Titolo della ricerca: Evaluation of the glycosylation process in the host-pathogen interactions in the *Caenorhabditis elegans* animal model.

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**DESCRIZIONE DELLA RICERCA**

Obiettivi della ricerca (max 4000 car.):  
This research will explore key interactions between the pathogen and the host cell surface; special focus will be placed on the host innate immune response. It should be noted that *Caenorhabditis elegans* does not have a humoral immune response so innate immunity study is simplified. Questions to be addressed include 1) Are the cell surface glycoconjugates in the tail region of *C. elegans* required for infection by *Microbacterium nematophilum*? 2) Is the innate immune response a dominating factor in *C. elegans bus* mutant’s resistance to *M. nematophilum* infection? 3) Are key innate immune system component expression levels good candidates to monitor *C. elegans* pathogen response state?  
The *bus* mutants are characterized by an absence of swelling in the tail region when exposed to the *M. nematophilum* leading to the bacterially unswollen (*bus*) phenotype. Included among the genes that have been cloned six (*bus-2*, *bus-4*, *bus-8*, *bus-12*, *bus-17* and *srf-3*) encode distinct genes required for, glycoconjugate biosynthesis [1,2].  
Innate immune system components identified as key in infection based on previous gene array and subsequent qPCR studies and the literature will be monitored in *C. elegans* after the exposure of these mutants to *M. nematophilum*. Thw upregulated genes will be candidates as markers to monitor immune response in subsequent experiments. RNA interference experiments of innate immune components will be carried out in *M. nematophilum* resistant *bus* mutants. If knock downs restore sensitivity then the targeted pathway is likely to be important in the observed *bus* resistance. If there is no observable difference in the systematic knock down experiments targeting innate immune system components then the altered innate immune system observed in the *bus* mutant(s) is less likely to be involved in *bus* resistance to *M. nematophilum*. As a control the nematode cell surface
will be monitored with ABA lectin to assess the glycosylation status of the animals during the experiments, in order to rule out aberrant glycosylation due to RNA interference conditions. Since transcriptional response to *Candida albicans* in *C. elegans* provided new information on the regulation of the innate immune response to different pathogens the expression of the candidate genes will be analyzed also in *C. elegans* bus mutants infected with other pathogens: *Staphylococcus aureus, Pseudomonas aeruginosa* or *C. albicans*.

Wild type N2 Bristol nematodes as well as transgenic animals carrying the gfp fused to the transcription factor daf-16, one of the key components of the innate immune response, will be subjected to RNA interference conditions targeting bus genes. Nuclear localization of daf-16, as a reporter of innate immune activation, and O-glycosylation will be monitored at the tail region using ABA lectin staining at specific time points. Glycosylation will also be analyzed at the protein level by release from glycoprotein and analysis by western blot. These assays will allow us to determine when glycosylation becomes affected and when key innate immune response genes are effected due to bus gene expression deficiency. Knock down experiments that penetrate the glycosylation and innate immune response differently will be used to further investigate the role of each in pathogen resistance.

This project can provide an opportunity to investigate glycosylation role in pathogen recognition as well as innate immune response. Key signaling pathways involved in bacterial pathogen response using the *M. nematophilum/C. elegans* interaction can be explored. Key expression patterns linked to bacterial infection will be revealed and studied.

**Stato delle conoscenze e referenze (max 4000 car.):**

*Caenorhabditis elegans* can be infected by over forty microbial pathogens [3]. Among these are the nematode specific pathogen, *Microbacterium nematophilum*, and the human pathogens *Yersinia pestis* and *Yersinia pseudotuberculosis*. *M. nematophilum* infects the anus, rectum and surrounding cuticle of the nematode causing localized swelling and constipation [4]. *Y. pestis* and *Y. pseudotuberculosis* do not directly infect *C. elegans*. Rather these bacteria secrete an exopolysaccharide that adheres to the head region of the nematode, causing starvation [5]. The bus and bah genetic screens have isolated mutants resistant to *M. nematophilum* and *Yersinia* spp., respectively, and there is significant genetic overlap between the screens demonstrating that a series of the same genes are required for both pathogenic processes [6]. Several advantages allow *C.elegans* to be used as *in vivo* infection model. *C. elegans* genome is completely sequenced. It has a defined number of somatic cells and all are visible in the intact living organism under the microscope making the observation of infection possible at the organism level. It is also an excellent genetic model as it has both hermaphrodite and male sexes and is a premiere organism for the use of RNA interference studies allowing rapid targeted investigation of gene function [7]. In recent years the *C. elegans* glycome has been described. Importantly, it has been characterized in the context of glycosylation’s role in the infective process during infection by several bacterial and fungal pathogens.

Glycosylation of lipids and proteins have been shown to be required for infection in this nematode. The *Bre* genes encode proteins required for glycolipid synthesis. *Bre* mutants deficient in glycolipid synthesis, are resistant to Cry toxins produced by Bacillus thuringiensis. Similarly *bah, srf*, and *bus* mutants are resistant to a number of bacterial infections. *Bah* mutants have an altered cuticle surface and are resistant to *Yersinia pestis* and *Y. pseudotuberculosis* biofilm adhesion. The *srf*
mutants, also have an altered cuticle surface as observed by ectopic lectin staining. Several of the srf mutants were co-isolated in bus screens. A series of these mutants, bus-2, -4, -8, -12, -17 as well as srf-2 and -3 genes encode putative glycosyltransferases or nucleotide sugar transporters. A minority of bus genes encode signaling proteins involved in the ERK map kinase signal transduction pathway. Previous studies have shown that deficiencies of bus-2, -4 and -17 diminish core 1 like O-glycan staining in the anal region of the worm, the site normally infected by *M. nematophilum*. Detailed fine structural analyses of the N- and O-glycans demonstrated that the former are altered significantly [8]. The implication here is that O-glycoconjugates in the tail region are required for *M. nematophilum* infection. However, these mutant’s bacterial resistance may not be as simple as loss of a pathogen’s target. Gene array studies of parent, bus-2, -4, -17 and srf-2 mutants have been performed and in the absence of pathogens all of these mutant strains, all deficient in glycosylation or a key cuticle glycoprotein of the cuticle, have greatly altered innate immune marker levels as conserved in the expression patterns. It should be noted that several of the bus mutants are also resistant to *Y. pestis*, infection in addition to *M. nematophilum*. Several key C-type lectins involved in bacterial response to infection are greatly up regulated as well as downstream effector molecules involved in resistance.

**Referenze**


