samples are likewise inhibited by aqueous betel nut extracts. Oral cancer development may be influenced by betel nut chewing, but the exact mechanism is unknown. Notably, changes in the oral microbiome were found in chewers who had premalignant lesions in their mouths, such as leukoplakia and submucous fibrosis. Oral lesions in betel nut chewers showed elevated levels of several distinct *Streptococcal* OTUs, as well as *Oribacterium* and *Actinomyces* OTUs, in addition to differences in both alpha and beta diversity indices. Notably, betel nut chewers with oral lesions had 16-fold higher levels of *Streptococcus*

anginosus than those without lesions.S. anginosus has previously been found in the tumour tissue, the adjacent normal tissue, and the dental plaque of individuals with oral squamous cell carcinoma. A notable example is the discovery that the anaerobic bacterium S. anginosus causes the creation of NO and inflammatory cytokines in mouse models, pointing to possible carcinogenic processes. Other Streptococcal species, such as S. salivarius, S. gordonii, and S. parasanguinis, have been found to be more prevalent in oral squamous cell carcinoma tissue than in non-tumor tissue. The impact of oral carcinogenesis on alterations in the oral microbiota in betel nut chewers is completely hypothetical. Despite the fact that there was no noticeably increased hazardous species in our research population, it is plausible that the decline in commensal species accelerates the establishment of dangerous bacteria. Alternately, alteration to the typical oral microbiota may impair its capacity to counteract betel nut-induced inflammation of the oral mucosa, increasing vulnerability to malignant transformation. Current betel nut chewing, cigarette smoking, and oral HPV were all considerably more common in individuals presenting with oral premalignant lesions, supporting the multifactorial aetiology of oral malignancies. But after controlling for smoking, HPV, levels of S. anginous and S. infantis, the only factor that continued to be a reliable indicator of oral lesions was current betel nut usage. This supports the independent risk factor for oral cancer that betel nut eating poses. Additionally, oral microbiota alterations brought on by betel nuts may not be connected to the development of mouth cancer. Oral cancer development in betel nut chewers and the microbiota. From a Guam dental clinic, study subjects were gathered. There were oral interrogations and structured interviews. By analysing the 4 region of the 16S rRNA bacterial gene and genotyping for HPV, oral swab and saliva samples were examined. A total of 122 persons were enrolled, including 21 individuals without a history of betel nut consumption and 64 individuals who now chew betel nuts. Ten chewers were found to have oral premalignant lesions,

158 such as leukoplakia and submucous fibrosis. Long-term (>10 years) chewers compared to non-
159 chewers and present chewers with oral lesions compared to non-lesioned subjects both had
160 considerably decreased within-sample bacterial diversity.

161 Numerous studies have revealed a strong link between regular chewing of AN and the
162 development and incidence of oral, esophageal, and pharyngeal malignancies.{12,13}

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