Glucanolytic enzyme production by *Schizophyllum commune* Fr. during mycoparasitism

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Schizophyllum commune S-25 was able to attack 16 out of 50 fungi tested, representing oomycetes, zygomycetes and hyphomycetes, which are either saprophytic, soilborne or foliar plant pathogens. In dual culture agar plates consisting of *S. commune* and its host *Rhizoctonia solani*, the production of extracellular endo-β-1,3(4)-glucanase was markedly enhanced, whereas chitinase, *N*-acetylglucosaminidase and glucosidase were not or barely detected. Coincidentally, the major constituents of the crude enzyme from dual culture broth was endo-β-1,3(4)-glucanase. Treatment of the young hyphae of *R. solani* by these crude enzyme preparations led to the release of protoplasts. The specific activity of the endo-β-1,3(4)-glucanase from crude preparations was increased 240 times by DEAE-Sephadex ion exchange chromatography, Sephadex G-100 gel filtration and isoelectric focusing. The purified endo-β-1,3(4)-glucanase was capable of hydrolysing purified cell walls of *R. solani*. It is suggested that endo-β-1,3(4)-glucanase in cooperation with trace amounts of certain wall-lytic enzymes, such as cellulase produced by *S. commune* may play a crucial role in host–parasite interactions.

INTRODUCTION

The term mycoparasitism was coined by Butler [*6*] to denote the parasitism of one fungus by another fungus. In accordance with the mode of parasitism, the mycoparasites may be separated into two major groups, biotrophic (balanced) mycoparasites and necrotrophic (destructive) mycoparasites [*4*]. Biotrophic mycoparasites are primarily contact and haustorial parasites, in which the absorption of nutrients, from their living hosts is via plasmodesmata, large pore connections, or haustoria [*4*]. The biotrophic mycoparasites usually show host specificity and cause little damage to their hosts [*4*]. In contrast, the necrotrophic mycoparasites usually have a broad host range, attack and invade their hosts by short lateral branches via appressoria, and concomitantly produce an array of wall-lytic enzymes, such as glucanase, chitinase, protease, lipase [*7*], and/or antibiotic metabolites, such as gliotoxin and glioviridin [*10*]. The chitinolytic [*23*] and glucanolytic enzymes [*24*], or the combination of lytic enzymes and toxins [*10*] of the mycoparasites usually act synergistically rather than alone. The invaded fungal hosts are usually lysed and destroyed over time. The wall-lytic enzymes and toxins are suggested to be major mechanisms responsible for biocontrol [*10*]. Due to the devastating effects, some of the necrotrophic mycoparasites, e.g. *Trichoderma* [*7*],

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Abbreviations used in text: SCM, *Schizophyllum* complete medium.

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and *Gliocladium* [*17*], have been extensively and successfully used for biocontrol of a wide range of plant pathogens including *Pythium ultimum*, *R. solani* and *Phytophthora cactorum* [*17*]. Recently it has been shown that lectin mediates host–parasite recognition, attachment and interactions during mycoparasitism [*2*, *26*]. For instance, the binding of the L-fucose and galactose residues to the cell wall surface of *Trichoderma harzianum*, and the agglutinin on the cell wall surface of *Rhizoctonia solani* leads to morphogenetic changes and interactions [*3*].

Previously *Schizophyllum commune* was shown by light, scanning and transmission electron microscopy to be a destructive mycoparasite on several phytopathogenic and nematophagous fungi [*33*]. *S. commune* was induced to form short lateral hyphal branches which clasped, entwined and attacked the host hyphae and fruiting structures. The invasion eventually resulted in degradation, disintegration and lysis of the host hyphae and implicated the involvement of enzymatic activity [*33*]. However, the nature of the wall-degrading enzymes remains unclear and merits further investigation. In this article, the purification and partial characterization of endo-β-1,3(4)-glucanase, a major wall-lytic enzyme responsible for the dissolution and disintegration of the host cell wall during mycoparasitism, is presented. A continuation of this study, by preparation of monoclonal antibody against β-1,3-glucanase to monitor the wall-lytic enzyme activity in mycoparasitism will be published elsewhere.

MATERIALS AND METHODS

S. commune *pure culture establishment*

Fruit-bodies of *S. commune* were collected, mostly from moribund trees from eight counties in Taiwan from September 1984–January 1986. The fruit bodies were attached by double adhesive tape inside the lids of 9·0 cm Petri dishes over 3% water agar. Discharged spore masses were then picked from the plates and streaked onto plates of *Schizophyllum* complete medium (SCM) [*30*]. After incubation for 3 days, agar discs were excised from the colony margins and subcultured onto SCM slants to establish pure cultures. A dikaryotic pure culture established from each fruit-body is designated as an isolate and their mycoparasitic capacity was determined.

Host range and mycoparasitic index

The mycoparasitic activity of 44 isolates of *S. commune* collected from Taiwan, was tested against *R. solani* and *Fusarium moniliforme* as index hosts on half-strength SCM agar plates. The host–parasite interactions were examined with a light microscope 5 days after inoculation. Isolate S-25 of *S. commune* showed the highest mycoparasitic capacity toward both *R. solani* and *F. moniliforme*. Isolate S-25 was also mycoparasitic against a range of other fungi, both plant pathogens and saprophytes. Isolate S-25 was therefore chosen for the present study.

Enzyme assays

Dual cultures of *S. commune* S-25 and *R. solani* on half-strength SCM were incubated at 25 °C for 10 days. Agar discs approx. 1 × 4 cm were excised from the host–parasite interaction zones and immersed in distilled water in a dialysis tube and dialysed against 1 l distilled water, with changes every 4 h at 4 °C overnight, until no reducing sugar was detectable. The enzyme activity of the dialysates was assayed as described below.

Endo-β-1,3(4)-glucanase. One millilitre reaction mixture contained 0·1 ml enzyme solution, 0·9 ml McIlvaine buffer (pH 5·5) and 1 mg of lichenin or 1 mg of $NaIO_4$ oxidized laminarin [*14*]. The reaction was conducted at 37 °C for 15 min with shaking at 100 r min^{-1}. The reaction mixtures were boiled for 5 min and the amount of sugar released by enzymatic hydrolysis was determined by the method of Nelson [*29*]. One unit of endo-β-1,3(4)-glucanase was defined as the quantity of enzyme required to catalyse the formation of 1 μg reducing sugar min^{-1}.

Chitinase. Chitinase was assayed by the method of Jeuniaux [*20*]. Pure chitin (Sigma, C-3641) or the colloidal chitin prepared from coarse chitin (Sigma, C-3132) was used as substrate. One unit of chitinase was defined as the quantity of enzyme required to catalyse the formation of 1 μg *N*-acetylglucosamine min^{-1}.

Cellulase. Cellulase was assayed by the same method as that for endo-β-1,3(4)-glucanase, except sodium carboxymethyl cellulose (Sigma, C-8758) was used as substrate and reacted at pH 4·5. The definition for one unit of cellulase was the same as that for endo-β-1,3(4)-glucanase.

β-D-glucosidase and β-N-acetylglucosaminidase. These two enzymes were assayed by the method of Agrawal & Bahl [*1*]. *p*-Nitrophenyl-β-D-glycopyranoside (Sigma, N-7006) and *p*-nitrophenyl-*N*-acetyl-β-D-glucopyranoside (Sigma, N-9376) were used as substrates. One unit of these two enzymes was defined as the quantity of enzyme required to catalyse the formation of 1 μmol of *p*-nitrophenol min^{-1}.

Protease. The assay of protease was performed by a spectrophotometric method [*32*] using gelatin (Sigma, G-6269) as substrate. One unit of protease catalysed the formation of trichloroacetic acid soluble reaction products with absorbency of 0·01 at 280 nm.

Phospholipase. Phospholipase activity was estimated by using the cup plate assay described by Doery *et al.* [*11*]. Soybean lecithin (Sigma, P-3644) was used as substrate. Enzyme activity which induced a 1 cm diameter clear zone around the hole was defined as one unit.

API ZYM enzyme diagnosis. To assay alkaline phosphatase, acid phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine acrylamidase, trypsin, chymotrypsin, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase, 65 μl enzyme preparations of *R. solani* or *S. commune* or both were added to each well of the diagnostic kit, and reacted for 4 h at 37 °C. The unit for each enzyme was as defined in the manufacturer's manual (API System S.A. Coop., France).

Wall-lytic enzyme. For preparation of the pure cell wall as substrate, *R. solani* was grown in potato sucrose broth at 30 °C for 15 h. The mycelium mats were collected on a filter, rinsed several times with distilled water and then homogenized in phosphate buffer (pH 7·0, 0·1 M) at 10000 r min^{-1} with a Virtis homogenizer for 10 min on ice. The homogenates were centrifuged at 8000 ***g*** for 20 min. The precipitates were homogenized

three times, each time for 5 min, again with bead (Bro Spec. Products) and then washed thoroughly with phosphate buffer until no cytoplasmic contents were visible by light microscopy. The purified cell walls were further subjected to homogenization at 15000 r min^{-1} for 5 min on ice before use. The crude enzymes from the dual culture broth or enzyme preparation partially purified by DEAE-Sephadex A-50 and Sephadex G-100 were assayed for wall-lytic activity by using purified cell walls as substrates in McIlvaine buffer, pH 5·5 (0·5 g/50 ml). The assay methods and units of wall-lytic enzyme activity were the same as that for chitinase, endo-β-1,3(4)-glucanase and protease.

Protein content. The protein content of the enzyme solution was determined by Lowry's method [25] by using bovine serum albumin (Sigma, A-4378) as a standard.

Lysis of hyphae of R. solani

One gram (fresh wt) of the mycelium of *R. solani* grown for 15 h in potato sucrose broth was harvested on Whatman No. 2 filter paper on a Büchner funnel, and washed with distilled water several times. The mycelium was suspended in 10 ml crude or partially purified enzyme solution (in McIlvaine buffer, pH 5·5) in a 50 ml flask, incubated at 37 °C for 6 h with shaking at 100 r min^{-1}, and then examined by light microscopy.

Wall lytic enzyme purification and partial characterization

S. commune and *R. solani* were grown separately in 100 ml half-strength broth at 30 °C in the dark. *R. solani* was incubated for 3 days, and *S. commune* for 5 days, after which mycelial mats of both fungi were aseptically transferred to fresh half-strength SCM broth minus glucose. After incubation for an additional 5 days, the dual culture was filtered through double-layered Miracloth; Chicopes Milles. Inc., U.S.A. and the filtrate used as a crude enzyme preparation. A 5000 ml crude enzyme preparation was condensed to approx. 500 ml by a minifiltration system built with a PT membrane (mol. wt 10000) (Millipore, Mini-pellicon. UF System) at 4 °C and the condensed crude enzyme was subsequently purified in four steps.

Step 1. Ammonium sulphate was gradually added to 250 ml of condensed crude enzyme solution with constant stirring on ice for 2 h, to make 20, 40, 60, 80 and 95 % saturated solutions. The precipitate of each fraction was dialysed against distilled water and the protein content and wall-lytic enzyme activity were assayed.

Step 2. Two millilitres of fractions from the $(NH_4)_2SO_4$ precipitation containing wall-lytic enzyme, endo-β-1,3(4)-glucanase and protease were applied to a 1·6 × 15 cm of DEAE Sephadex A-50 ion exchange column equilibrated with 0·05 M phosphate buffer (pH 7·0) at 1 °C. The column was eluted with 200 ml of 0·05 M phosphate buffer (pH 7·0) followed by a buffered NaCl gradient until 0·5 M was reached. Five millilitre fractions were collected for enzyme activity and protein content assays.

Step 3. Gel filtration (Sephadex G-100 column). One millilitre of enzyme solution from ion exchange column was applied to a 1·6 × 50 cm column of Sephadex G-100 equilibrated with 0·05 M phosphate buffer (pH 7·0) at 1 °C. The column was eluted with the same buffer. Five millilitre fractions were collected and assayed for enzyme activities and protein content.

Step 4. Isoelectric focusing. The peak activity fraction of wall-lytic enzyme, eluted from the Sephadex G-100 column was subjected to isoelectric focusing in a LKB S101 ampholine electrofocusing system at 4 °C for 48 h. A stable pH gradient formed and 5 ml fractions were collected and their values were measured. Each fraction was dialysed in distilled water at 4 °C for 6 h and then assayed for protein content, enzyme activity and isoelectric point.

RESULTS

Host range and wall lytic enzyme activity

Of the 44 local isolates of *S. commune*, a strain assigned as S-25 collected from a dead mango tree (*Mangifera indica* Linn) showed the highest mycoparasitic capacity toward two indexing-hosts, *R. solani* and *F. moniliforme*. *S. commune* S-25 was able to parasitise 16 out of 50 oomycetes, zygomycetes, and hyphomycetes tested (data not shown).

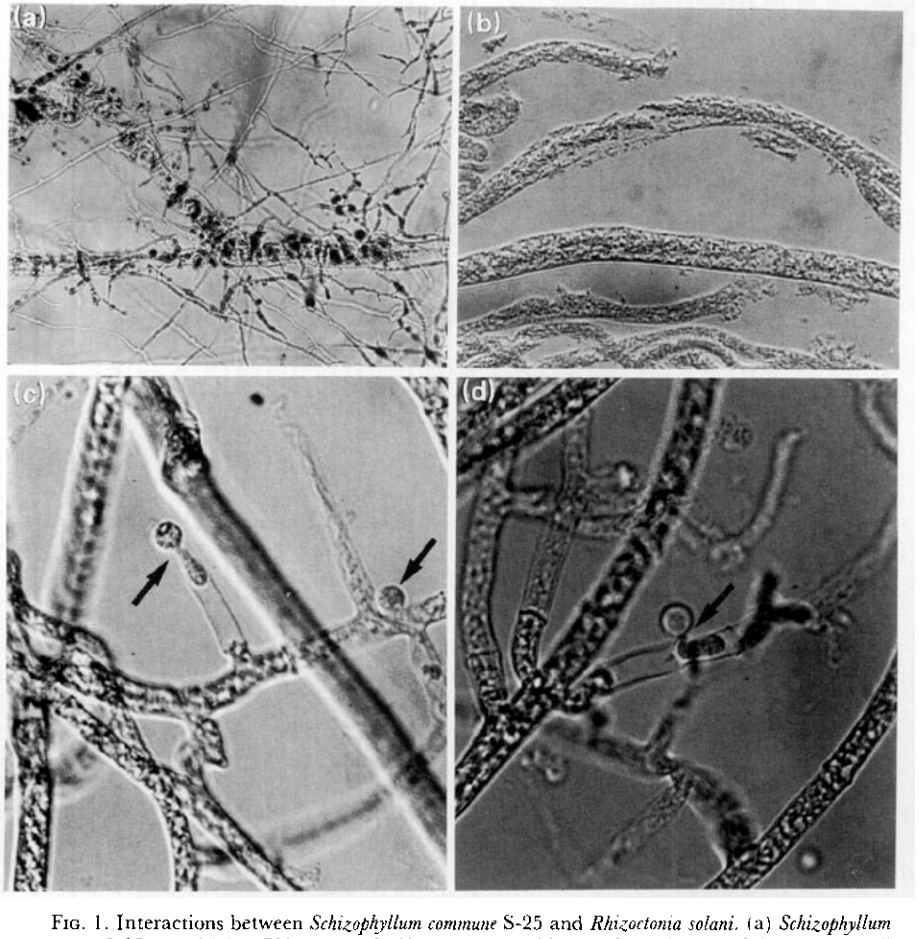

FIG. 1. Interactions between *Schizophyllum commune* S-25 and *Rhizoctonia solani*. (a) *Schizophyllum commune* S-25 parasitizing *Rhizoctonia solani* by short lateral hyphal branches which intensively coil round and entwine the host hyphae. (×400). (b) Hyphae of *R. solani* lysed after treatment with concentrated crude enzyme preparation of *S. commune*. (×400). (c) and (d) Release of protoplasts (arrows) from the hyphae or lateral hyphal tips of *R. solani* after treatment with concentrated crude enzyme preparation of *S. commune* for 6 h.

Some of the susceptible hosts, e.g. *F. solani* f.sp. *pisi* (Jones) Synd. and Hans., and *Pythium aphanidermatum* (Edson) Fitzp., are soilborne plant pathogens, whereas some hosts are fruit or foliar pathogens, such as *Penicillium digitatum* (Pers.: Fr.) Sacc., *Bipolaris maydis* (Nisikado & Miyake) Shoemaker and *B. oryzae* (B. de Haan) Shoemaker. On the other hand, *Colletotrichum musae* (Berk. et Curt.) v. Arx and a *Mycotypha* sp. were very resistant to invasion by *S. commune* S-25 and only slight parasitism occurred. *S. commune* S-25 attacked its hosts by short lateral branches which entwined, sometimes produced appressoria, and occasionally penetrated to form trophic hyphae which ramified and assimilated the host cytoplasmic contents. The growth of the mycoparasite was more profuse when parasitism took place. Conversely, the hyphal elements of the invaded hosts exhibited signs of constriction, disintegration, and lysis in 10–15 days (Fig. 1), implicating the involvement of enzymatic activity.

A time-course study indicated that in a dual culture comprising *S. commune* S-25 and *R. solani* at 10 days the endo-β-1,3(4)-glucanase activity was enhanced approximately three and 30-fold compared with monoculture of *S. commune* or *R. solani*, respectively (Table 1). After 20 days incubation, though the activity of endo-β-1,3-glucanase in *R.*

TABLE 1

Variation of enzyme activity in the 10-day-old pure cultures of Schizophyllum commune *(S),* Rhizoctonia solani *(R) and (S+R) dual cultures of* R. solani *plus* S. commune *(S+R)*

Enzymes*	Culture		
	S	R	S+R
β-D-Glucosidase	1·89	0·83	1·39
β-1,3(4)-Glucanase	8·30	0·80	23·20
Chitinase	0·00	0·00	0·00
Cellulase	1·18	0·00	0·16
Lipase	0·80	0·20	0·60
β-*N*-Acetylglucosaminidase	1·49	0·32	1·42
Protease	10·20	8·80	21·20
Phospholipase	3·25	3·25	3·25

*One unit of enzyme activity is defined as: β-D-glucosidase and β-*N*-acetylglucosaminidase, the quantity of enzyme catalysing the formation of 1 μmol *p*-nitrophenol min^{-1}, β-1,3(4)-glucanase and cellulase, the quantity of enzyme catalysing the formation of 1 μg reducing sugar; chitinase, the amount of enzyme catalysing the formation of 1 μg *N*-acetylglucosamine min^{-1}; phospholipase, enzyme activity required to induce a 1 cm diameter clear zone to form in a substrate agar plate; protease, the quantity of enzyme required to form trichloroacetic acid soluble reaction products sufficient to increase the absorbency at 280 nm by 0·01; lipase, adapted from the definition of the API ZYM manufacturer's manual (API system S. A. Coop., France).

solani monoculture increased significantly (approx. nine times), however it still accounted for approx. one-ninth of the activity of *S. commune* plus *R. solani* in dual culture (data not shown). On the other hand, other wall-lytic enzyme activities, i.e. β-glucosidase, *N*-acetylglucosaminidase, chitinase and cellulase, were very low or undetectable (Table 1). Appreciable protease activity was detected in dual culture, but also in monoculture of *R. solani* and *S. commune*. The small increase in protease activity in dual culture appears not to be significant (Table 1). There was also no

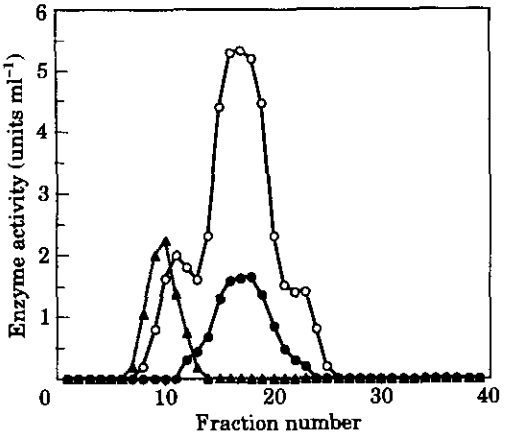

FIG. 2. Ion exchange chromatography profiles (DEAE-Sephadex A-50) of concentrated *Schizophyllum commune–Rhizoctonia solani* dual culture supernatant. (—●—), Wall lytic enzyme activity; (—○—), endo-β-1,3(4)-glucanase activity; (—▲—), protease activity.

distinctive difference between mono- or dual culture supernatants in 19 additional enzymes, e.g. α-mannosidase, α- and β-galactosidase, β-glucosidase, and β-glucuronidase as revealed by API ZYM multiple enzyme diagnosis kit.

Cell wall lytic enzyme purification and partial characterization

No chitinase activity was present in any of the fractions precipitated with ammonium sulphate from the dual culture filtrates. Similarly with phospholipase, except for trace activity (12 units) in the fraction precipitated by 40–60% ammonium sulphate (Table 2). However, appreciable amounts of cellulase, protease, and wall lytic enzyme were

TABLE 2
Enzyme activities in the Schizophyllum commune *and* Rhizoctonia solani *dual culture supernatant fractioned with varied saturations of ammonium sulphate*

Enzymes*	Ammonium sulphate saturation (%)			
	20–40	40–60	60–80	80–95
Endo-β-1,3(4)-Glucanase	256	97	3058	187
Chitinase	0	0	0	0
Cellulase	230	247	0	0
Protease	0	32	590	24
Phospholipase	0	12	0	0
Wall lytic enzyme*	32	34	513	11

*Activity determined by using the partially purified cell wall of *R. solani* as substrate.

present in the crude enzyme preparations. The former was primarily fractionated by 20–60% saturated ammonium sulphate while the latter two were fractionated by the 60–80% (Table 2). *S. commune* S-25 apparently possessed the highest endo-β-1,3(4)-glucanase activity among the enzymes fractionated by ammonium sulphate. The total

units of activity (3598) was almost six to seven times that of cellulase, protease and wall lytic enzymes that were present in crude enzyme preparations (Table 2). The enzyme preparation obtained with 60–80% saturated ammonium sulphate fractionation was subjected to purification by DEAE-Sephadex A-50 ion exchange chromatography (Fig. 2), Sephadex G-100 gel filtration (Fig. 3), and isoelectric focusing (Fig. 4). The

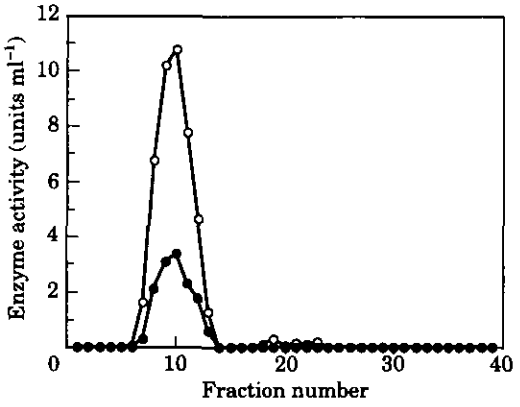

FIG. 3. Gel filtration chromatograph profiles (Sephadex G-100) of concentrated *Schizophyllum commune–Rhizoctonia solani* dual culture supernatant fractionated by ion exchange chromatography. (—●—), Wall-lytic enzyme activity; (—○—), endo-β-1,3(4)-glucanase activity.

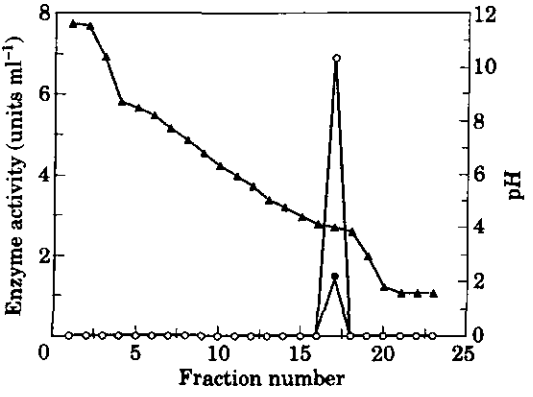

FIG. 4. Column isoelectric focusing profiles (carrier ampholytes pH 3·5–10·0) of concentrated *Schizophyllum commune–Rhizoctonia solani* dual culture supernatant fractionated by anion exchange (DEAE-Sephadex A-50) and gel filtration (Sephadex G-100) chromatography. (—●—), Wall-lytic enzyme activity; (—○—), endo-β-1,3(4) glucanase activity; (—▲—), pH gradient.

final specific activity of the endo-β-1,3(4)-glucanase was 240 times that of crude enzyme solution (Table 3). The purified enzyme hydrolysed the purified cell wall of *R. solani* and released reducing sugar. However, it neither lysed the cell wall of the living host nor induced the discharge of protoplasts even when augmented with the purified protease. In contrast, the crude enzyme preparations lysed the host hyphae and released protoplasts after treatment for 6 h (Fig. 1). The optimum temperature for activity of the endo-β-1,3(4)-glucanase was 40 °C. It was heat-labile, completely losing

TABLE 3

Purification of endo-β-1,3(4)-glucanase from the Schizophyllum commune–Rhizoctonia solani *dual culture supernatant by ion exchange, gel filtration chromatography and column isoelectric focusing*

Purification step	Recovery (%)	Protein (mg ml^{-1})	Specific activity (unit ug^{-1} protein)	Purification fold
Crude enzyme	100	75	72	1
Condensed enzyme (10-fold)	98	66·1	80	1
$(NH_4)_2SO_4$	57	4·73	647	9
DEAE-Sephadex A-50	28	0·30	5027	70
Sephadex G-100	10	0·05	10920	152
Isoelectric focusing	9	0·03	17275	240

its activity when heated at 60 °C for 8 min but it still retained 82% of its activity after storage at 4 °C for 8 days. Its pI value was 4·0 (Fig. 4), over an optimum pH ranging from 5·7–6·2 and it was stable between pH 4·6 and 7·1.

DISCUSSION

The roles played by chitinolytic and glucanolytic enzymes in the growth, development, morphogenesis or parasitic behaviour in fungi are well documented. The autolysis of *Coprinus* basidiocarp, the growth and expansion of the pileus in *Schizophyllum*, the parasitism of one fungus on another, or on insects, or on other microfauna are correlated with lytic enzyme activity [*5, 8, 19, 23, 24, 36*]. Under the present experimental system *R. solani* produced a substantial amount of β-1,3-glucanase but only in old cultures (20-day-old) undergoing autolysis. During mycoparasitism, particularly in the early stages, the possibility that the lytic enzyme, β-1,3-glucanase, primarily originated from *R. solani* as a result of more rapid aging due to stress induced by *S. commune* cannot be ruled out. The disintegration and lysis of *R. solani* hyphae does not occur randomly, but is restricted to certain specific sites where parasitism occurs as revealed by scanning and transmission electron microscopy [*33*]. Furthermore, later experiments proved that β-1,3-glucanase activity in dual culture of *R. solani* and a weak *S. commune* mycoparasite differed from that using a virulent strain of *S. commune*. The former produced only about one-third to one-fourth the enzyme activity of the latter in 8 to 12-day-old cultures [*18*]. This evidence implied that under the same circumstances, the glucanolytic enzyme activity was closely related to virulence of the mycoparasite, *S. commune*.

Endo-β-1,3(4)-glucanase produced by *S. commune* seems to play a crucial role in the parasitization of *R. solani*, *F. moniliforme* and other fungi. This conclusion is based mainly on the following observations. Firstly, the crude enzymes collected from the dual culture broth could lyse the host hyphae and lead to the release of protoplasts. Secondly, the crude enzyme concentrated and purified by Sephadex ion exchange chromatography and gel filtration resulted in an increase of 240 times in specific

activity. The purified enzyme lacked cellulase, protease, lipase, phospholipase, β-D-glucosidase, *N*-acetylglucosaminidase and chitinase activity but was still capable of degrading laminarin (β-1,3-glucan) and lichenin [1-3(4)glucan]. Also, the purified enzyme could digest laminarin when both terminal sugars had been previously oxidized by $NaIO_4$. Thirdly, the endo-β-1,3(4)-glucanase was capable of lysing the purified cell wall of *R. solani*. The endo-β-1,3(4)-glucanase of *S. commune* was adaptive and constitutive, because its activity was greatly enhanced in dual culture in the early parasitic phase (10 days). Recently, using a monoclonal antibody raised against β-1,3-glucanase conjugated with fluorescein isothiocyanate (FITC) to probe the interface between *S. commune* and *R. solani* we have observed the presence of a specific fluorescence, particularly in the interaction sites where mycoparasitism was taking place but fluorescence was absent on control *R. solani* or *S. commune*. The mycoparasitic activity was also considerably reduced when the host and parasite were treated with the monoclonal antibody immediately before making contact with each other [*18*]. Moreover, a virulent strain of *S. commune*, produced three to four times more β-1,3-glucanase in dual culture supernatant in 8–12 days than a less virulent strain. The virulent strain completely destroyed the host, *R. solani* in 14 days, whereas the less virulent strain did not; the host in this instance was still alive and produced abundant sclerotia [*18*]. The results suggest a causal relationship between endo-β-1,3-glucanase production and mycoparasitic behaviour by *S. commune*.

Although *S. commune* commonly grows as a saprotroph or as a weak parasite on wood substrates [*9*], other habitats also have been documented such as the rhizosphere of banana in Honduras [*15*], or forest and cultivated soils in Georgia, U.S.A. [*28*]. Soils are inhabited by a wide variety of basidiomycetes [*35*]. *R. solani* is usually recognized as a soilborne plant pathogen. Nevertheless, it can also attack stems or foliage. For instance, rice sheath blight, peanut and soybean leaf scorch caused by *R. solani* are responsible for the yield decline and inferior quality of these crops [*37*]. Therefore in terms of niche or habitats, the interaction between *S. commune* and *R. solani* described here is likely to occur in nature. In other situations, *S. commune* also can attack foliar or fruit pathogens, e.g. *Bipolaris maydis*, *Penicillium digitatum* (cf. Table 1). In the light of these events, the potential for biocontrol of air- or soilborne fungal plant pathogens cannot be ruled out.

S. commune was able to invade a wide range of fungal hosts with varied cell wall structures and constituents. The failure of the purified endo-β-1,3(4)-glucanase to dissolve the living host mycelium indicates a requirement for additional cell wall-lytic enzymes to assist penetration. Chitinase may be excluded, but cellulase (endo-β-1,4-glucanase) may have a synergistic effect with endo-β-1,3(4)-glucanase in the dissolution of *R. solani* cell walls by *S. commune*. This is supported by the finding that Onozuka Cellulase can induce a high release of protoplasts from young mycelium of *R. solani* [*16*]. When major skeletal elements like glucan are degraded, the degradation will in turn result in changes in the chemistry and structure of the fungal cell wall and lead to its eventual dissolution [*21*, *22*]. Some other lytic enzymes such as chitinase [*31*], pustulanase [*36*], exo-, and endo-1,3-glucanase [*21*], α-1,3-glucanase [*34*], β-1,6-glucanase [*36*], chitinase and β-1,3-glucanase [*10*, *12*, *13*, *23*, *24*] have been claimed to play some roles in mycoparasitic host–parasite interactions or in fungal cell wall degradation.

Recent evidence suggests that lectins and some proteinaceous mucilages coating the outer layer of fungal host cell walls may account for specific recognition in the mycoparasitic interactions between *Trichoderma* spp. and *Sclerotium rolfsii* [*3*], and *Pythium nunn* during interactions with host fungi [*13*]. In biotrophic mycoparasitism by *Piptocephalis virginiana* and *Choaneophora cucurbitarum* the cell wall chitosan and the cellular r-linolenic acid of the mucoraceous hosts are determinants for specificity and resistance [*27*]. The factors determining host range, specificity and resistance in *S. commune* are still unclear; however involvement of lectins seems possible and merits further exploration.

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