The Effect of Preservatives on the Development of Penicillium.

By Katherine Golden Bitting.

In examining ketchup for the organisms present, it was noted that the hyphæ of moulds in preserved ketchup were swollen and distorted. In many of the brands of ketchup, the mould present was the common blue mould, *Penicillium*. As this mould is apparently omnivorous in habit, thriving and fruiting on many media, has been used in many physiological investigations to determine the nutritive value of many compounds, grows normally in liquid media, and fruits normally in a saturated atmosphere, is regular in its germinative power, and, so far as known, constant in form, it was selected to determine the effect of sodium benzoate, used in varying quantities, on its structure and development. The media used in the experiments were tomato bouillon, tomato gelatin, and tomato pulp, and were selected because the tomato juice and pulp are present in ketchup, and also because they do not alter the toxic properties of the agents used toward the fungus. Afterwards the condiments used in ketchup were tested and also the ordinary food preservatives, though not so extensively as the sodium benzoate. In these latter experiments tomato bouillon was the only medium used.

The bouillon was made by adding to a can of tomatoes an equal volume of water, boiling for about half an hour, and then filtering. The filtrate is clear, and a good medium for growth. It has an acidity of approximately .2% calculated as citric acid. For the tomato gelatin, 10% of gelatin was added to the tomato bouillon, cleared with egg, and filtered. The tomato pulp was obtained from a factory, and was made from whole tomatoes. To these media the sodium benzoate was added in the various amounts used in factory practice. Before sterilizing the media, calendered paper was tied closely over the cotton plug to prevent the distillation of the benzoate. After sterilization and cooling, the media were inoculated with spores from a vigorously growing culture of the mould. During development, the cultures were kept at room temperature, unless otherwise stated. The method of culture was by moist chambers and flasks for the bouillon and gelatin, and Petri dishes for the pulp. The moist

chambers had a few drops of the culture medium placed in the bottom, so as to keep the vapor tension unaltered.

The cultures were examined at regular intervals, as indicated in the tables, those in the flasks having specimens taken for examination with the miscroscope. The points noted were the swelling of the spores preceding germination, the length of hyphæ, and the earliest appearance of conidiophores, for the cultures in the moist chambers. For all other cultures a hand lens was used to determine the first appearance of germination. The appearance of the conidia was shown by the blue color, and the maturing by the change in color from the blue to green, and then to olive. The volume of mycelium and conidia was noted to determine the extent of development.

PENICILLIUM GROWN IN TOMATO BOUILLON 500 CC. 70° F.

Per Cent Sod. Benz.	Time to Germ. Hours.	Development.
——	24	Spores germinated. 48 hours—surface covered. 72 hours—spores developed, surface blue. Hyphæ uniform in outline, protoplasm homogeneous, many vacuoles. 120 hours—fully matured.
1–12	48	Thin ring at edge, small colonies submerged. 120 hours—surface covered, blue. Hyphæ, uneven outlines, protoplasm granular, walls broken easily. 240 hours—fully matured.
1–10	48	Slightly less developed than in the preceding, otherwise alike. 240 hours—fully matured.
1–5	120	Thin interrupted ring at edge. 168 hours—spores swollen, irregular in outline, filled with coarsely granular protoplasm, walls broken by cover glass. 336 hours—surface dotted with colonies, showing blue spots. 348 hours—fully matured.
1–2	——	——

The effect of the sodium benzoate on the development is shown in a retarded and abnormal development, these being accentuated as the amount of the salt was increased, to a point where no development occurred.

PENICILLIUM GROWN IN 10% TOMATO GELATIN. 100 CC. 70° F.

Per Cent Sod. Benz.	Time to Germ. Hours.	Development.
——	24	White colonies dotting surface. 96 hours—surface covered, green, mature.
1–12	24	Same as control.
1–10	24	Same as control.
1–8	24	Same as control.
1–6	24	Less developed than control. 96 hours—surface covered, nearly all green. 432 hours—mycelium curled up round edges.
1–4	48	Small white colonies dotting surface. 96 hours—surface covered, nearly all green. 342 hours—mycelium curled from edges to center.
1–2	72	Small white colonies dotting surface. 96 hours—surface about two-thirds covered, center green. 342 hours—mycelium curled up so as to enclose the spores.

In this experiment in which a solid medium was used, the effect of the sodium benzoate on the development of the mould was not marked, except in the cultures containing the larger amounts. In these there was a slight retardation, and also a curling up by the mycelium from the substratum.

PENICILLIUM GROWN IN TOMATO BOUILLON IN MOIST CHAMBERS. 70° F.

Per Cent Sod. Benz.	Time to Germ. Hours.	Development.
——	24	Short tubes formed. 48 hours—well developed colonies formed.
1–12	24	Short tubes formed. 48 hours—colonies smaller than in the control.
1–10	24	Tubes just forming. 48 hours—less development than in the 1–12th solution.
1–8	24	Less than in the 1–10th solution. 48 hours—less than in the 1–10th solution.
1–6	48	Spores germinated, shorter tubes than in the 1–8th solution.
1–2	96	Spores germinated, short tubes.

NOTE.—In 120 hours the control was exhausted, having empty hyphæ; the other cultures, with the exception of the $\frac{1}{2}\%$ solution, have hyphæ with many vacuoles in the protoplasm, the conidiophores formed are apparently normal.

The effect of the antiseptic on the development of the mould grown in the moist chambers was not so pronounced as when a larger quantity of solution was used. Neither was the effect always uniform; sometimes the spores in the $\frac{1}{4}$% and the $\frac{1}{2}$% solutions merely swelled, but no development of hyphæ occurred; in others short tubes developed from some of the spores, while still other spores showed no changes whatever.

To test the effect of the larger quantity of solution, inoculations were made into flasks containing 100 and 500 cc., respectively, of the solutions. The results indicated that the effect of the antiseptic on the mould development was greater when grown in the larger quantity of the solution.

PENICILLIUM GROWN IN TOMATO PULP, IN PETRI DISHES, 65° F.

Per Cent Sod. Benz.	Time to Germ. Hours.	Development.
- - -	72	White colonies dotting surface. 96 hours—spores formed. 192 hours—surface covered, green.
1-12	144	White colonies growing up on side. 192 hours—spores formed on one side, colonies starting in center.
1-10	192	Colonies started in center. 312 hours—spores formed.
1-8	144	White colonies growing up one side. 192 hours—spores formed.
1-6	—	—
1-4	—	—
1-2	—	—

The pulp used in the experiments was of fine quality, and without any added ingredients such as are used in ketchup, and was used so as to determine the action of the sodium benzoate alone in the pulp. During the early stages of development, the mould grows down into the pulp, so that the whole surface of the hyphæ acts as an absorbent and would thus be affected to a greater extent than where only a part of the surface was in contact. This may serve to explain the more pronounced action of the sodium benzoate when in the pulp, and also the fact that after the mould has developed sufficiently to grow out of the pulp the development becomes more nearly normal.

The experiments were repeated many times and show slight variations, but the results as shown in the tables given are fairly representative.

BENZOIC ACID IN CRANBERRIES.

The occurrence of benzoic acid in cranberries has been cited so often, and in a manner that is often misleading, figures obtained by Lafar[1] on the low-bush cranberry, *Vaccinium Vitis Idaca*, being given for the common cranberries, *Vaccinium macrocarpon* and *Vaccinium Oxycoccus*. *Vac. Vitis Idaca* is a common form in Europe, growing wild, and also in this country in Nova Scotia, and though it is imported into the United States, it is not the form which is used to any extent as compared with *Vac. macrocarpon*, the large cranberry and *Vac. Oxycoccus*, the small cranberry. The amount of benzoic acid in *V. Vitis-Idaca*, as quoted by Lafar, varies from .64–.86 grams per liter.

Testimony[2] given before the committee on interstate and foreign commerce of the House of Representatives on the pure food bills in February, 1906, gave the amount occurring in raw cranberries as ½%, and that half of this was volatilized in the cooking. It was not stated which of the two American species was used for the determination. These figures have not been verified, so far as known to the writer, though diligent search has been made in many chemical and food journals.

There is undoubtedly an antiseptic present in cranberries, a fact known to any one who has made either cranberry jelly or sauce, as these can be kept without spoiling for a long time, even when exposed to the germs in the air.

Experiments were made to determine the effect of growth in cranberry juice on the development of the organism used in the previous experiments.

The cranberries selected were the small oval ones, said to contain the largest amount of the antiseptic and were tested in three ways:

1. 200 grams were crushed in a mortar, then covered with 200 cc. water, and allowed to stand for 12 hours, after which the juice was filtered.

2. 200 grams placed in an open vessel in the sterilizer and steamed until the cranberries were soft, after which they were crushed in a mortar,

[1] Lafar, F., Technical Mycology, Vol. I, p. 117, 1898.
[2] The Canner and Dried Fruit Packer, Vol. XXVI, No. 8.

had 200 cc. water added, then stood for 12 hours, after which the juice was filtered.

3. This was similar to 2, but the vessel was covered closely during the steaming.

For the experiments, 50 cc. of the filtrate from each set were placed in flasks. They were inoculated with the mould without any previous sterilization. The following table shows the time required for, and the effect on, development:

PENICILLIUM GROWN IN CRANBERRY JUICE.

MEDIUM.	Days to Germinate.	Development.
Raw juice	4	Short tubes. 7 days—only small white colonies.
Juice cooked, open	2	Short tubes. 7 days—colony green.
Juice cooked, closed	2	Surface nearly covered, white. 7 days—surface green.
Raw juice + 10cc. water	3	Small white colony. 7 days—surface green.
Juice cooked, open + 10cc. water	2	Surface nearly covered. 7 days—surface green.
Juice cooked, closed + 10cc. water	2	Surface nearly covered. 7 days—surface green.

After two weeks' development, the color of the spores of Penicillium was a yellowish green, instead of the normal bluish green, and the mycelium was very scantily developed. The surface had a somewhat granular appearance, instead of the smooth, even appearance of a normal culture. The filaments, when seen with the microscope, were thin, shrunken, and clear, with distorted outlines. The cultures were kept for months, remaining scanty and granular looking, and a peculiar feature was that no development of bacteria occurred, even in the uninoculated ones, though no sterilization had been done, and the uninoculated were exposed to the

air at times. Sometimes the cultures become infected with yeast, which will develop in a normal manner, seemingly not affected as is the mould.

The antiseptic in the cranberries was weakened by the cooking, and it made little difference whether the vessel in which they were cooked was open or closed, development occurring in the same time in both. It is probable that the contained acid would evaporate to a greater extent if the cooking had been done on a stove, as they are cooked ordinarily, instead of in the enclosed sterilizer. It is also probable that some of the antiseptic property is due to the astringent present, which is said to be destroyed in the cooking[1], and which gives the raw cranberry its unpleasant taste. This is further borne out by the fact that the effect produced on the mould is different from that produced by the benzoate, used either as a salt or acid.

In nearly all the experiments with other media, in which sodium benzoate was used, in the lesser amounts, the organisms though delayed in germination, and at first forming an abnormal development, apparently became accustomed to their environment, and later developed fairly normally, which is different from the result in the cranberry juice, in the latter the restrictive effect persisted.

CONDIMENTS.

The condiments used were those which are used in ketchup—salt, sugar, celery, cinnamon, cloves, garlic, ginger, mace, mustard, paprika, black, white, and red pepper, and vinegar. Along with these acetic acid and alcohol were also tested. With the exception of the cinnamon and cloves, the other spices showed slight antiseptic properties, so are not reported. They were tested in the form of infusions, made according to the method of the U. S. pharmacopoeia[2], also as acetic acid and oil extracts. The ordinary table salt and sugar were used. The quantities of the condiments used in the report were determined after a series of experiments had been made to locate their point of inhibition.

[1] Willis, C. R., Practical Flora, p. 174, 1894.
[2] U. S. Dispensatory, 19th ed., p. 651.

EFFECT OF CONDIMENTS ON DEVELOPMENT OF PENICILLIUM.

Moist chamber cultures, capacity 1.23 cc.

Solutions.	20 Hours.		26 Hours.	48 Hours.		Description.
	Germination.	Hyphæ, Length in μ.	Hyphæ, Length in μ.	Germination.	Hyphæ, Length in μ.	
Tomato bouillon	100%	56.0	.....	.....	∝	
Tomato bouillon + salt, 5%	——	——	——	100%	∝	
" " " 10%	——	——	——	——	——	
" " " 25%	——	——	——	——	——	
Tomato bouillon + sugar, 25%	15%	30.7	.....	75%	.....	Hyphæ distorted, appear empty.
" " " 50%	5%	24.0	.....	75%	.....	" " " "
" " " 75%	——	——	——	——	——	
Tomato bouillon + cinnamon, 5%	100%	136.0	369.1	.....	∝	Nearly normal, spores blue, vertical sterigmata.
" " " 10%	30%	40.0	230.7	.....	∝	Like the 5% but less development.
" " " 25%	20%	32.0	200.0	.....	∝	" 10% " " "
Tomato bouillon + cloves, 5%	100%	88.0	569.1	.....	∝	Hyphæ like the 5% cinnamon, heads close to germinated spore, few conidia formed.
" " " 10%	25%	48.0	369.1	50%	∝	Hyphæ well developed, no conidiophores.
" " " 25%	——	——	——	10%	722.8	
Tomato bouillon + alcohol, 5%	——	——	——	100%	Swollen	
" " " 10%	——	——	——	10%	"	
" " " 15%	——	——	——	——	——	
" " " 20%	——	——	——	——	——	

EFFECT OF CONDIMENTS ON DEVELOPMENT OF PENICILLIUM.

Flask cultures, 50 cc. medium; 70° F.

Solutions.	48 Hours.	72 Hours.	96 Hours.	120 Hours.
Tomato bouillon	Ring, heavy colonies, blue spots, many submerged colonies.	Ring $\frac{1}{2}''$ wide, older part blue.	Ring, $\frac{3}{4}''$, green, surface dotted.	Ring thick, surface covered olive.
Tomato bouillon + salt, 5 %	Few small, submerged colonies.	Many small, submerged colonies.	Few surface colonies	Surface colonies curled, light green.
" " " 10 %	———	———	———	———
" " " 15 %	———	———	———	———
Tomato bouillon + sugar, 25 %	Thin ring at edge	Ring $\frac{1}{4}''$ wide, edge blue	Small surface colonies	Ring thick, surface nearly covered, green.
" " " 50 %	———	———	Ring barely perceptible	Thin ring at edge, green.
" " " 75 %	———	———	———	———
Tomato bouillon + cinnamon, 5 %	Large colonies at edge	Colonies enlarged, blue center, many sub.	Colonies increased in number, older ones green.	Surface nearly covered, thick, wrinkled, olive.
" " " 10 %	———	One small colony	Colony enlarged, center green.	More colonies formed, oldest one olive.
" " " 25 %	———	———	———	———
Tomato bouillon + cloves, 5 %	Thin ring at edge	Ring $\frac{3}{8}''$, edge blue, many sub. col.	Ring $\frac{1}{2}''$, edge green	Ring thick, wrinkled, olive. Surface colonies.
" " " 10 %	———	Tiny colonies at edge	No change	Tiny colonies curled.
" " " 25 %	———	———	———	———
Tomato bouillon + alcohol, 5 %	Tiny colonies on surface	Colonies slightly enlarged, many sub. col.	Few colonies $\frac{3}{8}''$, curled	Colonies thick, wrinkled, blu
" " " 10 %	———	———	———	———
" " " 15 %	———	———	———	———
" " " 20 %	———	———	———	———
Tomato bouillon + acetic acid, $\frac{1}{2}$ %	———	———	Few surface colonies	Slight increase.
" " " " 1 %	———	———	———	———
" " " " 2 %	———	———	———	———

The tables show the germinative power and also the gross effect in development. The moist chamber cultures gave closer results on the germination and the earlier effects on growth, but were not as satisfactory as the flask cultures in showing the general effect on development. In the flasks the amount of development, the method of formation, and the color in maturing could be seen to better advantage.

The 5% salt had a retarding effect, and also induced an abnormal development, the growth being confined to a small amount of curled surface mycelium not spreading normally over the surface, and some submerged colonies. The sugar caused a delayed, stunted development, sometimes the growth in the 50% consisting of a scanty, submerged mycelium. In lesser amounts than 25% a thin surface mycelium forms, with a thick layer of spores. The cinnamon and cloves in the 5% solutions were stimulating, while stronger solutions retarded the development, the cloves being stronger in action than the cinnamon. In the 5% solution of alcohol in the moist chambers the conidia became swollen as they do previous to germination, but no further development took place. In the flask cultures the action of the alcohol was weaker, the conidia germinating and forming small colonies, which was probably due to the evaporation of the alcohol, causing the solution to become weaker on standing. The $\frac{1}{2}$% acetic acid retarded growth, and caused the mycelium to wrinkle. In all the flask cultures with the exception of the alcohol the effect of the condiment of corresponding per cent. was stronger than in the moist chambers.

PRESERVATIVES.

The preservatives are those which have been used in foods, and used in approximately the same amounts. The results show that they have a retarding effect on the development of the mould, even when in small amounts, and that most of them become inhibitive when the amounts are increased, the increase not exceeding the amounts which have been used in foods.

EFFECT OF PRESERVATIVES ON DEVELOPMENT OF PENICILLIUM.

Moist chamber cultures, 80° F.

Solutions.	21 Hours.		28 Hours.	48 Hours.	Description.
	Germination.	Hyphæ, length in μ.	Hyphæ, length in μ.	Hyphæ, length in μ.	
Tomato bouillon	100%	307.6	999.7	∞	Conidiophores formed.
Tomato bouillon + sodium benzoate, 1–10%	100%	46.1	169.2	∞	Conidiophores formed.
" " " " 1–5%	——	——	30.8	∞	Conidiophores starting. Only about 50% spores have developed.
" " " " 1–2%	——	——	——	——	
Tomato bouillon + benzoic acid, 1–10%	25%	30.8	46.1	∞	Only about 35% spores have developed.
" " " " 1–5%	——	——	15.4	769.0	" " 50% " "
" " " " 1–2%	——	——	——	——	
Tomato bouillon + borax, 1–10%	100%	276.8	461.4	∞	Conidiophores formed.
" " " 1–5%	100%	15.4	30.8	.....	No change after 28 hours.
" " " 1–2%	——	——	——	——	
Tomato bouillon + boric acid, 1–10%	50%	76.9	307.6	492.2	
" " " " 1–5%	50%	15.4	30.8	.....	No change in hyphæ after 28 hours. 50% spores swollen.
" " " " 1–2%	——	——	——	——	About 10% of the spores swollen.
Tomato bouillon + sod. salicylate, 1–10%	100%	46.1	307.6	∞	Conidiophores formed, but have few sterigmata.
" " " " 1–5%	100%	123.0	169.2	∞	Thin mycelium, conidiophores formed close to germinated spore
" " " " 1–2%	10%	15.4	76.9	∞	
Tomato bouillon + salicylic acid, 1–10%	50%	30.8	123.0	∞	Like the 1–5% sod. salicylate.
" " " " 1–5%	——	——	——	——	
" " " " 1–2%	——	——	——	——	

EFFECT OF PRESERVATIVES ON DEVELOPMENT OF PENICILLIUM—Continued.

Solutions.	21 Hours.		28 Hours.	48 Hours.	Description.
	Germination.	Hyphæ, length in μ.	Hyphæ, length in μ.	Hyphæ, length in μ.	
Tomato bouillon + sod. sulphite, 1-10%	100%	30.8	92.3	769.0	Like the 1-5% sod. salicylate.
" " " " 1-5%	——	——	——	153.8	
" " " " 1-2%	——	——	——	——	
Tomato bouillon + saccharin 1-10%	10%	15.4	61.5	∝	Conidiophores formed.
" " " 1-5%	——	——	30.8	∝	Conidiophores small, few.
" " " 1-2%	——	——	15.4	507.5	
Tomato bouillon + cop. sulphate, 1-10%	100%	30.8	169.2	∝	
" " " " 1-5%	——	——	15.4	276.8	
" " " " 1-2%	——	——	——	——	
Tomato bouillon + sod. formate, 1-5%	10%	19.0	.....	.....	
" " " " 1-2%	2%	11.4	.....	.....	
Tomato bouillon + formic acid, 1-5%	2%	11.4	.....	.....	
" " " " 1-2%	50%	11.4	.....	.....	

EFFECT OF PRESERVATIVES ON DEVELOPMENT OF PENICILLIUM.

Flask cultures, 50cc. medium; 80°.

Solutions.	24 Hours.	48 Hours.	72 Hours.	18 Days.	*
Tomato bouillon	Tiny colonies at edge.	Edge colonies $\frac{3}{8}''$	Ring $\frac{1}{2}''$, center blue, many sub. col.	Surface covered, wrinkled, olive. Liquid nearly black.	1
Tomato bouillon + sod. benzoate, 1–10 %	———	Tiny colonies at edge, few submerged.	Slight increase	Surface covered, wrinkled, olive Liquid darkened.	2
" " " " 1–5 %	———	———	———	Surface nearly covered, green, mycelium still growing.	8
" " " " 1–2 %	———	———	———	———	
Tomato bouillon + benzoic acid, 1–10 %	———	———	———	Surface nearly covered, wrinkled, green, mycelium growing.	6
" " " " 1–5 %	———	———	———	———	—
" " " " 1–2 %	———	———	———	———	—
Tomato bouillon + borax, 1–10 %	Tiny colonies at edge	Edge colonies $\frac{1}{4}''$, many submerged.	Ring $\frac{3}{8}''$	Surface covered, wrinkled, drab, thin spore layer. Liq. dark.	1
" " " 1–5 %	———	———	Few tiny submerged colonies	Few surface col., curled, drab, thin spore layer, many sub.	3
" " " 1–2 %	———	———	———	———	—
Tomato bouillon + boric acid, 1–10 %	Tiny colonies at edge	Edge colonies $\frac{1}{4}''$, many submerged.	Few surface colonies curled.	Surface partly covered, curled, thin spore layer, many sub.	1
" " " " 1–5 %	———	———	Few submerged colonies	Same general appearance as 28, but less developed.	3
" " " " 1–2 %	———	———	———	Tiny submerged colonies.	14
Tomato bouillon + sod. salicylate, 1–10 %	———	———	Few colonies at edge	Surface nearly covered, wrinkled, olive, liquid darkened.	3

*No. days to germinate.

EFFECT OF PRESERVATIVES ON DEVELOPMENT OF PENICILLIUM—Continued.

Solutions.	24 Hours.	48 Hours.	72 Hours.	18 Days.	*
Tomato bouillon + sod. salicylate, 1–5 %	———	Few edge colonies, few submerged.	Larger than the 1–10 %....	Same general appearance as the 1–10 % but less devel. Liquid slightly darkened.	2 2
" " " " 1–2 %	———	——— ———	———	Colony on side, green, many sub. Liquid darkened slightly.	12
Tomato bouillon + salicylic acid, 1–10 %.	———	Few edge colonies, few submerged.	Colonies curled.....	Surface ⅔ covered, dark olive. Liquid darkened.	2
" " " " 1–5 %.	———	———	———	Like the 1–10 %, but less developed, liquid lighter.	4
" " " " 1–2 %.	———	———	———	———	—
Tomato bouillon + sod. sulphite, 1–10 %.	———	———	———	Surface nearly covered, olive, many sub. col. Liq. darkened.	4
" " " " 1–5 %.	———	———	———	Ring at edge 1″, blue to olive, many sub. col. Liq. darkened.	10
" " " " 1–2 %.	———	———	———	Few edge colonies, green, few submerged colonies.	15
Tomato bouillon + saccharin, 1–10 %....	Tiny colonies at edge.....	Edge colonies ¼″, many submerged.	Interrupted ring ½″, center blue.	Surface nearly covered, wrinkled, olive. Liq. dark slightly.	1
" " " 1–5 %....	———	Edge colonies ⅛″, many submerged.	Ring ¼″, blue spots.	Slightly less development than the 1–10 %.	2
" " " 1–2 %....	———	Tiny colonies at edge, many submerged.	Interrupted ring, surface colony curled.	Same as the 1–5 %.	2
Tomato bouillon + cop. sulphate, 1–10%.	———	Ring of colonies, many submerged.	Surface nearly covered, older parts blue.	Surface nearly covered wrinkled, olive, many sub.	2
" " " " 1–5 %.	———	Ring of colonies, many submerged.	Surface nearly covered, older parts blue.	Surface nearly covered, wrinkled, olive, many sub.	2

" " " " 1–2 %.	————	Thin ring.....	Surface colonies curled.....	Enlarged blue spots, ring olive...	2
Tomato bouillon + sod. formate, 1–5 %..	————	White ring, few submerged..	Thick ring, blue at edge, many sub.	Surface covered, olive, many-submerged.	2
" " " " 1–2 %..	————	Thin interrupted ring, few submerged.	Many surface colonies.....	Surface covered, olive, many submerged.	2
Tomato bouillon + formic acid, 1–5 %...	————	Thin ring, few submerged colonies.	Ring enlarged, blue at edge.	Surface covered, olive, many submerged.	2
" " " " 1–2 %...	————	————	————	Surface nearly covered, green, many submerged.	4

*No. days to germinote.

The results indicate the acid to be stronger in its effect than the corresponding salt, though *Penicillium* is a plant which grows luxuriantly on acid fruits. The sodium sulphite bleached the solutions, ½% being a pale straw color. The copper sulphate solutions were also changed in color, the ½% solution was a decided green.

In all cases microscopic examination was made of material from the flask cultures, and indicated more conclusively than the gross appearance the effect on the development. Submerged colonies have been used for the reports in the table, as they are more uniform. The surface colonies have the characteristics of the submerged in their earlier growth, but as development proceeds and the hyphæ grow away from the medium, the characteristics may change, sometimes more nearly approaching the normal, or they may develop characters more pronounced than the submerged. In a few instances, only submerged colonies, and in the raw cranberry and cinnamon solutions only surface colonies, developed. In making measurements the germinated spores were used, and only the average sizes; the extreme in size was avoided, as not giving a fair estimate of the effect of the preservative. Where only one measurement is given, it indicates that the spores were fairly uniform; where two measurements are given, the spores showed such strong variation that an average was taken of the smaller and also of the larger instead of taking the average of the two sets. The hyphæ were measured but varied so much that it was thought a better estimate could be obtained from the photographs.

Microscopic Appearance of Penicillium Grown in Preservative Solutions.

Preservative.	Size of Germinated Conidia in μ.	Characteristics of Development.
Control	8.5	Hyphæ somewhat irregular in outline near germinated conidia, tapering tips, homogeneous protoplasm, many large round vacuoles.
Salt, 5%	7.6	Hyphæ short, distorted, homogeneous protoplasm, no vacuoles, blunt tips.
Sugar, 50%	7.6	Hyphæ shrunken, distorted, homogeneous protoplasm, vacuoles show as pink spots, giving a beaded appearance.

MICROSCOPIC APPEARANCE OF PENICILLIUM—Continued.

PRESERVATIVE.	Size of Germinated Conidia in μ.	Characteristics of Development.
Cinnamon, 10%	15.2	Hyphæ swollen, blunt tips, protoplasm finely granular, without cohesion, walls break with weight of cover-glass. Few sepia in some, in others prominent. Few side branches. Hyphæ disorganized when placed in water.
Cloves, 10%	13.3	Hyphæ swollen, blunt tips thicker than older part, short thick side branches, finely granular protoplasm, not so badly disorganized as in cinnamon.
Cranberry, raw	7.6	Hyphæ shrunken, distorted, tendency to develop conidiophores close to germinated conidia.
Cranberry, cooked, open	6.7	Hyphæ thin, tapering, protoplasm finely granular.
Cranberry, cooked, covered	7.6	Hyphæ slender, tapering to threads, protoplasm reduced to lining of walls, coarse granules, many septa.
Alcohol, 5%	15.2	Hyphæ swollen, distorted, walls tough, protoplasm clear.
Acetic acid, 1–5%	11.4	Hyphæ enlarged, blunt tips, few septa, short side branches, proto plasm finely granular.
Sodium benz., 1–5%	15.2 38.0	Hyphæ and conidia swollen and distorted, no uniformity in formation of septa, some hyphæ, few, others many; protoplasm coarsely granular, filling tubes; walls break readily.
Benzoic acid, 1–10%	15.2 49.4	Hyphæ larger than in benzoate, more easily broken, distorted. Less swollen hyphæ have less distortion and less disorganization.
Borax, 1–5%	9.5 15.2	Hyphæ short, distorted or long and swollen, blunt ends, protoplasm clear, homogeneous or finely granular.
Boric acid, 1–5%	15.2 19.0	Hyphæ swollen, short thick side branches, blunt ends, protoplasm finely or coarsely granular.
Sodium salicylate, 1–5%	9.5 15.2	Hyphæ as wide as germinated conidia, few septa, granular protoplasm.
Salicylic acid, 1–5%	15.2 30.4	Hyphæ and conidia swollen, some of the conidia much elongated, hyphal ends blunt, few septa, protoplasm yellow, coarsely granular, protoplasm and walls disorganized.
Sodium sulphite, 1–5%	11.4	Hyphæ enlarged, few septa, protoplasm coarsely granular.
Saccharin, 1–5%	13.3	Hyphæ enlarged, some much swollen, slight distortion, clear, homogeneous protoplasm, thick, stunted conidiophores.
Copper sulphate, 1–5%	11.4	Hyphæ enlarged, slight distortion, protoplasm yellow, finely granular, dirty appearance.

Microscopic Appearance of Penicillium—Continued.

Preservative.	Size of Germinated Conidia in μ.	Characteristics of Development.
Sodium formate, 1–5%.....	13.3	Hyphæ swollen, coarsely granular protoplasm, short side branches, blunt ends, disorganized, or normal size with fine granules and many vacuoles, some cells empty.
Sodium formate, 1–2%.....	13.6	Hyphæ swollen, coarsely granular, short side branches which do not develop, blunt ends, disorganized, break easily.
Formic acid, 1–5%.....	14.0	Hyphæ swollen coarsely granular, blunt ends, many broken, or normal size, finely granular, many vacuoles.
Formic acid, 1–2%.....	11.4 41.8	Hyphæ swollen, coarsely granular, yellow, distorted, badly disorganized, break easily. Nearly all germinated conidia broken.

The sugar and salt caused the hyphæ to shrink and to assume distorted shapes when in sufficient amounts to cause a retardation. The cranberry juice, both raw and cooked, also caused shrinkage, and the raw juice a distortion. All of the others caused the conidia and hyphæ to swell and some of them also caused a distortion. The mould grown in the alcohol solution had tough walls in spite of the swelling, and a clear, sharp appearance. The borax and boric acid also produced a clear appearance. The sodium benzoate, benzoic acid, sodium salicylate, salicylic acid, sodium formate, formic acid, acetic acid, and cinnamon produced swelling, distortion, a disorganization of both the protoplasm and cell wall, and a yellowing of the protoplasm. The cell wall had no elasticity nor toughness, so that the placing of the cover-glass gently on a mount was sufficient to break the walls of the more distended hyphæ and to allow the protoplasm to flow out. The protoplasm appeared to be without coherence; when the wall gave way, it flowed in all directions, as if it were composed of loose particles having no cohesion. The sodium sulphite, saccharin, cloves, and copper sulphate growths had similar characteristics to those enumerated for the other preservatives, but not so strongly developed.

In summarizing the results, there seem to be two different actions induced by the action of the substances on the protoplasm, in one case a plasmolyzing effect causing a shrinkage and distortion, as in the salt and sugar, and in the other case a toxic effect producing a disorganization of both the protoplasm and wall, and a discoloration of the protoplasm, the substances showing varying degrees of toxic power.

GERMINATION FROM PRESERVATIVE MATERIAL.

To determine if there were a permanent deleterious effect produced on the plant through the toxic effect of the chemicals, inoculations were made from two weeks' old cultures into tomato bouillon. The result is shown in the table:

GERMINATION OF PENICILLIUM GROWN IN PRESERVATIVE SOLUTIONS 14 DAYS.

PRESERVATIVE.	Number Days to Germinate.	Stage of Development in 5 Days.
Control	1	Surface covered, green.
Sodium benz. 1–10%	2	Surface covered, green.
" " 1–5%	2	" " "
" " 1–2%	4	Thin ring, having blue dots.
Benzoic acid, 1–10%	2	Surface nearly covered, green.
" " 1–5%	4	Small surface colonies, blue.
" " 1–2%	–	———
Borax, 1–10%	2	Surface covered, green.
" 1–5%	2	One surface colony, green.
" 1–2%	–	———
Boric acid, 1–10%	1	Surface covered, green.
" " 1–5%	2	" nearly covered, green in center.
" " 1–2%	5	Few submerged colonies.
Sod. salicylate, 1–10%	1	Surface covered, green.
" " 1–5%.	2	" " "
" " 1–2%	1	" " "
Salicylic acid, 1–10%	1	" " "
" " 1–5%	1	" "
" " 1–2%	–	———
Sod. sulphite, 1–10%	3	Colonies on surface, green.
" " 1–5%	2	Surface covered, "
" " 1–2%	–	———
Saccharin, 1–10%	2	Surface covered, green.
" 1–5%	2	" " "
" 1–2%	1	" " "
Copper sulphate, 1–10%	3	Colonies on surface, green.
" " 1–5%	2	Surface covered, "
" " 1–2%	2	" " "

The germination and subsequent development indicate that the preservative affected the conidia deleteriously, as some were retarded, while the conidia from the solutions showing the strongest effects on the previous development, did not germinate, except from the ½% boric acid solution which formed a few submerged colonies, no surface development taking place. Lafar[1] states that the waterproof character of the conidial walls has a value in preventing the entrance of poisons to the protoplasm, but in the cases noted it is either dissolved by the chemicals or powerless to prevent their passage, for the results indicate that they exercised a decided toxic effect on the protoplasm.

SUMMARY.

Salt and sugar injure the plant by preventing normal action of the protoplasm through plasmolysis.

Alcohol hardens the protoplasm and walls and prevents development.

Cranberry juice, both raw and cooked, retards development and causes shrinkage, though not having the appearance of the shrinkage due to plasmolysis.

All of the other chemicals tested acted as poisons on the protoplasm, retarding development and causing abnormal swelling and disorganization of varying degrees of intensity on both the protoplasm and cell membrane.

Lafayette, Ind.

EXPLANATION OF PHOTOGRAPHS.

The photographs have the same magnification, ×395, so that comparisons may be made as to the effect of the preservatives. The specimens were submerged colonies in all cases except the raw cranberry and cinnamon, and no submerged colonies developed in these solutions. The endeavor was to have all of the same age, but this was impossible, as some developed much more rapidly than others, and in those which were slow in developing it was impossible to determine the changes which the conidia may have been undergoing before the development had attained the colony stage. The submerged colonies were used as soon as they made their appearance. In some of the specimens that show little or no swelling the disorganization can be seen in the collapsed ends of the hyphæ and the floating fragments of protoplasm.

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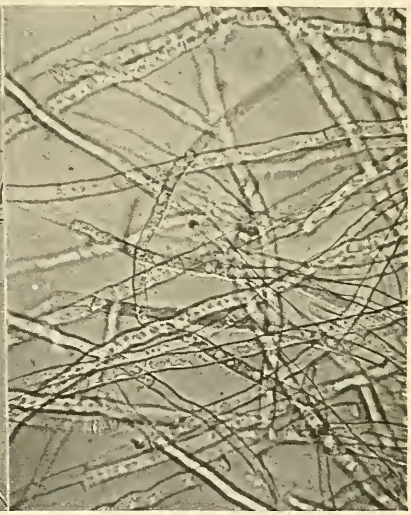
[1] Lafar, F., Technical Mycology, Vol. II, Part 1, p. 40.

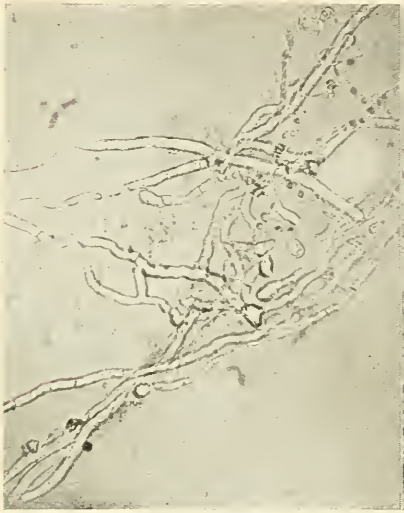
1. Control - conidiophore and hyphæ.



2. Mycelium, grown in tomato pulp.

3. Hyphæ from ketchup preserved with sodium benzoate. The label gave amount as 1-10%.

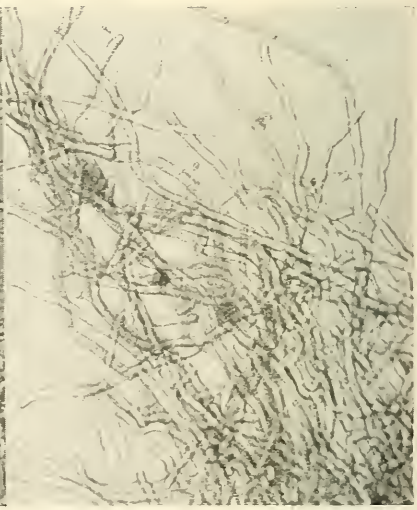
4. Mycelium from 5% salt solution.



5. Mycelium from 50% sugar solution.

6. Mycelium and conidiophores from 10% cinnamon solution.

7. Mycelium showing disorganized hyphæ from 10% clove solution.



8. Mycelium from 10% vinegar (50 grain.)

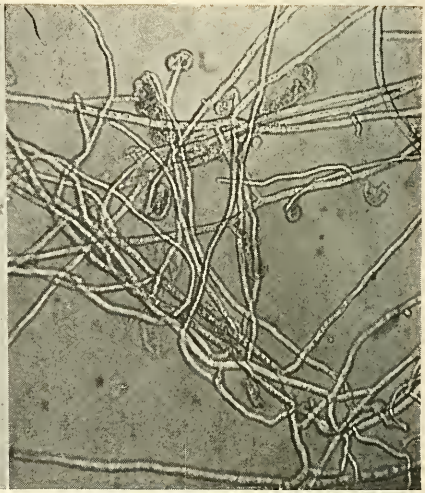
9. Mycelium from 1-5% acetic acid solution.

10. Conidia from 9.6% alcohol solution.

11. Mycelium and enlarged conidium from 1-5% sodium benzoate.

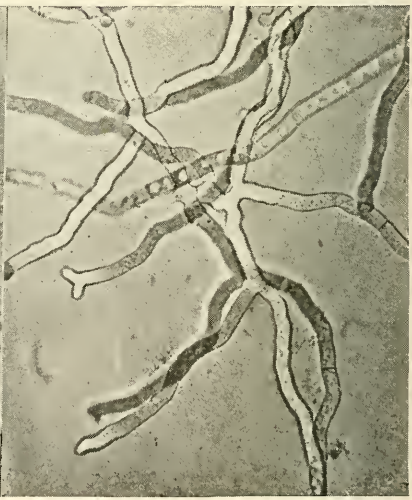
12. Mycelium swollen and disorganized from 1-5% benzoic acid solution.

13. Colony from raw cranberry solution.



14. Mycelium with disorganized hyphal ends from cooked (open) cranberry solution.

15. Mycelium with swollen conidia from cooked (closed) cranberry solution.

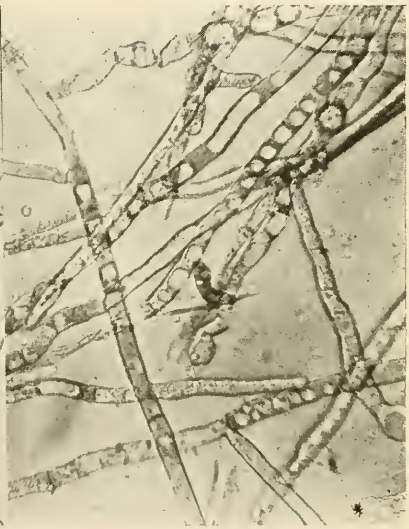


16. Mycelium from 1-5% borax solution.

17. Mycelium from 1-5% boric acid solution.

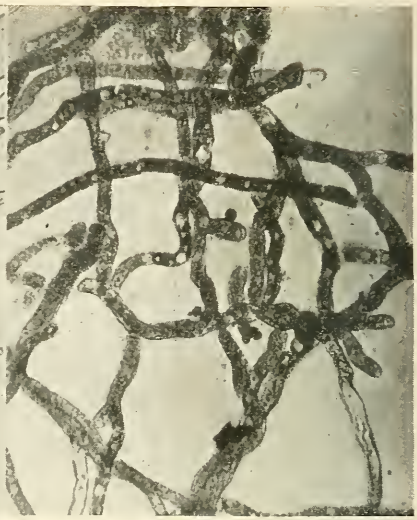
18. Mycelium from 1-5% sodium salicylate solution.

19. Germinated conidia and hyphæ from 1-5% salicylic acid solution.

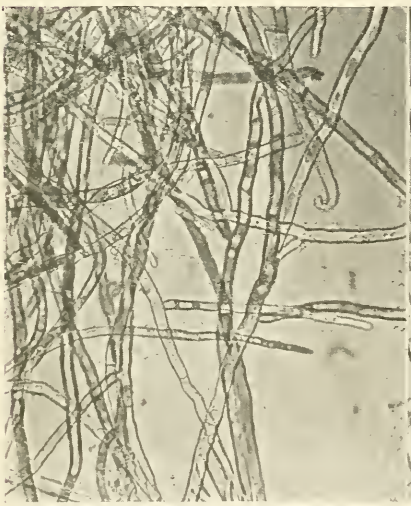


20. Mycelium from 1-5% sodium sulphite solution.

21. Mycelium from 1-5% saccharin solution.



22. Mycelium from 1-5% copper sulphate solution.



23. Mycelium with disorganized hyphal ends from 1-5% sodium formate.

24. Mycelium with disorganized hyphæ from 1-5% formic acid.