

Isolation of Thermophilic Fungi From Snuff

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Fourteen samples of retail purchases of snuff were examined for the presence of viable thermophilic fungi; four species were found.

Snuff is a tobacco product which is used by inhalation through the nose or by tucking it between the lower lip and gum. Processing of tobacco leaf for manufacture of snuff includes a stage or stages of natural fermentation during which elevated temperatures compatible with growth of thermophilic fungi are attained (7). In view of the human pathogenicity of several species of thermophilic fungi, it was considered worthwhile to examine retail purchases of snuff for the presence of viable propagules of thermophilic fungi.

Thermophilic fungi have been isolated from curing and cured tobacco leaves (3-5; J. T. Fletcher, G. B. Lucas, and R. E. Welty, *Phytopathol.* 57:458-459, 1967); and from retail purchases of cigars (6). I know of no studies of thermophilic fungi in snuff.

Snuff (Table 1) was purchased at retail stores in Chicago, Ill., in May 1974, and was contained in packages which were apparently airtight and undamaged. All analyses were initiated within 9 days of purchase.

The pH value of a 1:1 (vol/vol) mixture of sample and distilled water was measured with an electronic pH meter.

Each sample was opened with precautions sufficient to avoid contamination of the sample, and 0.5 and 0.1 ml of each sample was transferred to plastic petri dishes (15 by 90 mm) by means of calibrated measuring spoons. Twenty-five milliliters of Emerson Yp Ss agar (Difco) (supplemented with trace element solution to add 0.02 μg of Mn per ml from $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 2.0 μg of Zn per ml from $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1 μg of Cu per ml from $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.2 μg of Fe per ml from $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 0.02 μg of Mo per ml from $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} + 4\text{H}_2\text{O}$, 0.2 μg of Co per ml from $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, and 0.01 μg of B per ml from H_3BO_3) plus 50 μg of gentamicin sulfate plus 60 U of penicillin G plus 50 μg of vancomycin hydrochloride per ml (the latter two were added to the molten agar in suspension in 50% ethanol immediately before pouring) at 50 C were poured into each plate, the samples were mixed

with the molten agar, and the plates were rapidly cooled to room temperature. The pH of uninoculated medium was 7.2. These primary plates were incubated at 50 C in an incubator which maintained the temperature within 0.1 C of 50 C at a relative humidity approaching 100%. The same procedures were followed for each sample with incubation at 45 C and also (at 45 C) with the medium adjusted to pH 8.6 by addition of 2 N NaOH immediately before pouring. Plates were examined at 24-h intervals for 7 days and irregularly thereafter. Colonies were transferred to fresh plates of the original medium for purification.

At the end of 12 days, each 50 C plate which contained 0.5 ml of inoculum and which was not overgrown with fungi was inoculated with spores of each of four species of thermophilic fungi (*Chaetomium thermophile* var. *coprophile*, *Humicola lanuginosa*, *Malbranchea pulchella* var. *sulfurea*, and *Thermoascus aurantiacus* sensu Cooney and Emerson [2; ascospores]), and reincubated at 50 C.

The pH value for each sample is given in Table 1. All species which occurred on plates at pH 8.6 also occurred at pH 7.2.

H. lanuginosa (Griffon and Maublanc) Bunce was isolated from SN5 and SN9; *Thielavia albomyces* (Cooney and Emerson) Malloch and Cain from SN13; *M. pulchella* var. *sulfurea* (Miehe) Cooney and Emerson from SN5; and *Talaromyces thermophilus* Stolk from SN5, SN9, and SN13. All of these isolates grew well at 50 C but did not grow at 20 C; they are therefore thermophilic fungi in the sense of Cooney and Emerson (2). Other species grew on 45 C primary plates, but did not grow when subcultured and incubated at 50 C and are not considered further here.

Growth was usually apparent within a few days if it occurred. Since the species found grew rapidly and covered large areas or the entire dish, quantitative measurements of the number of viable propagules per volume of inoculum are meaningless. Based upon experience with quan-

TABLE 1. *Samples examined and their pH values*^a

Designation	pH	Sample
SN1	10.1	Dr. Rumney's Pure Tobacco Snuff, Mentholypus, Illingworth's Snuffs Ltd. Kendal., made in England. 1/2 oz (14.175 g).
SN2	6.8	Bruton Scotch Snuff, Superior Quality, Bruton of Nashville, Tennessee, manufactured by United States Tobacco Co., Nashville, Tenn., 1.15 oz (about 32.60 g), marked 0.14.
SN3	6.3	Scotch Snuff, originated by W. E. Garrett & Sons, Philadelphia, manufactured by American Snuff Div., Conwood Corp., Memphis, Tenn., 1.25 oz (about 35.43 g).
SN4	7.7	Skoal, Wintergreen Flavored Chewing Tobacco, manufactured by United States Tobacco Co., Franklin Park, Ill., packed 15 April, 1.2 oz (about 34.02 g).
SN5	6.6	DeVoe Sweet Scotch Snuff, Eagle Mills, Mild, High Grade, Sweet, manufactured by United States Tobacco Co., Nashville, Tenn., 1.15 oz, marked 014.
SN6	8.5	Seal, Blandning Svenskt Snus, Swedish Snuff, manufactured by United States Tobacco Co., Franklin Park, Ill., packed 18 May, 1.2 oz.
SN7	8.6	Copenhagen Snuff, manufactured by United States Tobacco Co., Franklin Park, Ill., packed 22 April, 1.2 oz.
SN8	9.1	Gallaher's Fine High Toast Snuff, made in United Kingdom, manufactured by Gallaher Limited, Belfast & London.
SN9	7.7	Ozona Snuff, Orange, Tabakfabriken Pöschul Landshut/Bay., W. Germany, imported by IBIS, New York, N.Y.
SN10	9.2	Smith's Snuff, Otterburn, Miniature, G. Smith and Sons, London, made in England, 3.5 drams (about 6.2 g).
SN11	9.2	Smith's Snuff, Town Clerk, Miniature, G. Smith and Sons, London, made in England, 3.5 drams.
SN12	9.5	Smith's Snuff, George IV, Miniature, G. Smith and Sons, London, made in England, 3.5 drams.
SN13	9.1	Smith's Snuff, Consort, Miniature, G. Smith and Sons, London, made in England, 3.5 drams.
SN14	8.8	Smith's Snuff, Carnation, Miniature, G. Smith and Sons, London, made in England, 3.5 drams.

^a SN4, SN6, and SN7 are wet snuffs; the remaining are dry snuffs.

titative and nonquantitative isolation of thermophilic fungi from several thousands of samples of various materials, I estimate that the number of propagules in even the most productive sample, SN5, was less than 10⁴/g.

All 50 C primary plates which were inoculated at day 12 with known species supported excellent growth of these fungi, indicating that the plates provided suitable conditions for detection of thermophilic fungi. No bacterial or actinomycete colonies were observed in primary plates.

The species isolated are typical inhabitants of self-heated organic matter; their presence in snuff is therefore not surprising. Although *H. lanuginosa* has been reported as a zoopathogen (1), it is not suspect as a serious public health hazard. Other thermophilic species of fungi which occur in curing and cured tobacco leaves are, however, of greater concern, and this report of occurrence of viable thermophilic fungi in snuff suggests that further studies, including

examination of different lots of the same product, should be conducted. Investigation of the possible role of thermophilic fungi in tobacco fermentation also deserves further attention.

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