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Proteomics in *Myzus persicae*: Effect of aphid host plant switch

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Abstract

Chemical ecology is the study of how particular chemicals are involved in interactions of organisms with each other and with their surroundings. In order to reduce insect attack, plants have evolved a variety of defence mechanisms, both constitutive and inducible, while insects have evolved strategies to overcome these plant defences (such as detoxification enzymes). A major determinant of the influence of evolutionary arms races is the strategy of the insect: generalist insect herbivores, such as *Myzus persicae* aphid, need more complex adaptive mechanisms since they need to respond to a large array of different plant defensive chemicals. Here we studied the chemical ecology of *M. persicae* associated with different plant species, from Brassicaceae and Solanaceae families. To identify the involved adaptation systems to cope with the plant secondary substances and to assess the differential expression of these systems, a proteomic approach was developed. A non-restrictive approach was developed to identify all the potential adaptation systems toward the secondary metabolites from host plants. The complex protein mixtures were separated by two-dimensional electrophoresis methods and the related spots of proteins significantly varying were selected and identified by mass spectrometry (ESI MS/MS) coupled with data bank investigations. Fourteen aphid proteins were found to vary according to host plant switch; ten of them were down regulated (proteins involved in glycolysis, TCA cycle, protein and lipid synthesis) while four others were overexpressed (mainly related to the cytoskeleton). These techniques are very reliable to describe the proteome from organisms such as insects in response to particular environmental change such as host plant species of herbivores.
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1. Introduction

Aphids are major crop pests and induce very high loss in crop yields notably by sucking plant sap and transmitting virus diseases. Among the 4000 worldwide aphid species, very different feeding behaviours can be observed, from strictly monophagous species spending all the year on a single host plant until very polyphagous species able to switch from one to plenty of host plants (Blackman and Eastop, 1994). *Myzus persicae* is one of the most generalist aphid species, infesting almost 400 host plant species in temperate weather regions. This capability to use a so large range of food sources has to be closely linked to high potential adaptation systems to cope with various defence mechanisms in host plant. Berenbaum (1995) presented the role of furanocoumarins in the Apiaceae plant species to illustrate the presence of secondary plant compounds to be one factor mediating co-evolution between plants and related phytophagous insects. The induction of detoxication enzymes such as glutathione *S*-transferases (GST) in response to the presence of plant allelochemicals in artificial diets has been previously reported in lepidopteran species such as *Sodoptera frugiperda* (Yu, 1984). Similar GST inductions were also observed in *M. persicae* when fed on Brassicaceae plants or directly exposed to glucosinolates (GLS) and ITC (Francis et al., 2005) but also in aphidophagous predators fed on aphids reared on Brassicaceae plant (Vanhaelen et al., 2001).

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Qualitative and quantitative changes of GST enzymes were related to the insect feeding preference, depending on the allelochemical presence in diets and host plants. An other enzymatic detoxification system, the cytochrome P-450, was also involved in the metabolisation by several herbivores of a broad range of secondary metabolites (SM) from the host plants (Cohen et al., 1992). The furanocoumarins in *Papilio polyxenes* were detoxified by the expression of multiple enzymes of cytochrome P450 monooxygenase and also of GST (Hung et al., 1995). More generally, polyphagous insects can selectively express a broad range of enzymes that assist in the detoxification of numerous xenobiotics including SM from plants (Li et al., 2000). Beside those particular enzymes, plenty of potential proteins could also be influenced by the presence of plant defence mechanisms. To broadly investigate the potential adaptation systems in herbivores, proteomics offers a great challenge to have biological information by a functional analysis of genes. To understand what is happening in an insect species when transferring to a new host plant, we have to study the insect proteins themselves. The aim of the present study was to obtain the proteome profiles and to identify the proteins in *M. persicae* aphid following host plant switch.

2. Materials and methods

2.1. Plants and insects

Broad beans (*Vicia faba* L.) were planted in a mixture of perlite and vermiculite (v:v, 50:50) in 12 cm diameter plastic containers and grown in a controlled environment room at 20±2 °C temperature and 16/8 photoperiod. One Brassicaceae species, *Brassica napus* L., and one Solanaceae, *Solanum tuberosum* L. were raised in separated rooms in an ordinary compost in the same environmental conditions as above.

Potato and rape plant species were selected according to known defence systems such as alkaloid and GLS SM, respectively, while broad bean was used as a control plant. Three treatments were studied here : aphids reared on bean and transferred either (1) on bean (which was considered as the control), or (2) on potato or (3) on rape. Five plants from each species were inoculated with 20 apterae adults of *M. persicae* Sultzer at the 5–6 leaf plant stage. Aphids used to inoculate the three host plant species belonged to the same clone reared on broad bean plants. After a 2-week duration, samples of aphids were collected in triplicate from their host plant to perform the proteomic analysis.

The aphids had been reared on broad bean plants in the laboratory for several years.

2.2. 2D polyacrylamide gel electrophoresis

2.2.1. Analytical 2D gel electrophoresis

Fresh aphids were collected from their host plant and 100 mg samples were crushed in triplicates in a 20 mM phosphate buffer including complete protease inhibitors (Roche), centrifuged at 15000*g*, 4 °C for 15 min. Supernatants were collected and proteins were extracted using the 2D Clean Up Kit according to the manufacturer's instructions (Amersham). Quantification of the precipitated proteins was realised using the 2D Quant kit from the same company.

The protein extracts (samples of 150 µg) were used to rehydrate 18 cm IPG strips (pH 3–10 NL from Amersham) for 12 h at 20 °C and constant voltage of 50 V. Isoelectric focusing (IEF) was carried out at 200 V for 200Vh, 500 V for 500Vh, 1000 V for 1000 Vh and 8000 V for 60,000 Vh at 20 °C and a maximum current setting of 50 µA/strip in an IEF unit from Biorad (Upsalla, Sweden). Following IEF, the IPG strips were equilibrated for 15 min in 375 mM Tris (pH 8.8) containing 6 M urea, 20% v/v glycerol, 2% w/v SDS, and 130 mM DTT and then for a further15 min in the same buffer except that DTT was replaced with 135 mM iodoacetamide. The IPG strips were then sealed with 0.5% agarose in SDS running buffer at the top of slab gels (280 × 210 × 1 mm) polymerised from 12% w/v acrylamide and 0.1% *N,N'*-methylenebisacrylamide. The second-dimensional electrophoresis was performed at 20 °C in a Dodeca Cell tank (Biorad, Upsalla) at 25 W/gel for 5 h. Gels were stained with laboratory made ruthenium(II) tris-bathophenantroline disulfonate (Rabilloud et al., 2001; Lamanda et al., 2004). Each gel was scanned with a Typhoon fluorescence imager (Amersham) at a wavelength corresponding ruthenium (II). Each experiment was conducted in triplicate. Images were analysed with Image master platinum 5.0 software (Amersham bioscience) according to the manufacturer.

2.2.2. Proteins identifications

Preparative gels were stained with a silver-stain compatible MS protocol (Shevchenko et al., 1996). Protein spots were manually excised from the gel. Excised gel plugs were first destained (Gharahdaghi et al., 1999) with a 30 mM potassium ferricyanide, 100 mM sodium thiosulfate solution. Then gel fragments were washed 3 times with water, cysteines were reduced with a 10 mM DTT solution for 45 minutes at 56 °C followed by alkylation with 50 mM iodoacetamide at room temperature in the dark. Digestion was performed overnight with 12.5 ng/µL of trypsin in 100 mM ammonium carbonate buffer, pH 8.4. The resulting peptides were extracted with 0.1% formic acid in acetonitrile, dried in a vacuum centrifuge, and resuspended in 30 µl 0.1% formic acid in water.

Peptide separation by reversed-phase liquid chromatography was performed on an Ultimate LC system (LC Packings) complete with Famos autosampler and Switchos II microcolumn switching device for sample clean-up and preconcentration. Sample (30 µl) was loaded at a flow rate of 200 nl/min on a micro-precolumn cartridge (300 µm i.d. × 5 mm, packed with 5 µm C18 100 Å PepMap). After 5 min, the precolumn was connected with the separating nano-column (75 µm i.d. × 15 cm, packed with

C18 PepMap100, 3 μm, 100 Å) and the gradient started. Elution gradient varied from 0% to 30% buffer B over 30 min, buffer A is 0.1% formic acid in acetonitrile/water 2:98 (vol/vol) and buffer B is 0.1% formic acid in acetonitrile/water 20:80 (vol/vol). The outlet of the LC system was directly connected to the nano electrospay source of an Esquire HCT ion trap mass spectrometer (Bruker Daltonics, Germany). Mass data acquisition was performed in the mass range of 50–1700 m/z using the Standard-Enhanced mode (8100 m/z/s). For each mass scan, a data-dependant scheme picked the 4 most intense doubly or triply charged ions to be selectively isolated and fragmented in the trap and the resulting fragments were mass analysed using the Ultra Scan mode (50–3000 m/z at 26,000 m/z/s).

Raw data were analysed and formatted (Data Analysis software, Bruker) for subsequent protein identification against the NCBI non-redundant protein database through the MS/MS ions search algorithm on the Mascot search engine (www.matrixscience.com). The mass tolerance of precursor and sequence ions were set at 0.5 and 0.3 Da, respectively, and carbamidomethylation of cysteines and methionine oxidation were set as fixed and variable modifications, respectively.

3. Results and discussion

Until now, plant–insect interactions were only investigated to determine the effect of the insect on the plant adaptation using mainly genomic approach. A lack of proteomic study focusing on the insect adaptation toward the plant defence is actually observed. This work is a first study on the aphid proteome change according to host plant switch. Although proteomics has emerged as an enormously powerful method for gaining insight to different physiological changes at cellular level, relatively no attempt have been made to apply this technique to study insect adaptation and response to toxic substances (Sharma et al., 2004). Only one study on a proteome analysis from insecticide treated brown planthopper was published and presented the modulation of 22 proteins at the expression level when compared to control samples (10 overexpressed and 8 underexpressed proteins, 4 proteins showed specific expression after insecticide treatment; Sharma et al., 2004). The differential expression of these proteins reflects the overall change in cellular structure and metabolism after insecticide treatment. In our work, 20 proteins varying in a significant way were observed following *M. persicae* transfer from one host plant to the others. As *M. persicae* as all the other aphid species are actually genome non-sequenced organism's, this situation leaded to meet some difficulties to identify the aphid proteins and implied that most of the identified peptides were related to Dipteran, and more particularly to *Drosophila* species. Fourteen proteins were identified and were related to metabolic changes in aphids infesting potato or rape host plants which develop pest defensive mechanisms. Similar protein profiles of aphids reared on the two former plants were generally observed despite their different SM. This observation requires more works using artificial diets including pure defensive molecules to compare the effect of specific SM and to understand this situation. The over- or under-expressed proteins in aphids infesting different host plant species (Figs. 1–3 and Table 1) can be grouped according to particular biological processes as presented below.

3.1. Mitochondrial proteins

Despite the fact that the major respiratory pathways were elucidated decades ago, relatively little is known about their regulation and control. Respiration can be divided into three main pathways: glycolysis, the mitochondrial tricarboxilic acid (TCA) cycle and mitochondrial electron transport (Fernie et al., 2004). A phosphoglycerate kinase (spot 24 Fig. 1) was identified to be down-regulated in *M. persicae* reared on rape and potato when compared to the aphid infested the bean control plant. Other glycolysis involved enzyme such as ATP synthase was already found to be modified in insect when treated by insecticide. Here, we found an under expression of four proteins related to the TCA cycle. The first one, a mitochondrial aconitase (spot LM Fig. 1), was 2.5 times less abundant in *M. persicae* reared on both potato and rape. Aconitase plays a key function in cellular energy production, and loss of its activity can have a major impact on cellular and aphid survival. Oxidative inactivation of aconitase has already been associated with decreased lifespan in *Drosophila* (Yan et al.,1997).

A pyruvate carboxylase (spot 107 Fig. 1) was a second TCA related enzyme down regulated (2.7–3.9 factor) in the potato—and rape—grown aphids compared to *M. persicae* reared on control bean plant. Expression perturbations in aphid of pyruvate carboxylase which has already been found in several tissues of ten insect species (Tu and Hagedorn, 1997) will imply modifications in the oxalate production which may be utilised in the synthesis of glucose, fat, some amino acids and several derivatives (Attwood, 1995; Wallace et al., 1998). Enzyme related to another CoA derivative, namely succinyl CoA (spot 109 Fig. 1) which is a high energy compound involved in ketone body metabolism in animals (Frazer et al., 1999) was also shown to be down regulated in *M. persicae* aphids reared mainly on potato but also on the rape host plant.

Transketolase (spot 108 Fig. 1) was also decreased in aphid reared on potato and rape host plant (2.14 and 2.95 factors, respectively). This enzyme occupies a pivotal place in metabolic regulation, providing a link between the glycolytic and pentose phosphate pathways by controlling the supply of ribose units for nucleotide biosynthesis (Turner, 2000).Changes in the oxidative pentose phosphate pathway in aphids would provide reduction of reductant (NADPH) for fatty acid synthesis, of the assimilation of inorganic nitrogen and would decrease the redox potential

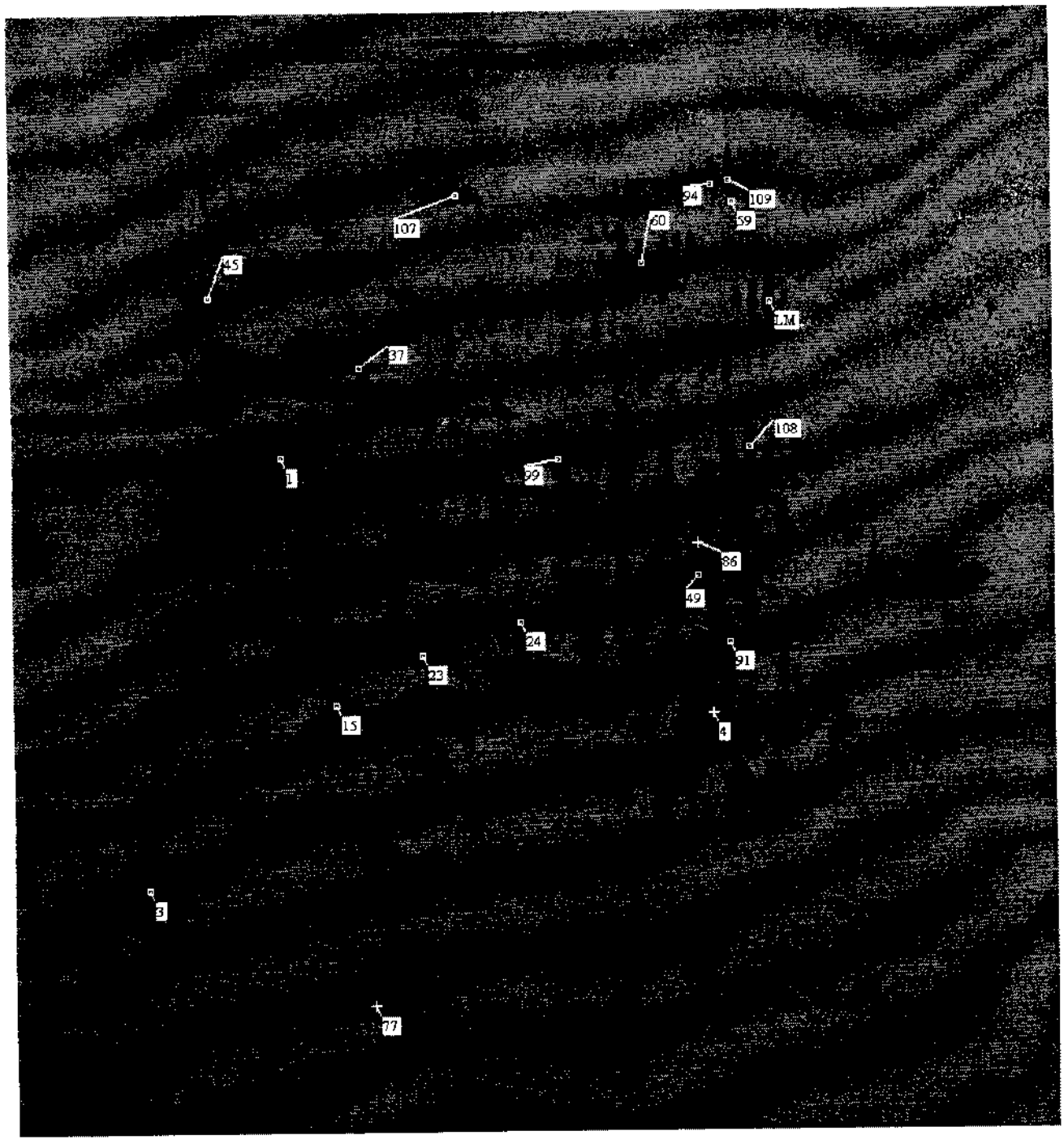

Fig. 1. A 2D-PAGE gel separation of proteins from *Myzus persicae* aphids reared on bean after ruthenium staining. Numbered spots corresponded to proteins significantly varying according to a host plant switch.

necessary to protect against oxidative stresses (Kruger and von Schaewen, 2003). Transketolases were already shown to be regulated by different kinds of xenobiotics such as paraquat and menadione in several biological systems (Akerman et al., 2003). A natural product isolated from *Bacillus megaterium* and *Streptomyces albus*, namely bacimethrin, was also demonstrated to inhibit several thiamine pyrophosphate-utilising enzymes, such as transketolases and be toxic to bacteria and yeast (Reddick et al., 2001).

3.2. Cytoskeleton proteins

Two proteins involved in the cytoskeleton, namely actin A4 and α2-tubulin (spots 15 and 1 Fig. 1, respectively), were under-expressed and up regulated respectively in the aphid reared on potato and rape when compared to bean control plants. Modification of tubulin production in aphids would lead to changes in all microtubules formation (Hachouf-Gherras et al., 1998; Kawasaki et al., 2003). Actin, a highly conserved protein of the contractile

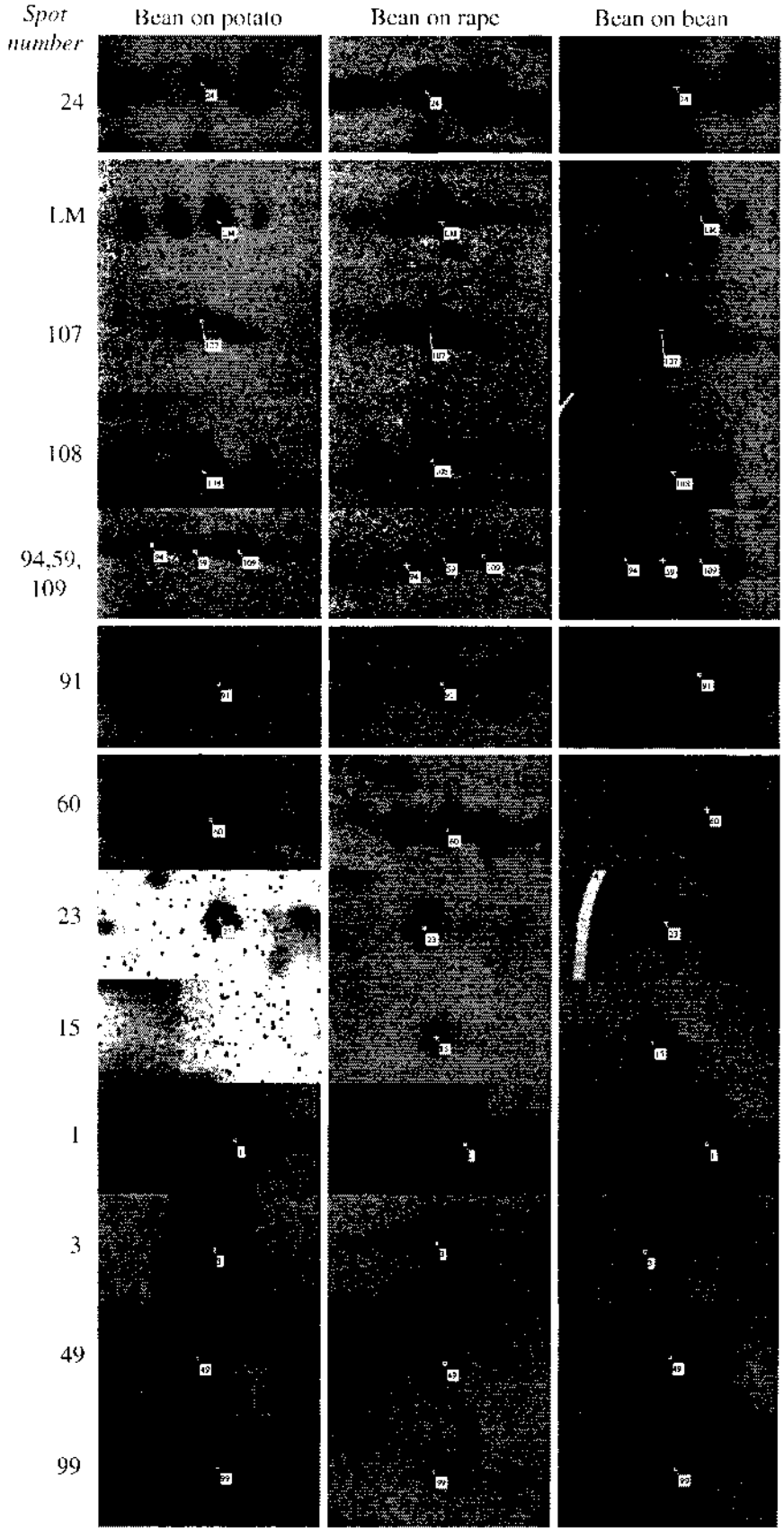

Fig. 2. 2D-PAGE gel areas related to *Myzus persicae* proteins differentially expressed according to host plant switch after ruthenium staining. Protein identifications related to spot numbers are presented in Table 1.

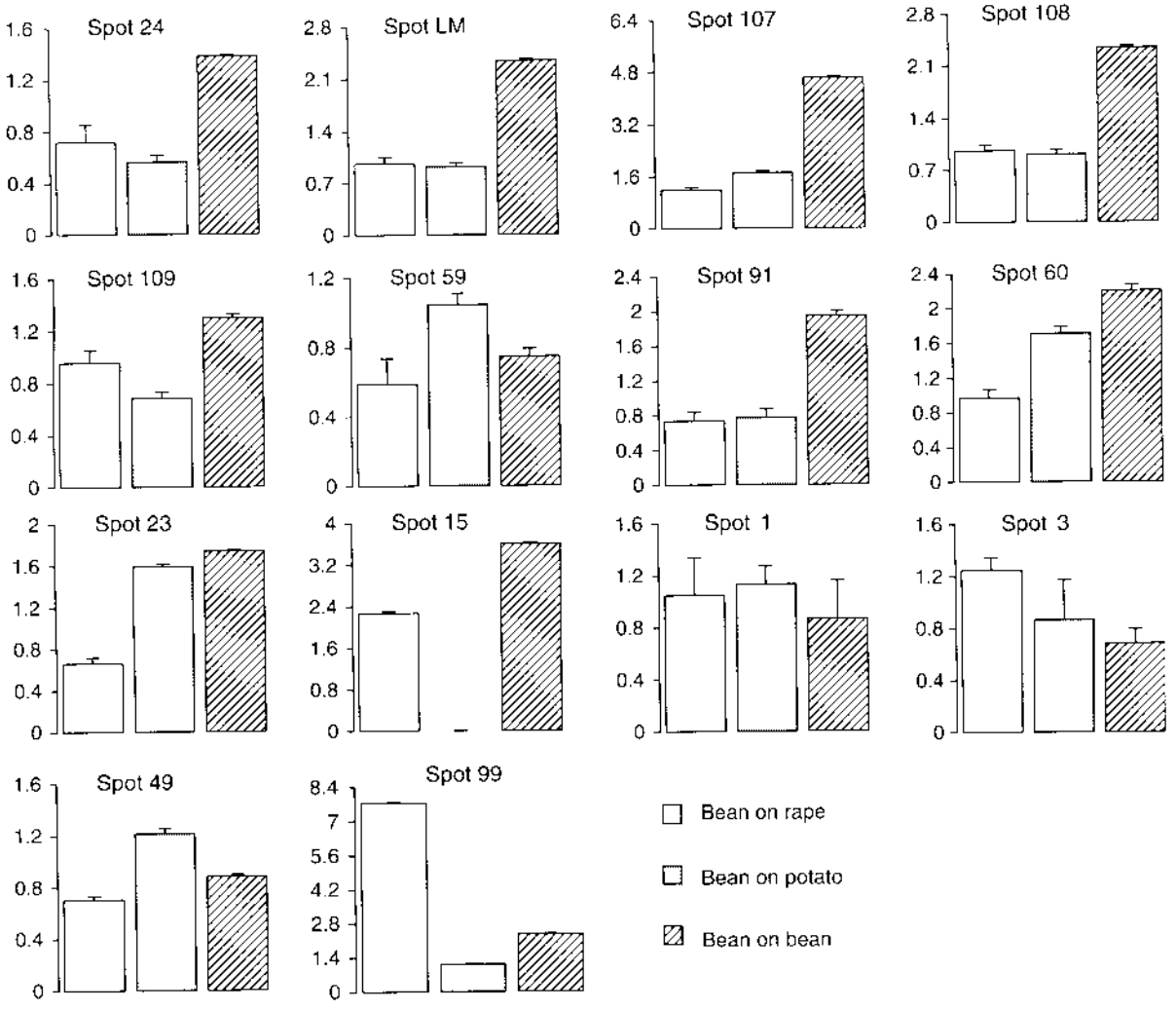

Fig. 3. Illustration of aphid protein differential expression (mean of spot volume density on gel with error bars representing standard deviations) in response to host plant switch. Protein identifications related to spot numbers are presented in Table 1.

machinery present in both cytoskeleton and muscle, contributes to a variety of cellular events such as organelle transportation, exo- and endocytosis. In this gene family, A4 actin isoform encodes a cytoskeletal actin which was characterised in *Bombyx mori* (Mangé et al., 1996) and which can be important target for neurotoxic chemicals (Damadoran et al., 2002). Moreover, consistent with the presence in nervous tissue, specific change in the α2 tubulin expression would disturb the efficient function of the sensory organs of the peripheral nervous system (Bo and Wensick, 1989).

3.3. Protein synthesis

Eukaryotic elongation factor 2 (eEF2) promotes ribosomal translocation as one of the three protein factors involved in eukaryotic polypeptide chain elongation (Bermek, 1978). Elongation factor 2 of *M. persicae* (spot 60 Fig. 1) was observed to be down regulated in a 2.3 factor when the aphid was reared on rape host plant. A decline in protein synthesis was already shown to take place, primarily due to inactivation of eEF2 along with oxidative stress and aging (Bektas et al., 2005) or by the ingestion or administration of xenobiotics (Parrado et al., 1999). One of the physiological consequences of slower rates of protein synthesis such as in *M. persicae* reared on rape but also in organisms exposed to several stress is a decrease in the availability of enzymes for the maintenance, repair and normal metabolic functioning in the cell (Rattan, 1996). Here are the potential negative consequences of aphid switching from a host plant to an other such as one from the Brassicaeae family including SM and related toxic degradation products (ITC).

A second protein involved in almost every process that involves nucleic acids, the initiation factor 4A (eIF-4, spot 23 Fig. 1) was under expressed by a 2.9 factor in aphids reared on rape plants compared with the two other plant species. Initiation factor 4A (eIF-4) is the prototype of the DEAD-box family of helicases which participates to replication, repair, recombination, transcription,

Table 1
Identification of aphid proteins showing differential expression in response to host plant switch

Metabolic pathways and protein identification	Mowse score	Spots[a]	Sequences of identified peptides	
Glycolysis				
Phosphoglycerate kinase *Toxoptera citricida*	354	24	VDFNVPLK	AAGILLNK
			YSMKPVAEELQK	ELTYFAK
			LFSEPIAR	FHIEEEGK
			IGDSLYDEAGAK	
TCA				
Mitochondrial aconitase - *Drosophila melanogaster*	59	LM	VADILTVK	
Pyruvate carboxylase—*Drosophila melanogaster*	80	107	VEYKPIR	AEELSEPK
			ADESYIVGK	IFLTGPK
Transketolase isoform D—*Drosophila melanogaster*	93	108	KIDSDLEGHPTPR	AFDQIR
			LGQSEAT SLQHKLDVYR	
Succinyl CoA ligase *Drosophila melanogaster*	93	109	AGPNYQEGLR	LILGIGHR VK
Lipid synthesis				
ATP citrate lyase *Drosophila melanogaster*	57	59	AGPNYQEGLR	
Citrate synthase *Drosophila melanogaster*	76	91	SGQVVPGYGHAVLR	
Protein biosynthesis				
Translation elongation factor 2 *Toxoptera citricida*	639	60	VNFTVDEIR	TILMMGR
			NMSVIAHVDHGK	TGTITTFK
			STLTDSLVSK	FSVSPVVR
			DLVFIK	NPADLPK
			QFAEMYAFK	YEYDVTEAR
			VFNSIMNYK	GVQYLNFIK
			FYAFGR	EGVLAEENMR
			IMGPNYTPGK	GGGQIPTAR
Translation initiation factor eIF-4A *Drosophila melanogaster*	81	23	AIPCVR	VLITTDLLAR
			EELTLEGIK	
Cytosqueleton				
Actin A4—*Bombyx mori*	176	15	DSYVGDEAQSK	EITALAPSTMK
			GYSFTTTAER	
Tubulin alpha-2 chain—*Drosophila melanogaster*	223	1	AVFVDLEPTVVDEVR	FDLMYAK
			EIVDVVLDR	LSVDYGK
			VGINYQPPTVVPGGDLAK	
Others				
ENSANGP00000009311 *Anopheles gambiae* str. PEST	343	3	SVTETGVELSNEER	VFYLK
			NLLSVAYK	YLAEVATGETR
			VISSIEQK	
Chlorophyll *a/b*-binding protein precursor-cucumber	108	49	LAMFSMFGFFVQAIVTGK	
Chaperonin - Buchnera sp.	59	99	AAVEEGVVAGGGVALVR	
			EMLPILESVAK	

Mowse scores algorithm (Pappin et al. 1993) is incorporated as a probability based implementation by Mascot software calculation. The total score is the absolute probability that the observed match (peptide masses for each entry in the sequence database with the set of experimental data) is a random event. The scores are reported as $-10 \log_{10}(P)$ where P is the absolute probability.

[a]Spot numbers are related to Fig. 1.

translation and splicing (Pause and Sonenberg, 1992). Perturbation of eIF-4 expression constitutes a proposed way to control insects by using inhibitors specific for these essential DNA/RNA helicase enzymes.

3.4. Lipid synthesis

Control of fatty acids biosynthetic pathways reflects changes in physicochemical properties of the membranes in response to the changing internal and external environments (Gibbons, 2003). An essential enzyme in lipid synthesis, the ATP citrate lyase (spot 59 Fig. 1) was found to be reduced in *M. persicae* reared on rape and will imply a reduction of cytosolic acetyl-CoA derived from citrate by the action of enzyme ATP citrate lyase. Acetyl-CoA, required for the synthesis of lipids, is too large to pass through biological membranes and two mechanisms have been proposed for its transport between cell compartments. The first requires ATP citrate lyase (ACL). Reduction of ACL expression in rape infested aphids would imply a reduced conversion of citrate by ACL into oxaloacetate and a lower release of acetyl-CoA in the cytosol (Adams et al., 2002). Citrate synthase (spot 91 Fig. 1) was also found to be down regulated by a 2.5 factor in aphids reared

on both rape and potato compared to the bean control plant.

The expression of three identified proteins not belonging to the previous metabolic pathways were found to be modified by the aphid host plant switch. One protein found to be originated from the aphid endosymbionts, namely from a *Buchnera* species, was over-expressed in *M. persicae* reared on rape. Particular aphid symbionts were already shown to be involved in *Acyrthosiphon pisum* specific host plant relations. This observation provides a further evidence that the presence of symbionts has to be considered when particular aphid–host plant interactions are investigated (Fukatsu et al., 2000; Leonardo and Muiru, 2003). For the last two proteins, some peptide sequences from the first one were identified to be similar to an *A. gambiae* protein sequence without any complementary information's and the second was found to be closed to a chlorophyll binding protein precursor. The presence of higher amount of this kind of proteins in aphids could perhaps be explained by a higher ingestion and accumulation in *S. tuberosum* infesting aphids due to a larger abundance of this kind of protein in the potato host plant.

In conclusion, the development of proteomic techniques contributes much in helping to refine and enhance our understanding of the regulation of various biosynthesis pathways in insects in relation to change of environmental conditions such as the switch of herbivore host plant. Whether many physiological and molecular functions are the sum of individual processes linked in sequence, proteomics allows consideration in a global approach, many individual processes that evolve in a close way. Here, we found that not only the expression of detoxification system enzymes are modified according to plant defense mechanisms. A more global metabolic change occurred when changing the feeding plant of herbivores such as aphids. Even very polyphagous insect such as *M. persicae* found on hundreds of plant species present broad change in their metabolism in protein, lipid and sugar production pathways. That kind of work on the insect response to their environment modification is of interest to entomological physiologists and biochemists focusing on insect adaptations and evolution.

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