Chapter 5
Plant Nematode Surfaces

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Abstract The outer surface of nematodes act as an external skeleton and is covered by a tough, but flexible, multi-layered, extracellular cuticle which protects them from the external environment, maintains body shape and is involved in locomotion and defence against their host or microorganism attack. This chapter highlights the role of the nematode surface cuticle, during the various life-stages, with their environment, including their host and other microorganism. A comprehensive appraisal is presented of the complex interactions between nematodes and microbial antagonists, as the surface cuticle is believed to be involved in the host-recognition events determining the specificity of such interactions.

5.1 Introduction

The nematode cuticle is an extracellular coating that is secreted by the hypodermis and has a variety of important roles in nematode biology. The cuticle maintains the body shape, provides a strong layer against which muscles can act during locomotion and protects the nematode from the external environment. The cuticle is overlaid with a fuzzy coating material – the surface coat (SC). Substances on the surface

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of the cuticle are in direct contact with the outside world and therefore, also have important roles in terms of the interactions of nematodes during the various life-stages with their environment, including their host and other microorganism. Nematodes eggs generally are the most resistant nematode life-stage and many have a remarkable capacity for survival in stressful environments; therefore, the eggshell is a most important barrier at this stage.

5.2 Eggshell

Most nematode eggs are morphologically very similar and are similar in size (average of 53–133 μm in length and 17–79 μm in width) irrespective of the size of the adult. They are ellipsoidal in shape with a transparent shell (Bird and Bird 1991). The eggshell in nematodes is formed after fertilization and usually contains four layers. The outermost layer (uterine) consists of material that is secreted by the uterine epithelial cells and can be absent in some nematodes. In plant-parasitic nematodes the composition of the uterine layer resembles the gelatinous matrix (gm) secreted from various organs (Mackintosh 1960; Maggenti 1962; Bird and Rogers 1965). The next layer, vitelline, originates from the vitelline membrane (oolemma) which is formed after fertilization of the oocyte. In many eggs this retains a unit membrane-like structure and forms the outer layer of the eggshell. Carbohydrate residues have been detected on the surfaces of the eggs (Rao et al. 1988; Taylor et al. 1986; Spiegel and McClure 1991).

The underlying chitinous layer is made-up of chitin microfibrils embedded in a protein coat (Wharton 1983). Chitin is formed from the polymerization of N-acetyl glucosamine which is itself synthesized from glycogen (Preston and Jenkins 1985). The chitinous layer is often the thickest layer and provides structural rigidity to the eggshell. Protein is frequently present in this layer; it has been estimated that the eggshell of *Globodera rostochiensis* contains 59% protein and 9% chitin while that of *Meloidogyne javanica* contains 50% protein and 30% chitin (Clarke et al. 1967; Bird and McClure 1976). Perry and Trett (1986) suggest the sub-division of the chitinous layers of cyst nematodes into distinctive outer and inner components due to differences in their chemical composition on the basis of ultrastructural studies that showed that the external outer layer is thin and amorphous followed by a predominantly tetra-pentalaminate inner layer. Chitin is synthesised by chitin synthase and the gene encoding this enzyme has been shown to be expressed in the egg-producing adult stages and fertilized eggs of various nematodes including *Caenorhabditis elegans*, *M. artiellia*, *Ascaris suum*, *Brugia malayi* and *Dirofilaria immitis* (Veronico et al. 2001; Harris and Fuhrman 2002; Dubinsky et al. 1986a, b). These observations are consistent with a role for this enzyme in producing chitin for the eggshell. This has been confirmed with RNAi-mediated ablation of the *C. elegans* chitin synthase gene function which resulted in sterile hermaphodites that lay defective eggs (Hanazawa et al. 2001). RNAi of a chitin synthase gene expressed in the eggs of root-knot nematode *M. artiella* was achieved by soaking intact eggs

within their gelatinous matrix in a solution containing dsRNA and led to a reduction in stainable chitin in eggshells and a delay in hatching of juveniles from treated eggs (Fanelli et al. 2005). So far, the eggshell is the only structure in nematodes in which the presence of chitin has been conclusively demonstrated (Bird and Bird 1991). A chitin synthase gene has been shown to be expressed in the cells that form the pharynx of *C. elegans* at a time that precedes a moult. It has also been suggested that this gene might be involved in the synthesis of the feeding apparatus which is replaced during each moult (Veronico et al. 2001). A secreted chitinase has been identified in the perivitelline fluid surrounding the infective larva of *A. suum* prior to hatching indicating that this enzyme might be responsible for the digestion of the eggshell during hatching of this nematode (Geng et al. 2002). The potential of using bacterial and fungal chitinases and chitin synthase inhibitors to control root-knot nematodes have been demonstrated (Spiegel and Chet 1985; Jung et al. 2002; van Nguyen et al. 2007).

The most internal layer is the lipid layer which is responsible for the extreme impermeability of the nematode eggshell. It is formed in the middle region of the uterus in *M. javanica* (Bird and McClure 1976) where proline-containing proteins are incorporated into both lipid and chitinous layers and the synthesis of the eggshell is then completed (Bird and Bird 1991). Nematode eggs are permeable to chemicals prior to the formation of the lipid layer and when this layer is broken down before hatching. Egg permeability changes are central to the hatching of cyst nematodes (Perry and Clarke 1981; Bird and McClure 1976) and work by Twomey et al. (2000) corroborated these studies and showed that the biological nematicide DiTera (fermentation product of killed *Myrothecium verrucaria*) induced a significant inhibition of hatch of *G. rostochiensis* by possibly preventing eggshell permeability change by competitively blocking the Ca^{2+} binding sites on the eggshell. DiTera showed a lack of inhibition of hatch on *M. incognita* and therefore do not affect second-stage juveniles (J2) directly during the hatch process or inhibit the action of enzymes. It has been suggested that the breakdown of the lipid layer is enzymatic and secretions have been shown to emanate from various structures of J2s of *M. incognita* and *M. javanica*, including the amphids, secretory-excretory pore and from around the mouth while still inside the egg. An increase in size of the nucleolus of the dorsal pharyngeal glands of *G. rostochiensis* J2 inside the eggs that were stimulated with root diffusate was demonstrated indicating that the oesophageal glands are activated and leucine amonipeptidase activity was identified in the supernatant of eggs of *Heterodera glycines* (Atkinson et al. 1987; Premachandran et al. 1988; Bird and Bird 1991). Perry and Trett (1986) observed that the eggs within *H. glycines* cysts internally contaminated with fungus, had no inner lipid layer and it was suggested that fungal lipases might have contributed to disrupt both inner and outer lipid layers.

As both the lipid layer and the juvenile epicuticle are derived directly from the secondary vitelline membrane of the embryo, both probably share the same protein moieties. Cross-reactivity of polyclonal and monoclonal antibodies (Fig. 5.1) produced against *M. incognita* J2 with eggshells support this suggestion (R. Curtis unpubl; Sharon et al. 2002, 2009a).

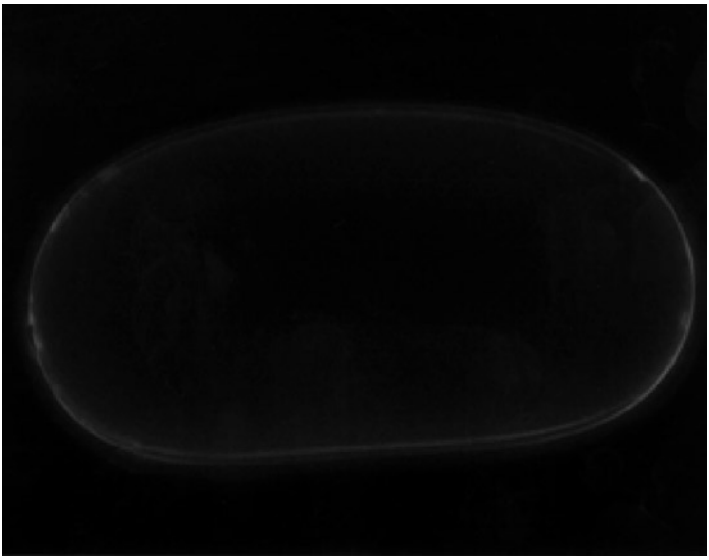

Fig. 5.1 Monoclonal antibody raised to second-stage juveniles of *Meloidogyne incognita* shows reactivity with the egg-shell

Cysteine proteinases are involved in a variety of biological functions and have been implicated in tissue remodeling in free-living and animal-parasitic nematodes. Cathepsin L has been shown to be present in the eggshell surrounding the embryos of *C. elegans*, *Onchocerca volvulus* and *B. pahangi* and is possibly involved in eggshell remodeling by processing of nutrients responsible for the synthesis and/or degradation of the eggshell in these nematodes (Hashmi et al. 2002; Guiliano et al. 2004). Transmission electron microscopy showed that the eggshells and cuticles layers of *C. elegans* and of the parasitic stages of *Haemonchus contortus* have an ABC transporter, P-glycoprotein. This might function as a membrane efflux 'pump' and may play a major role in the transport of antihelmintic drugs in parasitic nematodes of ruminants (Riou et al. 2005).

5.3 Cuticle Structure and Function

A detailed review describing the structure of the cuticle and contrasting the cuticular features of a wide range of nematode species has been published previously (Lee 2002) and only the salient points are summarized here. The cuticle of most nematodes can be subdivided into three main zones covered with an epicuticle. In addition, the cuticle is overlaid with a surface coat composed of mucins and other proteins (Fig. 5.2). The innermost cuticle zone, the basal zone, often has a striated appearance when sections through the cuticle are viewed under an electron microscope and is thought to be composed largely of collagens (see below), arranged in oriented layers of fibrils. The median zone varies in appearance

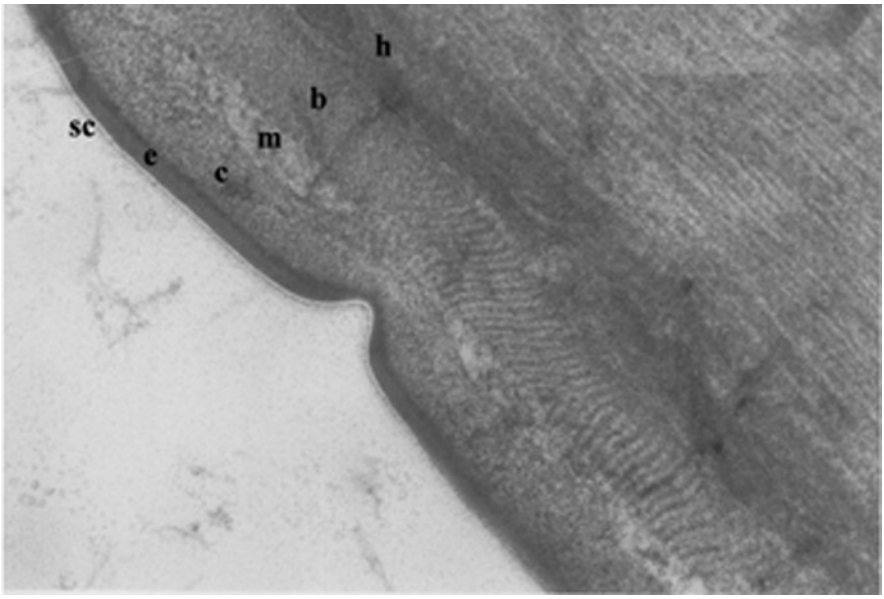

Fig. 5.2 Transmission electron micrograph of a longitudal section of *Meloidogyne javanica* second-stage juvenile (X 40,000), showing surface coat (*sc*), epicuticle (*e*), cortical zone (*c*), median zone (*m*), basal zone (*b*) and hypodermis (*h*)

in electron micrographs but often appears less electron-dense than the basal and cortical zones. It may appear to contain vacuoles and struts and has often been described as fluid-filled or gel-like. The appearance of this layer sometimes varies between nematode life-stages. For example, the median zone of unhatched second-stage juveniles (J2s) of *G. rostochiensis* appears to contain an electron dense material which is lost on hatching (Jones et al. 1993). It has been suggested that this zone is composed of a combination of collagens and soluble proteins, possibly destined for the nematode surface (Blaxter and Robertson 1998). The cortical zone varies enormously in thickness and may have regions that have different electron densities when viewed under an electron microscope. The cortical zone is composed of collagens and extremely insoluble cuticulins. The cuticle of many nematodes is annulated and may also carry a wide range of projections including hooks, bristles and papillae. Many of these projections are associated with mechanoreceptive neurons (Jones 2002). Lateral projections (alae) may also be present that extend along the length of the nematode body.

The best characterised, and most abundant, of the cuticle proteins are the collagens. Collagens from a wide range of animal species have a conserved and characteristic triple helical tertiary structure. This region is conserved to such an extent that the first nematode collagen gene was identified using a probe derived from a vertebrate sequence (Kramer et al. 1982). The triple helical region of the protein is formed from large Gly-X-Y repeat regions in which every third amino acid is a glycine residue and where Y is frequently proline, which forces the helical turn.

Collagen genes are present in extremely large gene families in a wide range of nematode species; over 170 genes are present in *C. elegans* (Page and Johnstone 2007) and 122 are predicted from the genome of *M. incognita* (Abad et al. 2008). This large suite of collagen proteins allows variation in the collagens present in different life-stages and thus variation in the structural and physical properties of the cuticle of different life-stages (Koltai et al. 1997; Liu et al. 2001). A number of conserved cysteine residues are present in the non Gly-X-Y, N- and C-terminal regions and within short stretches of amino acids that interrupt the Gly-X-Y regions. The number and spacing of these residues, along with other conserved residues, has been used to subdivide the *C. elegans* collagens into subfamilies (Johnstone 1994). The other major protein component of the cuticle is formed by cuticulins. Cuticulins contain cysteine rich regions and are extensively cross linked, particularly in the outer cortical layers and in the dauer cuticle, through tyrosine (Page and Johnstone 2007). It has been suggested on the basis of expression patterns of various cuticulin genes in *C. elegans* that cuticulins are also important in formation of lateral alae and other cuticular annulations and ridges (Sapio et al. 2005). Studies on the expression patterns during the development of *M. artiellia* indicate that there is a burst of expression of the cut-1 gene during moulting. Then, the expression rate is reduced in the infective juveniles, which migrate in the soil. In the sedentary females, in contrast, no expression is detected, while in the males which move freely through the soil, the gene is expressed and the transcript fully processed. These data strongly suggest that the gene is developmentally regulated. It is proposed that the production of cuticlin plays an important role in determining the mechanical properties of the cuticle (de Giorgi et al. 1997).

The cuticle itself is synthesized during a series of moults in the hypodermis, a syncytial cell layer immediately below the cuticle. The moulting process can be subdivided into several distinct phases and has been analysed in detail in *C. elegans*. Once the new cuticle has been resynthesised the nematode shows a decrease in activity and feeding (lethargus) followed by separation of the old cuticle from the new structure (apolysis). The nematode then moves rapidly in order to loosen the old cuticle which is shed during ecdysis. The new cuticle is synthesised at the end of each juvenile stage and the timing of the expression patterns of collagen genes during this process has been examined in detail (Johnstone and Barry 1996). Cuticle collagen genes are expressed in distinct waves and in an order that is repeated at each moult, with some genes always expressed early in the moulting cycle and others at a later stage. It is possible that these waves of gene expression exist to ensure that the collagen components required for each cuticle zone or substructure are expressed simultaneously, with different zones synthesised at different times in the moulting cycle.

5.4 The Surface Coat and Excreted/Secreted Antigens

The surface coat (SC) of nematodes contains various proteins, carbohydrates and lipids, as individual components or as glycoproteins/mucins, glycolipids, or lipoproteins. Studies have used antisera, lectins, biotin and neoglycoproteins to characterize and

localize surface components and to learn about its nature. Binding of lectins indicated the presence of specific carbohydrates on the surface and amphids of different life-stages of plant-parasitic nematodes. Carbohydrate-recognition domains (CRDs) were demonstrated on the surface of plant-parasitic nematodes, suggesting the presence of lectins on the surface of the nematodes (Spiegel and McClure 1995; Sharon and Spiegel 1996). Carbohydrates or CRDs on nematodes surface and/or secretions might be involved in nematode-plant and nematode-microorganisms interactions (Spiegel and McClure 1995; Koltai et al. 2002). Surface coat proteins and glycoproteins from pre-parasitic J2s and adult females of *Meloidogyne* were labeled, extracted and partially characterized (Robinson et al. 1989; Lin and McClure 1996; Spiegel et al. 1997). Specific antibodies were raised against surface antigens and excretory-secretory (E-S) products of plant-parasitic nematodes and have been used to localize and characterize antigens on SC regions on the nematodes (Fig. 5.3), on amphids and secretory-excretory products. Antibodies served as a tool for studying interactions with plant hosts (*e.g.* Curtis 1996; Gravato-Nobre and Evans 1998; Lopez de Mendoza et al. 1999) and microorganisms (*e.g.* Spiegel et al. 1996; Davies and Danks 1992; Sharon et al. 2009a).

One of the most interesting features of the nematode SC is its labile and dynamic nature and there is a continuous turn-over of surface-associated antigens that involves shedding and replacing of the antigens (Blaxter and Robertson 1998). Studies have shown that surface coat of plant-parasitic nematodes is shed (Fig. 5.4) both in vitro (Bird et al. 1988; Lin and McClure 1996; Spiegel et al. 1997; Robertson et al. 2000) and in the host (Curtis 1996; Gravato-Nobre et al. 1999;

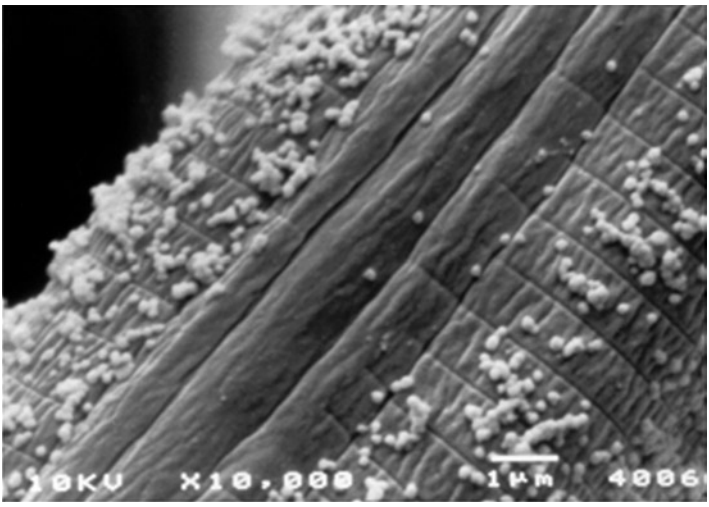

Fig. 5.3 Scaning electron micrograph of immunogold-labeled surface of *Meloidogyne javanica* second-stage juvenile; labeling was visualized by silver enhancement reaction, bar = 1 µm

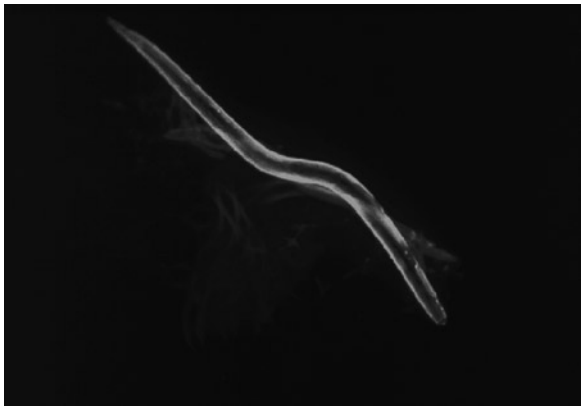

Fig. 5.4 Antiserum (anti-rGR-TpX) binds to the surface of *Globodera rostochiensis* and to material shed in great quantities from the parasite surface

Sharon et al. 2002). Unlike other cuticle proteins surface coat molecules are readily secreted/released into the environment and this was demonstrated for the preparasitic juveniles of *Meloidogyne* spp., as the surface coat proteins of *M. incognita* were released when the J2 were incubated in water (Lin and McClure 1996). Also, when J2 of *M. javanica* were treated with detergents there was a reduction in the binding of red blood cells to the nematode surface but the binding properties were completely renewed after 24 h at 25°C, but not at 4°C, indicating that the sloughing-off and replacement of the nematode's surface coat is an active event. This phenomenon was visualized also with detergent-extracted SC proteins, using gel-electrophoresis (Spiegel et al. 1997).

Interestingly, binding of antibodies to *M. javanica* and *G. pallida* J2s affected their normal movement pattern on agar plates, regardless the binding pattern (i.e. SC regions, head, amphids, E-S products); this was reversed within several hours, probably due to renewal of the SC. However, continuous binding of antibodies did not enable the recovery and nematodes stopped moving after 2–3 days, subsequently inhibiting plants inoculation by the nematodes (Fioretti et al. 2002; Sharon et al. 2002). Some antibodies caused even more drastic lethal effects after less than one day of contact with antibodies (Sharon et al. 2009a).

There is evidence to suggest that some components of the surface coat are synthesised in the hypodermis (*e.g.* Jones et al. 2004). Further evidence to support this idea comes from the fact that antibodies reactive with the SC of *M. incognita* and *M. javanica* also show reactivity with the hypodermis (Sharon et al. 2002). The origin of surface-associated antigens on nematodes may differ for various antigens and is still not clear in most cases (Spiegel and McClure 1995; Blaxter and Robertson 1998). These non-structural proteins can originate from gland cells such as excretory cells, pharyngeal glands, amphids and phasmids as well as from the hypodermis (Blaxter and Robertson 1998; Page and Johnstone 2007).

In *Meloidogyne* spp., as well as in other species like *Rotylenchulus*, *Tylenchulus* and *Heterodera*, eggs are enveloped with a gelatinous matrix (gm) that contains glycoproteins (Sharon and Spiegel 1993; Agudelo et al. 2004). The eggs and the emerging J2s are exposed to these components and some of it probably attach to the surface of the J2s and affect their interactions with the environment. Labelling of *M. javanica* egg mass-originated eggs and J2s with the monoclonal antibody (MAb), MISC, presented different patterns and was more intense than on gm-free ones (from hypochlorite-treated eggs); labellings were inhibited by fucose (Sharon et al. 2009a). This MAb had also labelled *M. incognita* J2s SC, the gm and the rectal glands, where the gm originates, which suggests that there are mutual epitopes in the gm and SC (Hu et al. 2000). Actually, when gm-originated J2s are used, some of the surface components can be of gm origin. The gm plays a key role in attachment and parasitism of microorganisms, such as *Trichoderma* (see Sect. 5.5.3 and Chap. 8) on *Meloidogyne* J2s and eggs. The role of gm-originated components on the surface of nematodes and their fate during SC turn-over should still be further investigated.

5.4.1 Role of the Surface Coat in Host-Nematode Interactions

Nematodes can rapidly change their surface composition in response to environmental signals, which may enable animal-parasitic nematodes to escape host immune responses and free-living nematodes to escape pathogenic infections (Grenache et al. 1996; Olsen et al. 2007; Proudfoot et al. 1993). A growing body of evidence indicates that some molecules present at the nematode surface of parasitic nematodes serve as an active defense against host responses and are therefore important for nematode survival (Blaxter et al. 1992; Jones et al. 2004; Olsen et al. 2007).

5.4.2 Changes in the Surface Coat in Response to Host Derived Signal

The idea that nematodes switch surface composition in response to environmental signals has been based on rapid changes in surface lipophilicity (Modha et al. 1995; Proudfoot et al. 1993) or surface antigenicity (Philipp and Rumjaneck 1984; Politz and Philipp 1992) that occur during parasitic nematode infections. Therefore, the surface composition can change within a single stage of the life-cycle, during the entry of parasitic nematodes into a new host or host tissue; these surface changes are different from the moulting process as they occur more rapidly (Modha et al. 1995; Proudfoot et al. 1993). Some surface proteic epitopes present in the pre-parasitic *Meloidogyne* J2s were shown to be abundantly shed during root invasion (De Mendoza et al. 2002; Sharon et al. 2002; Curtis 2007b). *In vitro*, plant signals

present in root exudates trigger a rapid modification of the surface cuticle of *M. incognita* and *G. rostochiensis* (de Mendoza et al. 2000; Akhkha et al. 2002). Increase in the surface lipophilicity was also induced by phytohormones, in particular indole-acetic acid (IAA) and kinetin in *M. incognita* but not *G. rostochiensis* (Akhkha et al. 2002, 2004). It has been suggested that the ability of *M. incognita* to respond to a general plant compound as opposed to a specific root diffusate is related to the broad host-range of this species. The increase in the lipophilicty of the SC of *M. incognita* J2, induced by plant signals might allow this nematode to adapt to and survive plant defence processes. By contrast, more specific host cues from root exudates of Solanaceous plants increase the lipophilicity of the surface cuticle of infective J2 of *Globodera* species (Akhkha et al. 2002; Curtis 2007a).

The composition of the nematode surface is also important for the survival of free-living nematodes (see chapter on *C. elegans* for more details) as specific surface-altered mutants of *C. elegans* are resistant to pathogen infections (Mendoza de Gives et al. 1999; Gravato-Nobre et al. 2005; Hodgkin et al. 2000; Ewbank 2002). *C. elegans* responds to environmental conditions by modifying its surface, a process similar to surface switching in parasitic nematodes and these environmental signals are detected by the nematode's chemosensory organs (Grenache et al. 1996; Olsen et al. 2007). These studies suggest that surface switching in plant-parasites might also rely on chemosensation and it can be speculated that free-living and parasitic nematodes use their sensilla to detect environmental signals that lead to changes in the surface composition. Olsen et al. (2007) hypothesized that, like dauer formation, surface antigen switching in *C. elegans* is guided by chemical signals sensed by the amphid neurons. This behavioral adaptation may protect the nematodes from biological attack.

5.4.3 *Protection of the Plant Nematode from Host Defence Responses*

Endoparasitic nematodes spend a significant portion of their life-cycles within their hosts and are therefore exposed to host defence responses. All endoparasites will aim to minimise the effects of defence responses. In addition, many plant-parasitic nematodes, including cyst and root-knot nematodes, are biotrophic and it is particularly important for these nematodes to mask their presence from their host, or suppress any defence response that is mounted, as detection of the pathogen will lead to destruction of the feeding site and death of the pathogen. The nematode surface is in intimate contact with the host. Materials present on the surface of the nematode are therefore targets for detection by the plant and substances can be secreted to the surface that mask the presence of the nematode from the plant, suppress host defences or modulate the effects of any defence response that is mounted.

The ability of nematodes to continuously shed and renew the SC may help the nematode avoid recognition in the host-plant. It has been suggested that shedding of SC components may cause a defence response to be mounted against a region

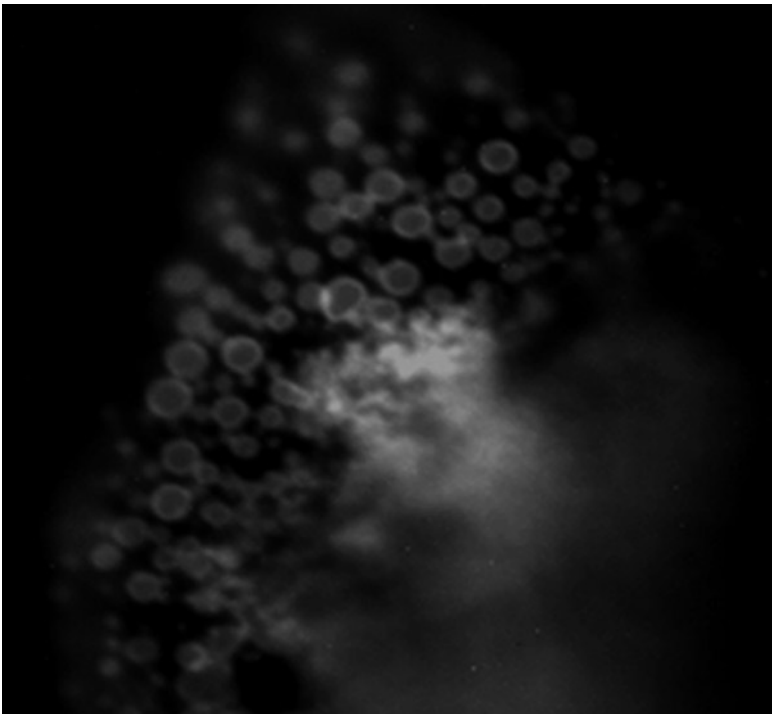

Fig. 5.5 Monoclonal antibody (IACR-CCNj.2a.15) shows reactivity with globules of different sizes exuded from the cuticle of adult female of *Meloidogyne incognita*

that the nematode has vacated, protecting the parasite from early host defences. However, this process of exudation continues once the nematode is established at the feeding site (*e.g.* Jones et al. 1993; de Mendoza et al. 2002; Curtis 2007b) as fibrillar exudates and secretion vesicles exuded as globules of different sizes (Fig. 5.5) have been observed on the cuticle surface of feeding nematodes, suggesting that other processes are also used by the nematode to mask its presence from the host. An abundant cuticular secretion was shown to envelop the adult females and the giant cells and it might have a role in protecting the nematodes from harmful root compounds (de Mendoza et al. 2002; Curtis 2007b).

Antibodies raised against nematode surface components cross react with host tissues; therefore it has been suggested that the SC may mimic host tissues in order to prevent a host reaction (Bird and Wilson 1994; Curtis 1996). Similarly, it has also been suggested that the presence of carbohydrate residues in glycoproteins of plant-parasitic nematodes SC, and therefore the binding of lectins to the nematode surface, is masking other components which may be recognised by the host. Numerous studies have shown that a range of lectins can bind to the surface of many plant-parasitic nematodes (reviewed by Lee 2002) but this idea has yet to be tested *in vitro*. The nematode surface may also play a role in suppressing or modulating

the effects of host defences and striking parallels have been found between the proteins used by plant- and animal-parasites to suppress defence signalling. For plant-parasites these processes have been most thoroughly investigated in cyst nematodes. A range of enzymes that may neutralise active oxygen species produced by the host have been described on the nematode surface. A peroxiredoxin (thioredoxin peroxidase) secreted in great quantities from the surface of the potato cyst nematode *G. rostochiensis* was found to catalyse the breakdown of hydrogen peroxide but, unlike most proteins of this type, did not metabolise larger lipid hydroperoxides (Robertson et al. 2000). It is known that hydrogen peroxide is produced as part of the host response to cyst nematode infection (Waetzig et al. 1999) and it is therefore possible that the nematode peroxiredoxin has become modified to allow it to target host-derived hydrogen peroxide efficiently (Robertson et al. 2000). Another peroxidase, glutathione peroxidase, was also found on the surface of this nematode and this protein was shown to metabolise a wider range of hydroperoxides, including larger hydroperoxides (Jones et al. 2004). Like animal-parasites, plant-parasitic nematodes therefore have a range of peroxidases within their surface secretions that can metabolise a wide range of active oxygen species produced as part of the host defence response.

In contrast to root-knot nematodes which migrate between root cells, minimising disruption to host tissues, cyst nematodes migrate destructively through root cells to their chosen feeding site. This process and the damage caused to plant tissues are likely to provoke defence pathways that provide protection against herbivores. These defence pathways lead to production of antifeedants and are triggered by the plant hormone jasmonic acid (Kunkel and Brooks 2002). Jasmonic acid is produced via the octadecanoid pathway and peroxidation of linoleic and linolenic acid by lipoxygenase is an early step in this process. A surface localised retinol and fatty acid binding protein (GpFAR1) has been described from *G. pallida* which binds both linoleic and linolenic acids and it has been shown that FAR1 inhibits lipoxygenase mediated peroxidation of these fatty acids, presumably due to sequestration of the ligands (Prior et al. 2001). Cyst nematodes, therefore, contain proteins at their surface that can inhibit jasmonate signalling pathways and which can also metabolise active oxygen moieties produced as part of any defence response that is mounted. The genome sequencing projects for *M. incognita* and *M. hapla* (Abad et al. 2008; Opperman et al. 2008) show that similar proteins are also present in root-knot nematodes but functional studies examining localisation of these proteins and biochemical activities remain to be carried out.

5.4.4 Cross Reactivity of Surface Coats of Animal- and Plant-Parasitic Nematodes

Monoclonal antibodies (MAb) produced to excretory-secretory (E-S) products of plant-parasitic nematodes were shown to cross-react with E–S products and the SC of the animal-parasites *Trichinella spiralis* and *Haemonchus contortus* (de Mendoza

et al. 1999). Glycosylated peptides have been reported to be present in abundance on the SC and in E–S products of several parasitic nematodes (Robertson et al. 1989; Schallig et al. 1994, 1995) and, in fact, most of the MAbs tested recognized carbohydrate epitopes. However, 2 out of 7 MAbs recognized proteic epitopes present in the SC and oral exudate of *M. incognita*, *T. spiralis* and *H. contortus*. One of these cross-reactive antigens was detected in the exudate present during ecdysis of *H. contortus* (de Mendoza et al. 1999). Whether this antigen plays any role in mediating ecdysis remains to be determined.

A cross reactive cuticular proteic epitope was identified in *T. spiralis* and *M. incognita* and this antigen might play a role in the interaction of *M. incognita* with its host-plant and in the interaction of *T. spirallis* inside the nurse cell as they are secreted *in planta* and *in vivo* (Lopez-Arellano and Curtis 2002). *In planta* this proteic antigen was immunolocalized surrounding the cuticle of the adult females of *M. incognita* and in the plasma membrane of root cells of *Arabidopsis thaliana*, close to the feeding cell formed during infection with *M. incognita*. *In vivo* this antigen was localised on the nematode surface and as secreted droplets close to the collagen capsule surrounding the nematode nurse cell (Curtis 1996; De Mendoza et al. 2002). These common antigens might represent immunodominant epitopes which are secreted inside the hosts and may perform related functions in these parasitic nematodes.

5.4.5 *The Role of the Surface Coat in Immune Evasion by Animal-Parasitic Nematodes*

Animal-parasitic nematodes have evolved a mutiplicity of evasive strategies to survive in immunologically competent host. The parasite's ability to exist for long periods of time in their host, has been attributed to a rapid turnover of their cuticle surface, shedding of surface antigens and membrane rigidity, which are likely to render the parasite less susceptible to immune attack (Simpson et al. 1984; Kusel and Gordon 1989). The mechanisms underlying surface antigen switching mechanisms are presently unknown but nematodes can alter their SC protein compositions at the moults between developmental stages or in response to host/environmental changes. As a rapid change in the surface lipophilicity of various animal-parasitic nematodes occurs during their transition from pre- to post-parasitic forms, these surface alterations may enable parasitic nematodes to evade host immune defenses during the course of infection (Jungery et al. 1983). Intracellular signalling and second messenger pathways involving cyclic nucleotides, calcium and intracellular alkalinisation participate in bringing about these surface changes (Modha et al. 1995, 1997).

Disguise of the parasite cuticle surface with the acquisition of host derived antigens (Smithers and Doenhoff 1982) and the action of parasite surface proteases that can cleave the Fc region of Immunoglobulins (Auriault et al. 1981) are some other mechanisms that may also help parasites to evade the host immune response by inhibiting important cellular functions.

Evasion of host immunity by *Toxocara canis* infective larvae is mediated by the nematode SC, as this nematode is able to shed the entire SC in response to binding antibodies or eosinophils, thus permitting parasites to physically escape immune attack (Maizels and Loukas 2001a). The major constituent of the SC of this nematode is the O-linked TES-120 (*Toxocara* excretory/secretory) glycoproteins series, which has a typical mucin domain and may explain a generally non-adhesive property of this parasite. Membrane associated mucins are closely concerned with the adhesion status of cells through electrostatic charge and due to steric effects of long chains protruding from the surface. It has been shown that the inhibition of T-cell adhesion can interfere with the ability of eosinophils to bind to the surface cuticle and kill schistosome parasites in *in vitro* tests (Hayes et al. 1990). TES-120 is secreted in internal excretory glands and ducted to the surface via the oesophagus and excretory pore and it is also released from *Toxocara* surface. The overexpression of some membrane-associated mucins suggests a possible model for the role of SC in immune evasion by parasitic nematodes, through changing the nematode surface cuticle adherence to defence cells and/or by releasing soluble mucins that might interact with host cells and blocks defence responses (Gems and Maizels 1996). *Toxocara canis* also secretes large quantities of a C-type lectin thought to compete with host innate immune system receptors (Loukas and Maizels 2000).

Another important group of surface proteins which may act to promote immune evasion in *B. malayi* are the anti-oxidant products glutathione peroxidase and superoxide dismutase. *Bm*-GPX-1 is the major 29 kDa surface glycoprotein of adult *Brugia* (Cookson et al. 1992; Maizels et al. 1989), which is believed to act as a lipid hydroperoxidase, protecting parasite membranes from peroxidation caused by free-oxygen radicals (Tang et al. 1996). A minor surface-associated protein of similar molecular weight is a superoxide dismutase, allowing the parasite to detoxify superoxide radicals (Tang et al. 1994). Many other surface-associated molecules may contribute to immune escape in a less obvious manner. For example, the polyprotein antigen (variously named gp15/400 or *Bm*-NPA-1) has a very high affinity for fatty acids (Kennedy et al. 1995; Smith et al. 1998) which could sequester substrate required for host leukotriene synthesis. Non-protein filarial products are also likely to play a significant role: a novel lipid found in the cuticle of *B. malayi* acts as a sink absorbing oxidative attack, perhaps protecting essential membrane lipids and proteins from degradation (Smith et al. 1998). A further component prominently expressed by *B. malayi* is phosphorylcholine (PC). Not only has this been suggested as an immunosuppressive moiety in lymphatic filariasis (Lal et al. 1990), but it has been possible to demonstrate direct down-regulation of both B (Deehan et al. 1998) and T cell (Harnett et al. 1998) function by a PC-bearing protein secreted from the filarial parasite *Acanthocheilonema viteae*.

A proteinase inhibitors member of the cystatin (cysteine protease inhibitor) family located on the surface of both L3 and adult *B. malayi*, and secreted by these parasites *in vitro* blocks conventional cysteine proteases but also the asparaginyl endopeptidase involved in the Class II antigen processing pathway in human B cells (Maizels et al. 2001b)

5.5 Interactions of Nematode Surfaces with Microorganisms

Complex interactions are formed between microorganisms, nematodes, plants and the environment. Some of the pant-parasitic nematodes microbial antagonists are also root colonizers and this may affect their activity against the nematodes (Kerry 2000; Bordallo et al. 2002; Sharon et al. 2007). Microorganisms have a wide range of suppressive activities on different nematode species. In direct interactions, nematode surface is believed to be important in recognition events and determining the specificity of interactions or the defence mechanisms involving microbial antagonists (Spiegel and McClure 1995; Kerry and Hominick 2001; Morton et al. 2004). Interactions between nematodes and microorganisms (fungi and bacteria) have been described in several reviews (Kerry and Hominick 2001; Bird 2004; Chen and Dickson 2004a; Chen and Dickson 2004b; Morton et al. 2004; Davies 2005; Tian et al. 2007; Lopez-Llorca et al. 2008). We will refer to some examples of such interactions, regarding attachment and penetration aspects.

5.5.1 Interactions with Fungi

Fungal antagonists of nematodes can be grouped into predacious fungi (nematode - trapping fungi), endoparasites of vermiform nematodes, parasites of sedentary females and eggs, and fungi that produce antibiotic substances. Nevertheless, some fungi can belong to more than one category. Attachment of fungi to nematodes is either specifically to head and tail regions, or all over the body, or very sparse. Zoospores usually attach near natural body openings (Chen and Dickson 2004a).

5.5.2 Predacious Fungi

Nematophagous fungi can capture, kill and consume their prey, and have evolved special devices for capturing vermiform nematodes: adhesive hyphae, branches, nets or knobs, non-constricting or constricting rings, and stephanocyts. Adhesive hyphae or branches are usually produced by lower fungi and Deuteromycetes such as *Arthrobotrys* and *Dactylaria* (Chen and Dickson 2004a), (see also Chap. 6).

In nematode-trapping fungi, parasitism begins with the induced formation of traps or other parasitism structures. Despite the variation in trap morphology, majorities of nematode-trapping fungi are closely related and the infection mechanism appears to be rather similar. Following traps development, the infection process proceeds through a sequence of events: attachment of the trap cells to nematode surface, penetration of the cuticle, digestion and assimilation of the nutrients from the killed nematode (Fekete et al. 2008). Free-living nematodes such as *Panagrellus*

redivivus and *C. elegans* serve as model host nematodes in research of these fungi. Involvement of a Gal-NAc-specific lectin of *A. oligospora* (Nordbring-Hertz and Mattiasson 1979) in nematode recognition has been suggested. Subsequently, a carbohydrate-binding protein from the capture organs of the fungi, not present on hyphae, was isolated and partially characterized (Borrebaeck et al. 1984). These early infection events lead to signaling cascades necessary for penetration and colonization of the nematode prey (Tunlid et al. 1992). After contact, an extracellular material, or adhesive, is formed which adhere the fungus to the nematode surface. The adhesive layer has a fibrillar structure containing residues of neutral sugars, uronic acid and proteins (Tunlid et al. 1991). The adhesive on the traps of *A. oligospora* changes from an amorphous to a fibrillar appearance after contact with a nematode (Jansson and Nordbring-Hertz 1988).

Initial contact with the host cuticle is probably followed by a cascade of interactions with specific receptors, reorganization of surface polymers to strengthen the adhesions, changes in morphology, and the secretion of specific enzymes. Trapped nematodes become immobilized after adhesion, when the fungus starts to penetrate the nematode cuticle (Dijksterhuis et al. 1994). A narrow penetration tube develops from the trap cells and the nematode cuticle is breached by a combination of physical force and enzymatic degradation (Kerry and Hominick 2001). The nematode-trapping fungus, *A. oligospora*, produces a subtilisin-like serine protease (designated PII) that immobilized *P. redivivus* and hydrolyzed proteins of purified cuticle (Tunlid et al. 1994; Åhman et al. 2002). Once inside the nematode, the penetration tube swells to form an infection bulb from which hyphae are growing inside the infected nematodes. At this stage the internal tissues of the nematode are rapidly degraded (Dijksterhuis et al. 1994). An extracellular 35 kDa alkaline serine protease (Ds1) was purified and characterized from the nematode-trapping fungus *Dactylella shizishanna*. The purified protease could degrade purified cuticle of *P. redivivus* and a broad range of protein substrates. It showed a high homology with Aozl and PII, two serine proteases purified from *A.oligospora* (Wang et al. 2006).

Monacrosporium haptotylum traps nematodes using a spherical structure called knob, which develops on the apex of a hyphal branch. The transcriptional response in the parasitic fungus *M. haptotylum* and its nematode host *C.elegans* were analyzed during infection using cDNA microarrays (Fekete et al. 2008). Among the infection-induced *C. elegans* genes were those encoding antimicrobial peptides, protease inhibitors and lectins. *C. elegans* mount protective responses against bacterial and fungal pathogens by activating several intracellular signalling pathways that lead to the production of compounds that limit the infection or destroy invading microorganisms (Gravato-Nobre and Hodgkin 2005). C-type lectin domains (CTLD) might act as pathogen recognition molecules, or may mask the virulence factors of the pathogen. CTLD-containing genes were induced by bacterial infections of *C. elegans* (Mallo et al. 2002; O'Rourke et al. 2006), but were down-regulated during infection with *M. haptotylum* (Fekete et al. 2008).

5.5.3 *Endoparasites of Vermiform Nematodes, Sedentary Females and Eggs*

The fungal species, most commonly isolated from sedentary stages, include *Acremonium*, *Fusarium*, *Gliocladium*, *Nematophthora*, *Paecilomyces*, *Penicillium*, *Phoma* and *Pochonia* (*Verticillium*) (Chen and Dickson 2004a). The infection of root-knot and cyst nematode eggs by parasitic fungi involves formation of appressoria when the hyphae encounter the eggshell. This depends on recognition of host surface, where the hydrophobicity of the surface is an important factor (Morton et al. 2004).

Pochonia chlamydosporia (*Verticillium chlamydosporium*), which colonizes cysts or egg-masses, can also attack J2s. The infection of nematode eggs by *Pochonia rubescens* and *P. chlamydosporia* starts with contact of the hyphae and formation of an appressorium. Extracellular material, or an adhesive, formed on the appressorium, could be labeled with the lectin Concanavalin A (Con A), indicating the presence of glucose/mannose residues (Lopez-Llorca et al. 2002). The fungus penetrates the nematode eggshell mechanically and by enzymatic hydrolysis. *P. rubescens* protease P32 was immunolocalized on appressoria that were infecting eggs of the beet cyst nematode *H. schachtii* (Lopez-Llorca and Robertson 1992).

Conidia of the endoparasitic fungus, *Drechmeria coniospora*, adhere to the chemosensory organs natural body openings of the nematodes (Jansson and Nordbring-Hertz 1983). Conidial adhesion to *P. redivivus* was suggested to involve a sialic acid-like carbohydrate since treatment of nematodes with the lectin Limulin, and treatment of the spores with sialic acid, decreased adhesion (Jansson and Nordbring-Hertz 1984). Adhesion of conidia to *C. elegans* was reduced after treatment of the nematodes with Pronase E. The process was reversible within 2 h, indicating that the proteinaceous material emanating from the sensory structures was rapidly replaced (Jansson 1994). Conidia of *D. coniospora* adhere to the chemosensory organs of *Meloidogyne* spp., but do not penetrate and infect the nematodes. Nevertheless, the fungus reduced root galling in tomato (Jansson et al. 1985), probably due to behavior interference. A chymotrypsin-like, was partially characterized from *D. coniospora* conidia (Jansson and Friman 1999).

The fungus *Clonostachys rosea* (syn. *Gliocladium roseum*) is a widely distributed facultative saprophyte in the soil. Observations reveal that the pathogenesis on *P. redivivus* started from the adherence of conidia to nematode cuticle for germination, followed by the penetration of germ tubes into the nematode body and subsequent death and degradation of the nematodes. The conidia excrete a mucous liquid that can stick to the cuticle of nematodes. Microscope observations suggested that the glutinous substance could prevent the nematodes escape from the adhesive areas formed by the secretions of the conidia (Zhang et al. 2008). Two extracellular serine proteases (Lmz1 and PrC) were isolated from *C. rosea* and identified as important factors in fungal pathogenicity. Chemicals isolated from the fungus showed strong nematicidal activities against several nematodes: *C. elegans*, *P. redivivus*, and *Bursaphelenchus xylophilus* (Zhang et al. 2008).

Fig. 5.6 Parasitism of *Trichoderma asperellum*-203 (constitutively expressing green fluorescent protein (GFP) construct) on *Xipinema index*. The image shows the posterior part of a female, colonized by the fungus, 4 days after inoculation with conidia, bar = 50 µm

Species of *Trichoderma* (see Chap. 8) are also facultative saprophytic fungi in soil and posses parasitism abilities against plant-pathogenic fungi and plant-parasitic nematodes, such as *Meloidogyne* spp. (Sharon et al. 2007, 2009b) and *Xipinema* spp. (Fig. 5.6) (Spiegel, Sharon, Chet unpubl.). Mechanisms involved in the attachment and parasitism processes on *M. javanica* were investigated, mainly with *T. asperellum*-203 and *T. atroviride*. It was found that the gelatinous matrix (gm) enables fungal attachment and enhances parasitic abilities of most isolates, which could also utilize it as a nutrient source. Fungal conidia can attach to nematode egg masses and to eggs and J2s that had contact with the gm, whereas gm-free J2s and eggs are almost unattached by fungal conidia; those were penetrated only by few fungal hyphae and colonized. Observations showed typical fungal parasitic behavior, including tight attachment of spores and hyphae to the J2s, coiling of hypae around J2s and appressoria-like structures formation upon egg penetration. Conidia were agglutinated by a gm suspension (enhanced in presence of Ca^{2+}) and their germination was improved. A model for fungal conidia attachment to nematodes suggests that carbohydrate-lectin-like interactions might be involved in this process (Sharon et al. 2007, 2009a).

Fungi of *Catenaria* spp. are known as obligate parasites of vermiform nematodes; however, *C. anguillulae* is a facultative parasite of females and eggs of root-knot nematodes (Wyss et al. 1990). Uniflagellate zoospores are attracted to natural

openings of the nematode and show "amoeboid movement" upon contact with the nematode cuticle, before encystment. During encystment a cell wall is formed, covered by an adhesive, and the flagellum is withdrawn. The encysted zoospore forms an infection peg which penetrates the nematode cuticle and the nematode content is digested.

5.5.4 Fungi That Produce Antibiotic Substances and Toxins

Toxic effects of fungal culture filtrates on vermiform nematodes and eggs have been reported for several fungi, such as species of *Paecilomyces*, *Verticillium*, *Fusarium*, *Aspergillus*, *Trichoderma*, *Myrothecium* and *Penicillium* (Chen and Dickson 2004a; Morton et al. 2004). Toxins are important for parasitic microorganisms because they facilitate infection by debilitating the host. The combination of lytic enzymes and nematicidal compounds can improve the efficacy of the biocontrol agent by increasing the permeability of the nematode surfaces and eggshells. In biocontrol, our interest is to avoid damage to non-target organisms, such as beneficial nematodes. For example, nematicidal activity of *T. atroviride* culture filtrates was restricted to several plant parasitic species, and did not harm non-target and beneficial nematode species (Spiegel, Sharon, Chet unpubl.). The wide differences among the structures and compositions of the various nematode surfaces might determine the different permeability of the nematodes.

5.5.5 Interactions with Bacteria

Due to the chemical nature of the nematode's cuticle, few bacteria and fungi could utilize and degrade the extracellular exoskeleton. The physical body design of plant-parasitic nematodes prevents bacteria from entering through body openings; therefore, to become an endoparasite of nematodes, the bacterium must overcome the nematode surface barriers (Chen and Dickson 2004b). Most nematophagous bacteria, except for obligate parasites, are saprophytes that can also penetrate the nematodes and use them as a nutrition resource. Members of the genera *Pasteuria*, *Pseudomonas* and *Bacillus* have shown great potential for biological control of nematodes (Tian et al. 2007).

5.5.6 Obligate Bacterial Parasites

Species of *Pasteuria* are bacterial obligate hyperparasites of nematodes. The attachment process of endospores and its host specificity have been extensively studied mainly with *P. penetrans* on root-knot nematodes (recently reviewed by Bird 2004; Davies 2005, 2009; Tian et al. 2007). The involvement of surface coat components in

the attachment has been demonstrated and antibodies raised against *Meloidogyne* surfaces, could inhibit spore attachment (Davies and Danks 1992; Spiegel et al. 1996). Monoclonal antibodies, raised against *H. cajani*, could also reduce spore attachment, whereas one antibody increased the attachment (Sharma and Davies 1997). There is high heterogeneity in bacterial surfaces, both within and among different *P. penetrans* populations (Davies and Redden 1997; Davies et al. 2000). The initial binding of endospores to their nematode hosts has been studied (Stirling et al. 1986; Persidis et al. 1991; Davies and Danks 1993; Spiegel et al. 1996). The results suggested a model in which a carbohydrate ligand on the surface of the endospore binds to a lectin-like receptor on the cuticle of the nematode host; however, binding of lectins to nematode surface also reduced the spore attachment (Spiegel et al. 1996). The fibres on the *Pasteuria* endospore are thought to be responsible for the adhesion to the host cuticle through a *Velcro*-like mechanism (Davies 2009). These fibres were shown to be glycoproteins, containing a high level of N-acetyglucosamine (Persidis et al. 1991). Collagen on the nematode's cuticle has been suggested to be involved in recognition process, because attachment was reduced by trypsin and endoglycosidase F, and because gelatin (denatured collagen) could inhibit spore attachment (Persidis et al. 1991; Mohan et al. 2001). However, treatments of J2s with collagenases did not inhibit endospore attachment (Davies and Danks 1993). Davies and Opperman (2006) identified several collagen-like proteins in *P. penetrans* that contain G-x-y repeats. Pretreatment of endospores with collagenase or the collagen-binding domain of fibronectin inhibited endospore binding to nematode cuticle (Mohan et al. 2001), suggesting that collagen-like proteins are also present on the *P. penetrans* exosporium surface and are involved in attachment.

5.5.7 Opportunistic Bacterial Parasites

Various isolates of *Brevibacillus laterosporus* have been reported as parasites of nematodes. The plant-parasites *Heterodera glycines*, *Trichostrongylus colubriformis* and *Bursaphelenchus xylophilus*, and the saprophytic nematode *P. redivius* could be killed by the bacterium (Tian et al. 2007). After attachment to the cuticle, the bacterium can multiply and form a single clone in the epidermis of the nematode cuticle. A circular hole can appear following the continuous degradation of the cuticle and tissue, until bacteria enter the nematode's body and digest it all for nutrition (Huang et al. 2005). The degradation of all the nematode cuticle components around the holes suggests the involvement of hydrolytic enzymes; the major pathogenic activity could be attributed to an extracellular alkaline serine protease, designated BLG4 (Huang et al. 2005; Tian et al. 2006).

A neutral protease (npr) (designated Bae16) of 40 kDa, toxic to nematodes, was purified to homogeneity from the strain *Bacillus nematocida*. The activity was tested against *P. redivivus* and *B. xylophilus*. This purified protease could destroy the nematode cuticle and its hydrolytic substrates included gelatin and collagen. The gene encoding Bae16 was cloned and expressed in *Escherichia coli*, confirming its nematicidal activity (Niu et al. 2006).

5.5.8 Other Bacterial Interactions

Bacterial antagonists that have been studied, such as *Telluria chitinolytica* (*Pseudomonas chitinolytica*) (Spiegel et al. 1991; Bowman et al. 1993) were able to attach to *M. javanica* juveniles, especially to head and tail regions, but no direct parasitism or degradation was observed. The bacteria and its culture filtrates could immobilize the nematodes and produce nematicidal compounds and hydrolytic enzymes such as chitinases, proteases and collagenases, which might contribute to the biocontrol process. These bacteria can colonize root surface and this might affect the nematode-plant interactions.

Bacillus cereus had also presented biocontrol activity against the root-knot nematode (Oka et al. 1993). An enzyme with collagenolytic/proteolytic activities that could degrade *M. javanica* cuticular collagens was purified from this bacterium (Sela et al. 1998).

Nematodes can serve as a vector of microorganisms that attach to their surface and enter the plants. Nematode species of *Anguina* are parasites of cereal grasses. They are vectors of *Rathaybacter* bacteria (formerly referred as *Clavibacter* and *Corynebacterium*). These bacteria produce toxins that can harm the animals feeding on the cereals. A positive correlation was found between a bacteriophage presence in *R. toxicus* and the toxin production (reviewed by Bird 2004). McClure and Spiegel (1991) showed that the bacteria are attached to the surface coat on the nematode's cuticle; however, the mechanism of the attachment remains unknown. Normal adhesion leads to fusion of the bacterium capsular material with the surface coat of the nematode. Slight swelling of nematode's epicuticle and cuticular damage, with loss of nematode's mobility may occur (Bird 2004).

Anguina funesta is also a vector of the fungus *Dilophosphora alopecuri*, a pathogen of cereals and grasses (reviewed by Bird 2004). The fungus has an inhibitory effect on nematode development in the plant. It shows also antagonism to *R. toxicus* and was suggested as a potential biocontrol agent. During adhesion, the fungal conidium secrets adhesive material that covers the surface of the nematode and fills the surface annulations, but no morphological changes were observed in the cuticle.

5.5.9 Role of Lytic Enzymes in Nematode Penetration

Enzymes degrade the host's barriers to infection and, therefore, play an important role in infection of nematodes by fungi and bacteria. The structure of nematode's cuticle and eggshells indicates that proteases and chitinases are necessary for their degradation. Such enzymes that have been identified and are associated with anti-nematode activity were described (Morton et al. 2004; Casas-Flores and Herrera-Estrella 2007; Lopez-Llorca et al. 2008; Tian et al. 2007; Gortari and Hours 2008). A cooperative effect of proteolytic and chtinolytic enzymes in eggshells degradation was demonstrated (Tikhonov et al. 2002). In nematophagous fungi, Pr1-like alkaline

serine proteases act on the collagen-like proteins of the nematode cuticle and the protein-containing elements of the eggshell; such enzymes have been identified in several fungi. The major proteases identified from nematophagous fungi belong to the proteinase K family of subtilases (from Peptidase S8 Subtilase family) (Morton et al. 2004). This family includes also the proteinase Prb1, produced by *T. atroviride* and is also active against nematodes (Sharon et al. 2009b). Bacterial serine protease genes from nematophagous bacteria (*Brevibacillus* and *Bacillus* strains) have shown high sequence identity, indicating that these genes are highly conserved in these bacteria (Tian et al. 2007). Collagenases might also be important in cuticle degradation, since the majority of the proteins in the nematode cuticle are collagens. Collagenase was identified in *Arthrobotrys* spp (Tosi et al. 2002). Galper et al. (1991) evaluated a collagenolytic fungus, *Cunninghamella elegans*, for biocontrol activity against plant-parasitic nematodes. Lipases have been implicated in the infection of nematode eggs: *Heterodera schachtii* eggs, infected by fungi, appeared to have their inner lipid layers degraded (Perry and Trett 1986).

5.6 Concluding Remarks and Future Directions

Plant nematodes must survive the hostile environment that runs from the bulk soil through to the rhizosphere which contains numerous microorganisms and then onto the host plant. Plant nematode surfaces are targets for passive and active environmental attack by the plant immune system, the attachment of bacterial spores and fungal traps. The nematode egg surface is a target for fungal attack and the understanding of this fungus-nematode interaction might lead to the development of biological control methods for soil nematodes. The cuticle and its surface coat have a central importance not only in the biosynthesis and maintenance, but also play a part in defence against their host innate immunity and pathogens. The cuticle, and in particular its surface, can be looked upon as a major part of the nematodes immune system as can be seen from the specifity observed between *Pasteuria penetrans* and root-knot nematodes. It is likely that this variation, as observed by endospore attachment to the nematode cuticle may also play an important role in nematode-plant interactions.

Nematodes respond to environmental conditions by modifying their surfaces, a process formally similar to surface antigen switching in animal-parasitic nematodes. Now that the full genome of various plant-parasitic nematodes and their host plants are available it should be possible to perform detailed study of the genes involved in these interactions and the molecular nature of the surface modification response. It is now possible to study the tritrophic interactions between both the plant and nematode, and the nematode and the microbe, so including all sides of the pathosystem (see Chap. 12). It is also important to identify the environmental factors in the soil, rhizosphere and plants inducing changes in the nematode surface and behaviour.

Another important aspect of research in plant nematology is the identification of nematode effector proteins present in the surface cuticle and also the plant compounds being suppressed by the nematode. There is a great interest in the development of high throughput method for identifying putative nematode effector proteins, which are essential parasitism genes.

This knowledge should lead to a better understanding of interactions between plants and would undoutdly contribute to the development of novel environmentally friendly methods to control plant nematodes which could be incorporated into an integrated pest management programme. Plant nematode surfaces are easily accessible to control measures and constitute an important target for any anti-nematode gene therapy.

References

Abad P, Gouzy J, Aury JM et al (2008) Genome sequence of the metazoan plant-parasitic nematode *Meloidogyne incognita*. Nat Biotechnol 26:909–915

Agudelo P, Robbins RT, JMcD S et al (2004) Glycoproteins in the gelatinous matrix of *Rotylenchulus reniformis*. Nematropica 34:229–234

Åhman J, Johansson T, Olsson M et al (2002) Improving the pathogenicity of a nematode-trapping fungus by genetic engineering of a subtilisin with nematotoxic activity. Appl Environ Microbiol 68:3408–3415

Akhkha A, Kusel J, Kennedy M et al (2002) Effects of phytohormones on the surfaces of plant-parasitic nematodes. Parasitology 125:165–175

Akhkha A, Curtis R, Kennedy M et al (2004) The potential signalling pathways which regulate surface changes induced by phytohormones in the potato cyst nematode (*Globodera rostochiensis*). Parasitology 128:533–539

Atkinson HJ, Taylor JD, Fowler M (1987) Changes in the 2nd stage juveniles of *Globodera rostochiensis* prior to hatching in response to potato root diffusate. Ann Appl Biol 110:105–114

Auriault C, Ouaissi MA, Torpier G et al (1981) Proteolytic cleavage of IgG bound to the Fc receptor of *Schistosoma mansoni* schistosomula. Parasite Immunol 3:33–44

Bais HP, Weir TL, Perry LG et al (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. Annu Rev Plant Biol 57:233–266

Bird AF (2004) Surface adhesion to nematodes and its consequences. In: Chen ZX, Chen SY, Dickson DW (eds) Nematology: advances and perspectives, vol I, Nematode morphology, physiology and ecology. CABI Publishing, Cambridge

Bird AF, Bird J (1991) The structure of nematodes, 2nd edn. Academic, San Diego

Bird AF, McClure MA (1976) Tylenchid (Nematoda) egg-shell, structure, composition and permeability. Parasitology 72:19–27

Bird AF, Rogers GE (1965) Ultrastructure of the cuticle and its formation in *Meloidogyne javanica*. Nematologica 11:224–230

Bird DM, Wilson MA (1994) Plant molecular and cellular responses to nematode infection. In: Lamberti F (ed) Advances in molecular plant nematology. Plenum Press, New York

Bird AF, Bonig I, Bacic A (1988) A role for the excretory-secretory in senernentean nematodes. J Nematol 20:493–496

Blaxter ML, Robertson WM (1998) The cuticle. In: Perry RN, Wright DJ (eds) The physiology and biochemistry of free-living and plant-parasitic nematodes. CABI Publishing, Wallingford

Blaxter ML, Page AP, Rudin W et al (1992) Nematode surface coats, actively evading immunity. Parasitol Today 8:243–247

Bordallo JJ, Lopez-Llorca LV, Jansson H-B et al (2002) Colonization of plant roots by egg-parasitic and nematode-trapping fungi. New Phytol 154:491–499

Borrebaeck CA, Mattlasson B, Nordbring-Hertz B (1984) Isolation and partial characterization of a carbohydrate-binding protein from a nematode-trapping fungus. J Bacteriol 159: 53–56

Bowman JP, Sly LI, Hayward AC et al (1993) *Telluria mixta* (*Pseudomonas mixta* Bowman, Sly, and Hayward 1988) gen. nov., comb. nov., and *Telluria chitinolytica* sp. nov., soil-dwelling organisms which actively degrade polysaccharides. Int J Syst Bacteriol 43:120–124

Casas-Flores S, Herrera-Estrella A (2007) Antagonism of plant parasitic nematodes by fungi. In: Kubicek CP, Druzhinina IS (eds) The mycota IV: environmental and microbial relationships, 2nd edn. Springer, Berlin

Chen S, Dickson DW (2004) Biological control of nematodes by fungal antagonists. In: Chen ZX, Chen SY, Dickson DW (eds) Nematology: advances and perspectives, vol II, Nematode management and utilization. CABI Publishing, Cambridge

Clarke AJ, Cox PM, Shepherd AM (1967) Chemical composition of egg shells of potato cyst-nematode *Heterodera rostochiensis* woll. Biochem J 104:1056–1060

Cookson E, Blaxter ML, Selkirk ME (1992) Identification of the major soluble cuticular glycoprotein of lymphatic filarial nematode parasites (gp29) as a secretory homolog of glutathione peroxidase. Proc Natl Acad Sci USA 89:5837–5841

Couillault C, Ewbank JJ (2002) Diverse bacteria are pathogens of *Caenorhabditis elegans*. Infect Immun 70:4705–4707

Curtis RHC (1996) Identification and *in vitro* and *in vivo* characterisation of secreted proteins produced by plant-parasitic nematodes. Parasitology 113:589–597

Curtis RHC (2007a) Do phytohormones influence nematode invasion and feeding site establishment? Nematology 9:155–160

Curtis RHC (2007b) Plant-parasitic nematodes and the host-parasite interactions. Brief Funct Genomics Proteomics 6:50–58

Davies KG (2005) Interactions between nematodes and microorganisms: bridging ecological and molecular approaches. Adv Appl Microbiol 57:53–78

Davies KG (2009) Understanding the interaction between an obligate hyperparasitic bacterium, *Pasteuria penetrans* and its obligate plant-parasitic nematode host, *Meloidogyne* spp. Adv Parasitol 68:211–245

Davies KG, Danks C (1992) Interspecific differences in the nematode surface coat between *Meloidogyne incognita* and *M. arenaria* related to the adhesion of the bacterium *Pasteuria penetrans*. Parasitology 105:475–480

Davies KG, Danks C (1993) Carbohydrate/protein interactions between the cuticle of infective juveniles of *Meloidogyne incognita* and spores of the obligate hyperparasite *Pasteuria penetrans*. Nematologica 39:53–64

Davies KG, Opperman CH (2006) A potential role for collagen in the attachment of *Pasteuria penetrans* to nematode cuticle. In: Raaijmakers JM, Sikora RA (eds) Multitrophic interactions in the soil. IOBC/WPRS Bull 29:11–16

Davies KG, Redden M (1997) Diversity and partial characterization of putative virulence determinants in *Pasteuria penetrans*, the hyperparasitic bacterium of root-knot nematodes (*Meloidogyne* spp.). J Appl Microbiol 83:227–235

Davies KG, Spiegel Y (2011) Biological control of plant parasitic nematodes: towards understanding field variation through molecular mechanisms. In: Jones J, Gheysen G, Fenoll C (eds) Exploiting genomics to understand plant-nematode interactions. Springer (in press)

Davies KG, Fargette M, Balla G et al (2000) Cuticle heterogeneity as exhibited by *Pasteuria* spore attachment is not linked to the phylogeny of parthenogenetic root-knot nematode (*Meloidogyne* spp.). Parasitology 122:111–120

De Giorgi C, De Luca F, Di Vito D et al (1997) Modulation of expression at the level of splicing of *cut-1* RNA in the infective second stage juvenile of the plant parasitic nematode *Meloidogyne artiella*. Mol Gen Genet 253:589–598

De Mendoza MEL, Curtis RHC, Gowen S (1999) Identification and characterization of excreted–secreted products and surface coat antigens of animal and plant-parasitic nematodes. Parasitology 118:397–405

De Mendoza MEL, Modha J, Roberts MC et al (2000) Changes in the lipophilicity of the surfaces of *Meloidogyne incognita* and *Haemonchus contortus* during exposure to host signals. Parasitology 120:203–209

De Mendoza MEL, Abrantes IMO, Rowe J et al (2002) Immunolocalisation *in planta* of secretions from parasitic stages of *Meloidogyne incognita* and *M. hispanica*. Int J Nematol 12:149–154

Deehan MR, Frame MJ, Parkhouse RME et al (1998) A phosphorylcholine-containing filarial nematode-secreted product disrupts B lymphocyte activation by targeting key proliferative signaling pathways. J Immunol 160:2692–2699

Dijksterhuis J, Veenhuis M, Harder W et al (1994) Nematophagous fungi: Physiological aspects and structure-function relationships. In: Rose AH, Tempest DW (eds) Advances in microbial physiology, vol 36. Academic, New York

Dubinsky P, Rybos M, Turcekova L (1986a) Properties and localization of chitin synthase in *Ascaris suum* eggs. Parasitology 92:219–225

Dubinsky P, Rybos M, Turcekova L et al (1986b) Chitin synthesis in zygotes of *Ascaris suum*. J Helminthol 60:187–192

Ewbank JJ (2002) Tackling both sides of the host-pathogen equation with *Caenorhabditis elegans*. Microb Infect 4:247–256

Fanelli E, Di Vito M, Jones JT et al (2005) Analysis of chitin synthase function in a plant parasitic nematode, *Meloidogyne artiellia*, using RNAi. Gene 349:87–95

Fekete C, Tholander M, Rajashekar B et al (2008) Paralysis of nematodes: shifts in the transcriptome of the nematode-trapping fungus *Monacrosporium haptotylum* during infection of *Caenorhabditis elegans*. Environ Microbiol 10:364–375

Fioretti L, Porter A, Haydock PJ et al (2002) Monoclonal antibodies reactive with secreted-excreted products from the amphids and the cuticle surface of *Globodera pallida* affect nematode movement and delay invasion of potato roots. Int J Parasitol 32:1709–1718

Galper S, Cohn E, Spiegel Y et al (1991) A collagenolytic fungus, *Cunninghamella elegans*, for biological control of plant-parasitic nematodes. J Nematol 23:269–274

Gems D, Maizels RM (1996) An abundantly expressed mucin-like protein from *Toxocara canis* infective larvae: The precursor of the larval surface coat glycoproteins. Proc Natl Acad Sci USA 93:1665–1670

Geng JM, Plenefisch J, Komuniecki PR et al (2002) Secretion of a novel developmentally regulated chitinase (family 19 glycosyl hydrolase) into the perivitelline fluid of the parasitic nematode, *Ascaris suum*. Mol Biochem Parasitol 124:11–21

Gortari MC, Hours RA (2008) Fungal chitinases and their biological role in the antagonism onto nematode eggs. Annu Rev Mycol Prog 7:221–238

Gravato-Nobre MJ, Evans K (1998) Plant and nematode surfaces: Their structure and importance in host-parasite interactions. Nematologica 44:103–124

Gravato-Nobre MJ, Hodgkin J (2005) *Caenorhabditis elegans* as a model for innate immunity to pathogens. Cell Microbiol 7:741–751

Gravato-Nobre MJ, McClure MA, Dolan L et al (1999) *Meloidogyne incognita* surface antigen epitopes in infected *Arabidopsis* roots. J Nematol 31:212–223

Gravato-Nobre MJ, Nicholas HR, Nijland R et al (2005) Multiple genes affect sensitivity of *Caenorhabditis elegans* to the bacterial pathogen *Microbacterium nematophilum*. Genetics 171:1033–1045

Grenache DG, Caldicott I, Albert PS et al (1996) Environmental induction and genetic control of surface antigen switching in the nematode *Caenorhabditis elegans*. Proc Natl Acad Sci USA 93:12388–12393

Guiliano DB, Hong XQ, McKerrow JH et al (2004) A gene family of cathepsin L-like proteases of filarial nematodes are associated with larval molting and cuticle and eggshell remodeling. Mol Biochem Parasitol 136:227–242

Hanazawa M, Mochii M, Ueno N et al (2001) Use of cDNA subtraction and RNA interference screens in combination reveals genes required for germ-line development in *Caenorhabditis elegans*. Proc Natl Acad Sci USA 98:8686–8691

Harnett MM, Deehan MR, Williams DM et al (1998) Induction of signalling energy via the T-cell receptor in cultured Jurkat T cells by pre-exposure to a filarial nematode secreted product. Parasite Immunol 20:551–563

Harris MT, Fuhrman JA (2002) Structure and expression of chitin synthase in the parasitic nematode *Dirofilaria immitis*. Mol Biochem Parasitol 122:231–234

Hashmi S, Britton C, Liu J et al (2002) Cathepsin L is essential for embryogenesis and development of *Caenorhabditis elegans*. J Biol Chem 277:3477–3486

Hayes DF, Silberstein DS, Rodrique SW et al (1990) DF3 antigen, a human epithelial-cell mucin, inhibits adhesion of eosinophils to antibody-coated targets. J Immunol 145:962–970

Hill DE, Fetterer RH, Urban JF (1991) *Ascaris suum*, stage-specific differences in lectin binding to the larval cuticle. Exp Parasitol 73:376–383

Hodgkin J, Kuwabara PE, Corneliussen B (2000) A novel bacterial pathogen, *Microbacterium nematophilum*, induces morphological change in the nematode *C. elegans*. Curr Biol 10:1615–1618

Hu GG, McClure MA, Schmitt ME (2000) Origin of a *Meloidogyne incognita* surface coat antigen. J Nematol 32:174–182

Huang XW, Tian BY, Niu QH et al (2005) An extracellular protease from *Brevibacillus laterosporus* G4 without parasporal crystal can serve as a pathogenic factor in infection of nematodes. Res Microbiol 156:719–727

Jansson H-B (1994) Adhesion of conidia of *Drechmeria coniospora* to *Caenorhabditis elegans* wild type and mutants. J Nematol 26:430–435

Jansson H-B, Friman E (1999) Infection-related surface proteins on conidia of the nematophagous fungus *Drechmeria coniospora*. Mycol Res 103:249–256

Jansson H-B, Nordbring-Hertz B (1983) The endoparasitic fungus *Meria coniospora* infects nematodes specifically at the chemosensory organs. J Gen Microbiol 129:1121–1126

Jansson H-B, Nordbring-Hertz B (1984) Involvement of sialic acid in nematode chemotaxis and infection by an endoparasitic nematophagous fungus. J Gen Microbiol 130:39–43

Jansson H-B, Nordbring-Hertz B (1988) Infection events in the fungus-nematode system. In: Poinar GO, Jansson H-B (eds) Diseases of nematodes, vol II. CRC Press, Boca Raton

Jansson H-B, Jeyaprakash A, Zuckerman BM (1985) Control of root-knot nematodes on tomato by the endoparasitic fungus *Meria coniospora*. J Nematol 17:327–330

Johnstone IL (1994) The cuticle of the nematode *Caenorhabditis elegans*, a complex collagen structure. Bioessays 16:171–178

Johnstone IL, Barry JD (1996) Temporal reiteration of a precise gene expression pattern during nematode development. EMBO J 15:3633–3639

Jones JT (2002) Nematode sense organs. In: Lee DL (ed) The biology of nematodes. Taylor & Francis, London

Jones JT, Perry RN, Johnston MRL (1993) Changes in the ultrastructure of the cuticle of the potato cyst-nematode, *Globodera rostochiensis*, during development and infection. Fundam Appl Nematol 16:433–445

Jones JT, Reavy B, Smant G et al (2004) Glutathione peroxidases of the potato cyst nematode *Globodera rostochiensis*. Gene 324:47–54

Jung WJ, Jung SJ, An KN et al (2002) Effect of chitinase-producing *Paenibacillus illinoisensis* KJA-424 on egg hatching of root-knot nematode (*Meloidogyne incognita*). J Microbiol Biotechnol 12:865–871

Jungery M, Clark NWT, Parkhouse RM (1983) A major change in surface-antigens during the maturation of newborn larvae of *Trichinella spiralis*. Mol Biochem Parasitol 7:101–109

Kennedy MW, Allen JE, Wright AS et al (1995) The gp15/400 polyprotein antigen of *Brugia malayi* binds fatty-acids and retinoids. Mol Biochem Parasitol 71:41–50

Kerry BR (2000) Rhizosphere interactions and exploitation of microbial agents for the biological control of plant-parasitic nematodes. Annu Rev Phytopathol 38:423–441

Kerry BR, Hominick WM (2001) Biological control. In: Lee DL (ed) Biology of nematodes. Taylor & Francis, London

Koltai H, Chejanovski N, Raccha B et al (1997) The first isolated collagen gene of the root-knot nematode *Meloidogyne javanica* is developmentally regulated. Gene 196:191–199

Koltai H, Sharon E, Spiegel Y (2002) Root-nematode interactions: recognition and pathogenicity. In: Waisel Y, Eshel A, Kafkafi U (eds) Plant roots: the hidden half, 3rd edn. Marcel Dekker, New York

Kramer JM, Cox GN, Hirsh D (1982) Comparisons of the complete sequences of 2 collagen genes from *Caenorhabditis elegans*. Cell 30:599–606

Kunkel BN, Brooks DM (2002) Cross talk between signaling pathways in pathogen defense. Curr Opin Plant Biol 5:325–331

Kusel JR, Gordon JF (1989) Biophysical studies of the *Schistosome* surface and their relevance to its properties under immune and drug attack. Parasite Immunol 11:431–451

Labrousse A, Chauvet S, Couillault C et al (2000) *Caenorhabditis elegans* is a model host for *Salmonella typhimurium*. Curr Biol 10:1543–1545

Lal RB, Kumaraswami V, Steel C et al (1990) Phosphocholine-containing antigens of *Brugia malayi* nonspecifically suppress lymphocyte function. Am J Trop Med Hyg 42:56–64

Lee DL (2002) Cuticle, moulting and exsheathment. In: Lee DL (ed) The biology of nematodes. Taylor & Francis, London

Lin HJ, McClure MA (1996) Surface coat of *Meloidogyne incognita*. J Nematol 28:216–224

Liu J, Koltai H, Chejanovski N et al (2001) Isolation of a novel collagen gene in *Meloidogyne javanica* and studies on its expression pattern. J Parasitol 87:801–807

Lopez-Arellano ME, Curtis RHC (2002) Immunolocalization of *Trichinella spiralis* L1 surface and excreted/secreted antigens *in situ*. Int J Nematol 12:55–58

Lopez-Llorca LV, Robertson WM (1992) Immunocytochemical localization of a 32-kDa protease from the nematophagous fungus *Verticillium suchlasporium* in infected nematode eggs. Exp Mycol 16:261–267

Lopez-Llorca LV, Olivares-Bernabeu C, Salinas J et al (2002) Prepenetration events in fungal parasitism of nematode eggs. Mycol Res 106:499–506

Lopez-Llorca LV, Macia-Vicente JG, Jansson H-B (2008) Mode of action and interactions of nematophagous fungi. In: Ciancio A, Mukerji KG (eds) Integrated pest and disease management and biocontrol of vegetable and grain crops nematodes. Springer Publishers, Dordrecht

Loukas A, Maizels RM (2000) Helminth C-type lectins and host-parasite interactions. Parasitol Today 16:333–339

Mackintosh GM (1960) The morphology of the *Brassica* root eelworm *Heterodera cruciferae* Franklin, 1945. Nematologica 5:158–165

Maggenti AR (1962) Production of gelatinous matrix and its taxonomic significance in *Tylenchulus* (Nematoda, Tylenchulinae). Proc Helminth Soc Washington 29:139–144

Maizels RM, Loukas A (2001) The surface and secreted antigens of *Toxocara canis*: genes, protein structure and function. In: Kennedy MW, Harnett W (eds) Parasitic nematodes – molecular biology, biochemistry and immunology. CABI Publishing, Wallingford

Maizels RM, Gregory WF, Kwanlim GE et al (1989) Filarial surface-antigens, the major 29 Kilodalton glycoprotein and a novel 17–200 Kilodalton complex from adult *Brugia malayi* parasites. Mol Biochem Parasitol 32:213–227

Maizels RM, Gomez-Escobar N, Gregory WF et al (2001) Immune evasion genes from filarial nematodes. Int J Parasitol 31:889–898

Mallo GV, Kurz CL, Couillault C et al (2002) Inducible antibacterial defense system in *C. elegans*. Curr Biol 12:1209–1214

McClure MA, Spiegel Y (1991) Role of the nematode surface coat in the adhesion of *Clavibacter sp*. to *Anguina funesta* and *Anguina tritici*. Parasitology 103:421–427

Mendoza de Gives P, Davies KG, Clark SJ, Behnke JM (1999) Predatory behavior of trapping fungi against *srf* mutants of *Caenorhabditis elegans* and different plant- and animal-parasitic nematodes. Parasitology 119:95–104

Modha J, Kusel JR, Kennedy MW (1995) A role for 2nd messengers in the control of activation-associated modification of the surface of *Trichinella spiralis* infective larvae. Mol Biochem Parasitol 72:141–148

Modha J, Roberts MC, Kennedy MW et al (1997) Induction of surface fluidity in *Trichinella spiralis* larvae during penetration of the host intestine: simulation by cyclic AMP *in vitro*. Parasitology 114:71–77

Mohan S, Fould S, Davies KG (2001) The interaction between the gelatin-binding domain of fibronectin and the attachment of *Pasteuria penetrans* endospores to nematode cuticle. Parasitology 123:271–276

Morton CO, Hirsch PR, Kerry B (2004) Infection of plant-parasitic nematodes by nematophagous fungi – a review of application of molecular biology to understand infection processes and to improve biological control. Nematology 6:161–170

Neuhaus B, Bresciani J, Peters W (1997) Ultrastructure of the pharyngeal cuticle and lectin labelling with wheat germ agglutinin-gold conjugate indicating chitin in the pharyngeal cuticle of *Oesophagostomum dentatum* (Strongylida, Nematoda). Acta Zool 78:205–213

Niu Q, Huang X, Zhang L et al (2006) A neutral protease from *Bacillus nematocida*, another potential virulence factor in the infection against nematodes. Arch Microbiol 185:439–448

Nordbring-Hertz B, Mattiasson B (1979) Action of a nematode-trapping fungus shows lectinmediated host-microorganism interaction. Nature 281:477–479

O'Rourke D, Baban D, Demidova M et al (2006) Genomic clusters, putative pathogen recognition molecules, and antimicrobial genes are induced by infection of *C. elegans* with *M. nematophilum*. Genome Res 16:1005–1016

Oka Y, Chet I, Spiegel Y (1993) Control of the root-knot nematode *Meloidogyne javanica* by *Bacillus cereus*. Biocontrol Sci Technol 3:115–126

Olsen DP, Phu D, Libby LJM et al (2007) Chemosensory control of surface antigen switching in the nematode *Caenorhabditis elegans*. Genes Brain Behav 6:240–252

Opperman CH, Bird DM, Williamson VM et al (2008) Sequence and genetic map of *Meloidogyne hapla*: A compact nematode genome for plant parasitism. Proc Natl Acad Sci USA 105:14802–14807

Page AP, Johnstone IL (2007) The cuticle. In: Wormbook. The *C. elegans* research community, doi/10.1895/wormbook.1.7.1, http://www.wormbook.org

Perry RN, Clarke AJ (1981) Hatching mechanisms of nematodes. Parasitology 83:435–449

Perry RN, Trett MW (1986) Ultrastructure of the eggshell of *Heterodera schachtii* and *Heterodera glycines* (Nematode, Tylenchida). Rev Nematol 9:399–403

Persidis A, Lay JG, Manousis T et al (1991) In: Wormbook. Characterization of potential adhesions of the bacterium *Pasteuria penetrans*, and of putative receptors on the cuticle of *Meloidogyne incognita*, a nematode host. J Cell Sci 100:613–622

Philipp M, Rumjaneck FD (1984) Antigenic and dynamic properties of helminth surface structures. Mol Biochem Parasitol 10:245–268

Politz SM, Philipp M (1992) *Caenorhabditis elegans* as a model for parasitic nematodes: a focus on the cuticle. Parasitol Today 8:6–12

Premachandran D, Vonmende N, Hussey RS et al (1988) A method for staining nematode secretions and structures. J Nematol 20:70–78

Preston CM, Jenkins T (1985) *Trichuris muris* – structure and formation of the egg polar plugs. Z Parasitenkunde – Parasitol Res 71:373–381

Prior A, Jones JT, Blok VC et al (2001) A surface-associated retinol- and fatty acid-binding protein (Gp-FAR-1) from the potato cyst nematode *Globodera pallida*: lipid binding activities, structural analysis and expression pattern. Biochem J 356:387–394

Proudfoot L, Kusel JR, Smith HV et al (1993) Rapid changes in the surfce of parasitic nematodes during transition from pre-parasitic to post-parasitic forms. Parasitology 107:107–117

Pujol N, Link EM, Liu LX et al (2001) Reverse genetic analysis of components of the Toll signaling pathway in *Caenorhabditis elegans*. Curr Biol 11:809–821

Rao UR, Chandrashekar R, Subrahmanyam D (1988) Developmental-changes in the surface carbohydrates of filariae. Indian J Med Res 87:9–14

Riou M, Koch C, Delaleu B et al (2005) Immunolocalisation of an ABC transporter, P-glycoprotein, in the eggshells and cuticles of free-living and parasitic stages of *Haemonchus contortus*. Parasitol Res 96:142–148

Robertson WM, Spiegel Y, Jansson H-B et al (1989) Surface carbohydrates of plant-parasitic nematodes. Nematologica 35:180–186

Robertson L, Robertson WM, Sobczak M et al (2000) Cloning, expression and functional characterisation of a peroxiredoxin from the potato cyst nematode *Globodera rostochiensis*. Mol Biochem Parasitol 111:41–49

Robinson MP, Delgado J, Parkhouse RMW (1989) Characterisation of stage-specific cuticular proteins of *Meloidogyne incognita* by radio-iodination. Physiol Mol Plant Pathol 35:135–140

Sapio MR, Hilliard MA, Cermola M et al (2005) The Zona Pellucida domain containing proteins, CUT-1, CUT-3 and CUT-5, play essential roles in the development of the larval alae in *Caenorhabditis elegans*. Dev Biol 282:231–245

Schallig H, Vanleeuwen MAW, Hendrikx WML (1994) Immune-responses of texel sheep to excretory/secretory products of adult *Haemonchus contortus*. Parasitology 108:351–357

Schallig H, Vanleeuwen MAW, Hendrikx WML (1995) Isotype-specific serum antibody-responses of sheep to *Haemonchus contortus* antigens. Vet Parasitol 56:149–162

Sela S, Schickler H, Chet I et al (1998) Purification and characterization of a B*acillus cereus* collagenolytic/proteolytic enzyme and its effect on *Meloidogyne javanica* cuticular proteins. Eur J Plant Pathol 104:59–67

Sharma SB, Davies KG (1997) Modulation of spore adhesion of the hyperparasitic bacterium *Pasteuria penetrans* to nematode cuticle. Lett Appl Microbiol 25:426–430

Sharon E, Spiegel Y (1993) Glycoprotein characterization of the gelatinous matrix in the root-knot nematode *Meloidogyne javanica*. J Nematol 25:585–589

Sharon E, Spiegel Y (1996) Gold-conjugated reagents for the labelling of carbohydrate-recognition domains and glycoconjugates on nematodes surfaces. J Nematol 28:124–127

Sharon E, Spiegel Y, Salomon R et al (2002) Characterization of *Meloidogyne javanica* surface coat with antibodies and their effect on nematode behaviour. Parasitology 125:177–185

Sharon E, Chet I, Viterbo A et al (2007) Parasitism of *Trichoderma* on *Meloidogyne javanica* and role of the gelatinous matrix. Eur J Plant Pathol 118:247–258

Sharon E, Chet I, Spiegel Y (2009a) Improved attachment and parasitism of *Trichoderma* on *Meloidogyne javanica in vitro*. Eur J Plant Pathol 123:291–299

Sharon E, Chet I, Bar-Eyal M et al (2009b) Biocontrol of root-knot nematodes by *Trichoderma* – modes of action. In: Steinberg C, Edel-Hermann V, Friberg H et al (eds) Multitrophic interactions in soil. IOBC/WPRS Bull 42:159–163

Simpson AJG, Payares G, Walker T et al (1984) The modulation of expression of polypeptide surface-antigens on developing schistosomula of *Schistosoma mansoni*. J Immunol 133:2725–2730

Smith VP, Selkirk ME, Gounaris K (1998) *Brugia malayi*: Resistance of cuticular lipids to oxidant-induced damage and detection of alpha-tocopherol in the neutral lipid fraction. Exp Parasitol 88:103–110

Smithers SR, Doenhoff MJ (1982) Schistosomiasis. In: Cohen S, Warren KS (eds) Immunology of parasitic infections. Blackwell Scientific Publications, Oxford

Spiegel Y, Chet I (1985) Chitin synthetase inhibitors and their potential to control the root-knot nematode, *Meloidogyne javanica*. Nematologica 31:480–482

Spiegel Y, McClure MA (1991) Stage-specific differences in lectin binding to the surface of *Anguina tritici* and *Meloidogyne incognita*. J Nematol 23:259–263

Spiegel Y, McClure MA (1995) The surface coat of plant-parasitic nematodes: chemical composition, origin and biological role: a review. J Nematol 27:127–134

Spiegel Y, Cohn E, Galper S et al (1991) Evaluation of a newly isolated bacterium, *Pseudomonas chitinolytica* sp. nov. for controlling the root-knot nematode *Meloidogyne javanica*. Biocontrol Sci Technol 1:115–125

Spiegel Y, Mor M, Sharon E (1996) Attachment of *Pasteuria penetrans* endospores to the surface of *Meloidogyne javanica* second-stage juveniles. J Nematol 28:328–334

Spiegel Y, Kahane I, Cohen L et al (1997) *Meloidogyne javanica* surface proteins: characterization and lability. Parasitology 115:513–519

Stirling GR, Bird AF, Cakurs AB (1986) Attachment of *Pasteuria panetrans* spores to the cuticle of root-knot nematodes. Rev Nematol 9:251–260

Tang L, Ou X, Henkleduhrsen K et al (1994) Extracellular and cytoplasmic superoxide dismutases from *Brugia* lymphatic filarial nematode parasites. Infect Immun 62:961–967

Tang L, Smith VP, Gounaris K et al (1996) *Brugia pahangi*: the cuticular glutathione peroxidase (gp29) protects heterologous membranes from lipid peroxidation. Exp Parasitol 82:329–332

Taylor DW, Goddard JM, McMahon JE (1986) Surface components of *Onchocerca volvulus*. Mol Biochem Parasitol 18:283–300

Tefft PM, Bone LW (1985) Plant-induced hatching of eggs of the soybean cyst nematode *Heterodera glycines*. J Nematol 17:275–279

Tian B, Li N, Lian L et al (2006) Cloning, expression and deletion of the cuticle-degrading protease BLG4 from nematophagous bacterium *Brevibacillus laterosporus* G4. Arch Microbiol 186:297–305

Tian B, Yang J, Zhang K-Q (2007) Bacteria used in the biological control of plant-parasitic nematodes: populations, mechanisms of action, and future prospects. FEMS Microbiol Ecol 61:197–213

Tikhonov VE, Lopez-Llorca LV, Salinas J et al (2002) Purification and characterization of chitinases from the nematophagous fungi *Verticillium chlamydosporium* and *V. suchlasporium*. Fungal Genet Biol 35:67–78

Tosi S, Annovazzi L, Tosi I et al (2002) Collagenase production in an Antarctic strain of *Arthrobotrys tortor jarowaja*. Mycopathologia 153:157–162

Tunlid A, Johansson T, Nordbring-Hertz B (1991) Surface polymers of the nematode-trapping fungus *Arthrobotrys oligospora*. J Gen Microbiol 137:1231–1240

Tunlid A, Jansson H-B, Nordbring-Hertz B (1992) Fungal attachment to nematodes. Mycol Res 96:401–412

Tunlid A, Rosen S, Ek B et al (1994) Purification and characterization of extracellular serine protease from the nematode-trapping fungus *Arthrobotrys oligospora*. Microbiology 140:1687–1695

Twomey U, Warrior P, Kerry BR et al (2000) Effects of the biological nematicide, DiTera, on hatching of *Globodera rostochiensis* and *G. pallida*. Nematology 2:355–362

Van Nguyen N, Kim Oh KT, Jung W et al (2007) The role of chitinase from *Lecanicillium antillanum* B-3 in parasitism to root-knot nematode *Meloidogyne incognita* eggs. Biocontrol Sci Technol 17:1047–1058

Veronico P, Gray LJ, Jones JT et al (2001) Nematode chitin synthases: gene structure, expression and function in *Caenorhabditis elegans* and the plant parasitic nematode *Meloidogyne artiellia*. Mol Genet Genomics 266:28–34

Waetzig GH, Sobczak M, Grundler FMW (1999) Localization of hydrogen peroxide during the defence response of *Arabidopsis thaliana* against the plant-parasitic nematode *Heterodera glycines*. Nematology 1:681–686

Wang RB, Yang JK, Lin C et al (2006) Purification and characterization of an extracellular serine protease from the nematode-trapping fungus *Dactylella shizishanna*. Lett Appl Microbiol 42:589–594

Wharton DA (1983) The production and functional-morphology of helminth egg-shells. Parasitology 86:85–97

Wyss U, Voss B, Jansson H-B (1990) *In vitro* observations on the infection of *Meloidogyne incognita* eggs by the zoosporic fungus *Catenaria anguilulae* Sorokin. Fundam Appl Nematol 15:133–139

Zhang L, Yang J, Niu Q et al (2008) Investigation on the infection mechanism of the fungus Clonostachys rosea against nematodes using the green fluorescent protein. Appl Microbiol Biotechnol 78:983–990