The Effect of Various Peptone Sources on Germ Tube Elongation in Uromyces phaseoli

R. M. Niles and Avery E. Rich

Graduate Research Assistant and Plant Pathologist, respectively, Department of Botany, University of New Hampshire, Durham 03824.

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ABSTRACT

The effect of different peptones on germ tube elongation in *Uromyces phaseoli* was studied. Six commercial peptones and plant extracts of pinto bean and lima bean were compared by adding them individually to a basal medium of Czapek's minerals and 2% agar, adjusted to pH 6.8. Uncontaminated bean rust uredospores were seeded onto the appropriate media and incubated at 17 C. The effect of increasing the concentration of peptone and yeast extract was studied. Lima bean extract was the most successful stimulator of germ tube elongation. Among the commercial peptones, only Difco peptone failed to yield tube lengths at least twice those recorded for the control. Increasing concentrations of Evans' peptone, lima bean extract, and yeast extract resulted in longer germ tubes up to a maximal point, after which higher concentrations yielded shorter germ tubes. A synergistic effect was noted in all cases when yeast extract was combined with peptones or plant extracts. Phytopathology 61:562-564.

Additional key words: bean rust, spore germination.

Uredospores from various rusts have been used as the starting material by many investigators trying to initiate saprophytic growth. Ezekiel (3) and Stock (9) observed germ tube lengths of 150-800 μ when uredospores were germinated on distilled water, depending on the isolate and species of rust studied. Ezekiel (3) observed a several-fold increase in germ tube length when he increased the uredospore inoculum density. Stock's (9) work with nutrient concoctions is largely inconclusive as he did not employ aseptic techniques. Sometime later, Yarwood (12) extended this type of work using nonnutrient agar and nutrient agar. Uredospore germ tubes from *Uromyces phaseoli* averaged 390 μ on nonnutrient agar and 1,000 μ on the nutrient agar.

In 1966, Williams et al. (11), starting with uredospores, produced the first axenic culture of a rust. Working on the assumption that rusts could be grown if provided the correct nutrition, they incubated their cultures for long periods of time and looked for germ tube elongation without concomitant infection structure formation. They used a medium containing Czapek's minerals, sucrose, 0.1% yeast extract, and 1% agar, which produced sparse mycelial growth of *Puccinia graminis tritici*. Later, Williams et al. (10), found that the addition of 0.1% Evans' peptone to the previously described medium greatly enhanced growth and stimulated uredospore production. Bushnell (1) repeated Williams' work using the same isolate of *P. graminis*. His findings were essentially the same, except that media without Evans' peptone did not support growth.

Singleton & Young (6) also grew *P. recondita tritici* in axenic culture from uredospores. They found the peptone sources of the medium to be very important. Growth continued for at least 3 weeks on media with Evans' peptone, but only 1 week on Difco peptone. The object of this study was to determine the effect of different peptone sources on germ tube elongation of uredospores of *Uromyces phaseoli* (Pers.) Wint.

MATERIALS AND METHODS.—Bean plants (*Phaseolus vulgaris* L. 'Pinto') were inoculated with uredospores of *Uromyces phaseoli* obtained from H. S. Chow, Crop Protection Inst., Durham, N.H. When white flecking on the leaves was observed, the leaves were removed, surface-sterilized by immersion for 4 min in 15% Clorox (sodium hypochlorite, 5.25%), washed with three changes of distilled water, and placed on nutrient agar at room temperature under continuous white fluorescent light. Uncontaminated uredospores were produced in 4 days and placed in a sterile petri dish in a desiccator to dry the spores. Uredospores were then placed in a sterile vial and stored at 4 C.

Uredospores were hydrated in sterile water and seeded onto plates containing a Czapek agar medium with one of the following commercial sources of peptone: Difco peptone (Difco); Multipeptone (Fisher); Soy peptone (Fisher); Phytone (BBL); Trypticase (BBL); and Evans' peptone (Evans' Medical Ltd., London, England). Trials were run with and without 0.1% yeast extract, and the final medium was adjusted to pH 6.8.

Extracts of pinto bean and lima bean leaves were also used as peptone sources. One hundred mg of leaf tissue was extracted, using the method of Smillie & Krotkov (7). The resultant nucleic acid polypeptide fraction was sterilized by passage through a Millipore filter, and 0.1% of the extract (0.1 ml extract/100 ml culture medium) was added to the culture medium just prior to solidification. The final extract contained nucleic acids and short-chained polypeptides. Trials were again run with and without 0.1% yeast extract. All plates were incubated at 17 C (4), and germ tube lengths were measured and recorded on days 1, 3, 7, 17, 21, and 28.

The effect of peptone concentration on germ tube elongation was determined also. Media containing 0.0%, 0.05%, 0.1%, 0.5%, 1.0%, 1.5%, and 2.0% of the

particular peptone or extract plus 0.1% yeast extract were seeded with uredospores, and germ tube lengths measured after 21 days' incubation at 17 C. The effect of increasing yeast extract concentration at a constant peptone level was tested in the same manner.

RESULTS AND DISCUSSION.—Table 1 shows the results for the entire range of peptone sources and the age at which tube lengths were measured. In all cases, the mean was determined by measuring 150 individual germ tubes/replicate, three replicates/trial.

A comparison graph (Fig. 1) was selected for day 21, by which time germ tube lengths on all peptone sources reached their max length. Soy peptone, Phytone, Multipeptone, Trypticase, and Difco peptone did not differ significantly as peptone sources. Germ tube lengths were about twice the length obtained on the control. Difco peptone gave the lowest germ tube length of all peptones tested, but the difference was not significant. Evans' peptone produced germ tubes significantly larger than the above group (Table 1). Pinto bean leaf extract yielded significantly higher germ tube lengths than any of the commercial peptones. Germ tubes on lima bean leaf extract were longer than on any other material tested, nearly one-third greater than the pinto bean leaf extract. Except for the basal Czapek medium, yeast extract significantly increased germ tube length obtained on all peptone and extract sources.

Figure 2 shows the effect of concentrations of a commercial peptone (Evans') and lima bean extract upon germ tube elongation. Increasing peptone concentration from 0.1% to 1.0% has a greater effect on germ tube length than does increasing yeast extract concentration. The longest lengths were obtained at the 0.5% level for lima bean leaf extract and 1.0% concentration level for Evans' peptone. Increasing yeast extract from 0.05 to 0.1% had a marked effect on germ tube length, but very little response was obtained from increasing the yeast extract level above 0.1%.

Various workers have investigated the physiology of resting uredospores. Caltrider (2) studied the enzymes involved in glucose catabolism in uredospores of *U. phaseoli typica*. He found that enzymes in the Embden-Meyerhof pathway and hexose monophosphate shunt were operative. However, the specific activities of these enzymes were lower than in conidia of *Penicillium oxalicum*. Shaw (5) reviewed much of the work on physiology of rust uredospores. He reported that almost all tricarboxylic acid intermediates have been found and identified. Shaw noted that uredospores were remarkably resistant to respiratory and protein synthesis inhibitors, and that a factor supplied or removed by interaction with the host permits protein synthesis to occur at a normal rate. Staples et al. (8) presented evidence for polysomes in extracts of bean rust uredospores. They report that polysome detection indicates that ungerminated uredospores have an intact mechanism for protein synthesis that is under internal restraint. If we accept the hypothesis that there is a restraint on protein synthesis in uredospores, then the results show that the peptones and especially the plant extracts partially removed this restraint, since germ tube elongation requires increased protein synthesis.

Although the action of the peptones and extracts upon uredospores metabolism is not known, insight may be gained by examining the concentration curves (Fig. 2). All the graphs resemble dose response or limiting nutrient curves that one finds in microbial nutrition studies. Therefore, the peptones and especially the plant extracts may be supplying one or more factors which release somewhat the internal restraint on protein synthesis. It may be an essential amino acid not manufactured by the uredospore or a coenzyme that

TABLE 1. Mean germ tube length (microns) of *Uromyces phaseoli* uredospores at intervals of 1 to 28 days when grown on media containing various sources of peptones

Medium	Days					
	1	3	7	14	21[a]	28
Water agar	326.8[b]	406.0	421.0	452.4	459.0a	440.4
Czapek	276.2	414.3	465.0	477.1	564.7c	504.3
Czapek + yeast	311.5	335.6	408.7	428.6	488.5b	405.5
Soy peptone	365.5	522.7	574.5	710.8	808.1d	748.1
Soy peptone + yeast	408.7	558.3	716.9	778.6	920.9e	901.1
Phytone	363.4	541.8	582.4	731.8	833.0d	741.5
Phytone + yeast	421.7	580.2	707.7	798.0	951.7e	862.5
Multipeptone	360.8	543.6	567.0	724.9	849.3d	754.6
Multipeptone + yeast	414.4	575.1	702.8	807.8	948.4e	863.1
Trypticase	346.9	554.9	572.8	715.3	845.5d	752.0
Trypticase + yeast	432.6	566.6	706.3	793.1	969.0e	866.2
Evans' peptone	433.7	608.0	621.5	785.7	904.6e	826.9
Evans' peptone + yeast	469.4	649.7	770.9	842.7	991.1e	926.9
Difco peptone	346.5	500.1	572.3	706.7	790.2d	750.1
Difco peptone + yeast	377.2	554.6	720.8	770.9	916.6e	863.5
Pinto bean extract	470.7	647.2	669.4	854.8	983.2e	875.1
Pinto bean extract + yeast	499.8	697.7	827.4	897.0	1,095.4f	1,002.0
Lima bean extract	564.3	714.6	803.7	988.5	1,194.9g	1,081.4
Lima bean extract + yeast	582.8	766.5	855.7	1,017.4	1,356.2h	1,142.9

[a] Means not followed by the same letter differ from each other at the 1% level of significance.
[b] Means of three trials.

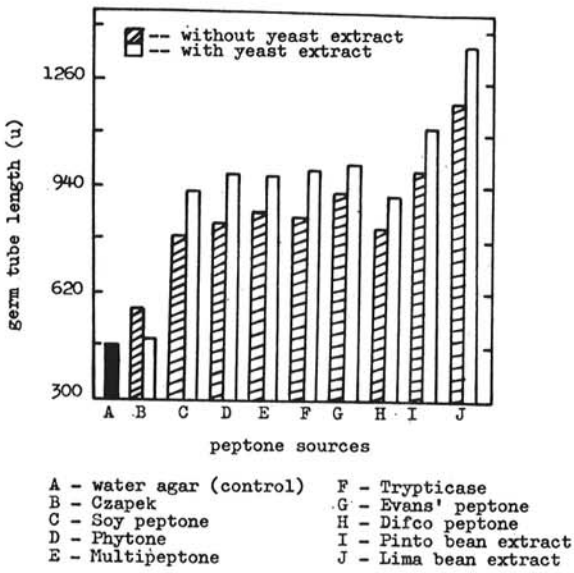

Fig. 1. Comparisons of bean rust (*Uromyces phaseoli*) uredospore germ tube lengths on various peptone sources after 21 days' incubation.

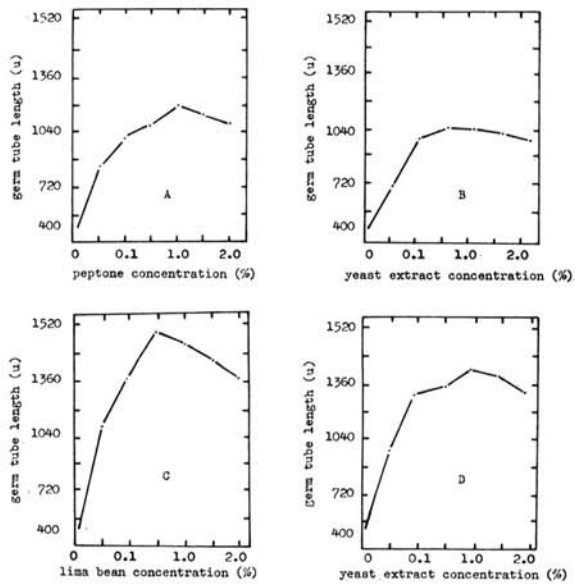

Fig. 2. Bean rust (*Uromyces phaseoli*) uredospore germ tube length as affected by concentrations of **A)** Evans' peptone; **B)** yeast extract with 0.1% Evans' peptone; **C)** lima bean extract; and **D)** yeast extract with 0.1% lima bean extract.

is needed before the uredospore can produce an active enzyme which will remove the block on protein synthesis. In this respect, it is interesting to note the synergistic action of the yeast extract when combined with peptones or bean extracts. Yeast extract is an excellent source of B-complex vitamins, which are essential cofactors for many microorganisms.

Based on the results presented here, more work is needed to elucidate the specific factor(s) present in the peptones, plant extracts, and yeast extract. This should aid in successfully culturing rust fungi such as *Uromyces phaseoli* in vitro.

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