(c) Obtaining a silk-free (and, optionally, essentially externally disinfected) pupa.

A method for producing a recombinant baculovirus comprising the steps of:

(a) Providing a pupa;

(b) Transfecting the pupa of step (a) with a transfer vector/bacmid suitable for producing a recombinant baculovirus derived from *Autographa californica multicapsid nucleopolyhedrovirus* (AcMNPV);

(c) Incubating the inoculated pupa of step (b) for a period of time sufficient for the recombinant baculovirus is produced;

(d) Obtaining the pupae comprising the recombinant baculovirus;

(e) Optionally, harvesting the recombinant baculovirus; and

(f) Optionally, purifying the recombinant baculovirus.

The present invention also relates to a device comprising a precision pump, a mobile mechanic arm and a (removable) needle/s suitable for injecting a fluid into a pupa belonging to the order *Lepidoptera*, preferably to the genera *Trichoplusia, Rachiplusia, Spodoptera, Heliothis, Manduca, Helicoverpa, Ascalapha* or *Samia,* more preferably to the genus *Trichoplusia, Rachiplusia, Spodoptera, Heliothis* or *Helicoverpa,* even more preferably to the species *Trichoplusia ni, Rachiplusia nu, Spodoptera frugiperda, Heliothis virescens, Helicoverpa armigera, Helicoverpa Zea, Manduca sexta, Ascalapha odorata* or *Samia cynthia.*

In an aspect, there is provided a pupa comprising a recombinant baculovirus and/or a bacmid derived from *Autographa californica multicapsid nucleopolyhedrovirus* (AcMNPV), wherein the pupa belongs to the genus *Trichoplusia, Rachiplusia, Spodoptera, Heliothis* or *Helicoverpa.*

In another aspect, there is provided a pupa comprising a nucleic acid sequence that allows for the expression above endogenous levels of the proteins IE-1, IE-0 and/or fragments thereof functioning as transcriptional regulators above endogenous levels obtained during baculovirus infection and a recombinant homologous region (*hr*) operably linked to any promoter that is suitable for driving the expression of a recombinant protein, wherein the pupa belongs to the genus *Trichoplusia, Rachiplusia, Spodoptera, Heliothis,* or *Helicoverpa*, wherein the nucleic acid sequence that allows for the expression of the proteins IE-1, IE-0 and/or fragments thereof is selected from the group consisting of:

(a) a nucleic acid containing the nucleotide sequence indicated in any of SEQ ID NOs: 1-5;

(b) a nucleic acid sequence having a sequence identity of at least 70% with the nucleotide sequence indicated in any of SEQ ID NOs: 1-5 and encoding a protein able to function as a transcriptional regulator in a recombinant baculovirus;

(c) a nucleic acid sequence encoding an amino acid containing the amino acid sequence indicated in any of SEQ ID NOs: 6-9; and

(d) a nucleic acid sequence encoding an amino acid sequence having a sequence similarity of at least 70% with the amino acid sequence indicated in any of SEQ ID NOs: 6-9 and able to function as a transcriptional regulator in a recombinant baculovirus.

In another aspect, there is provided the use of the pupa for the expression of recombinant proteins.

In another aspect, there is provided a method for producing at least one recombinant protein comprising the steps of:

(a) Providing a pupa;

(b) Inoculating the pupa of step (a) with a recombinant baculovirus derived from *Autographa californica multicapsid nucleopolyhedrovirus* (AcMNPV);

(c) Incubating the inoculated pupa of step (b) for a period of time sufficient for the at least one recombinant protein to be expressed; and

(d) Obtaining the pupae comprising the at least one recombinant protein,

wherein the pupa belongs to the genus *Trichoplusia, Rachiplusia, Spodoptera, Heliothis,* or *Helicoverpa.*

In another aspect, there is provided a method for producing a silk-free pupa belonging to the genus *Trichoplusia*, *Rachiplusia*, *Spodoptera*, *Heliothis*, or *Helicoverpa*, comprising the steps of:

(a) Providing a pupa contained in a silk cocoon;

(b) Treating the silk cocoon containing a pupa with a solution of a salt of hypochlorous acid; and

(c) Obtaining a silk-free and externally disinfected pupa.

In another aspect, there is provided a method for producing a recombinant baculovirus comprising the steps of:

(a) Providing a silk-free pupa belonging to the genus *Trichoplusia, Rachiplusia, Spodoptera, Heliothis* or *Helicoverpa*;

(b) Transfecting the pupa of step (a) with a bacmid suitable for producing a recombinant baculovirus derived from *Autographa californica multicapsid nucleopolyhedrovirus* (AcMNPV);

(c) Incubating the inoculated pupa of step (b) for a period of time sufficient for the recombinant baculovirus is produced; and

(d) Obtaining the pupae comprising the recombinant baculovirus.

In another aspect, there is provided a device comprising a precision pump, a mobile mechanic arm and a needle suitable for injecting a fluid into a pupa belonging to the genus *Trichoplusia, Rachiplusia, Spodoptera, Heliothis,* or *Helicoverpa.*

In another aspect, there is provided a silk-eliminating device comprising at least one container which contains a silk-dissolving solution, wherein the device comprises a second container, which is a wash-container and comprises and/or disperses a solution suitable for removing traces of silk and silk-dissolving solution from the pupae belonging to the genus *Trichoplusia, Rachiplusia, Spodoptera, Heliothis*, or *Helicoverpa.*

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: Massive single-use *T. ni* insect rearing module. Fifth instar stage larvae growing in the insect rearing module.

Figure 2: Comparison of cocoons (left part of the image) and pupae free of silk (right part of the image) formed by *Bombyx mori* and *Trichoplusia ni* Lepidoptera.

Figure 3: Semi-automatic device for silk removal from the *T. ni* cocoons. Schematic representation of the machine containing two containers and a mechanic arm in which the single-use rearing module is allocated. The first container contains hypochlorous acid and a system to project the liquid through the rearing modules containing the cocoons (it helps to dissolve more efficiently the silk surrounding the pupa). The second container is to wash the pupae, and it sprays water over the chrysalises. On the top of this container there is a system that dispenses air to dry the pupae. At the end of the process, the pupae are free of silk and ready to be used in infection or to be stored refrigerated until use.

CLAIMS

1. A pupa comprising a recombinant baculovirus and/or a bacmid derived from *Autographa californica multicapsid nucleopolyhedrovirus* (AcMNPV), wherein the pupa belongs to the genus *Trichoplusia, Rachiplusia, Spodoptera, Heliothis* or *Helicoverpa.*

2. The pupa according to claim 1, wherein the recombinant baculovirus and/or bacmid comprises a nucleic acid sequence encoding a recombinant protein.

3 The pupa according to any one of claims 1 or 2, wherein the baculovirus and/or bacmid comprises a nucleic acid sequence that allows for the expression above endogenous levels of the proteins IE-1, IE-0 and/or fragments thereof functioning as transcriptional regulators above endogenous levels obtained during baculovirus infection and a recombinant homologous region (*hr*) operably linked to any promoter that is suitable for driving the expression of a recombinant protein,

wherein the nucleic acid sequence that allows for the expression of the proteins IE-1, IE-0 and/or fragments thereof is selected from the group consisting of:

(a) a nucleic acid containing the nucleotide sequence indicated in any of SEQ ID NOs: 1-5;

(b) a nucleic acid sequence having a sequence identity of at least 70% with the nucleotide sequence indicated in any of SEQ ID NOs: 1-5 and encoding a protein able to function as a transcriptional regulator in a recombinant baculovirus;

(c) a nucleic acid sequence encoding an amino acid containing the amino acid sequence indicated in any of SEQ ID NOs: 6-9; and

(d) a nucleic acid sequence encoding an amino acid sequence having a sequence similarity of at least 70% with the amino acid sequence indicated in any of SEQ ID NOs: 6-9 and able to function as a transcriptional regulator in a recombinant baculovirus.

4. A pupa comprising a nucleic acid sequence that allows for the expression above endogenous levels of the proteins IE-1, IE-0 and/or fragments thereof functioning as transcriptional regulators above endogenous levels obtained during baculovirus infection and a recombinant homologous region (*hr*) operably linked to any promoter that is suitable for driving the expression of a recombinant protein, wherein the pupa belongs to the genus *Trichoplusia, Rachiplusia, Spodoptera, Heliothis,* or *Helicoverpa,*

wherein the nucleic acid sequence that allows for the expression of the proteins IE-1, IE-0 and/or fragments thereof is selected from the group consisting of:

(a) a nucleic acid containing the nucleotide sequence indicated in any of SEQ ID NOs: 1-5;

(b) a nucleic acid sequence having a sequence identity of at least 70% with the nucleotide sequence indicated in any of SEQ ID NOs: 1-5 and encoding a protein able to function as a transcriptional regulator in a recombinant baculovirus;

(c) a nucleic acid sequence encoding an amino acid containing the amino acid sequence indicated in any of SEQ ID NOs: 6-9; and

(d) a nucleic acid sequence encoding an amino acid sequence having a sequence similarity of at least 70% with the amino acid sequence indicated in any of SEQ ID NOs: 6-9 and able to function as a transcriptional regulator in a recombinant baculovirus.

5. The pupa according to any one of claims 2 to 4, wherein the promoter that drives the expression of said recombinant protein is, selected from the group of nucleic acids comprising:

(a) a nucleic acid containing the nucleotide sequence indicated in any of SEQ ID NOs: 10-14; and

(b) a nucleic acid sequence able to function as a promoter in a recombinant baculovirus and having a sequence identity of at least 70% with the nucleotide sequence

indicated in any of SEQ ID NOs: 10-14; and/or

wherein the recombinant homologous region (*hr*) is the sequence indicated in SEQ ID NO: 21 (*hr1*).

6. The pupa according to any one of claims 2 to 5, wherein the nucleic acid sequence that comprises combinations of recombinant promoters, sequences encoding transcriptional regulators and enhancer regions are selected from the group comprising SEQ ID NOs: 15-20.

7. The pupa according to any one of claims 3 to 6, wherein the pupa further comprises a nucleic acid sequence encoding a recombinant protein.

8. Use of the pupa as defined in any one of claims 1 to 7 for the expression of recombinant proteins.

9. A method for producing at least one recombinant protein comprising the steps of:

(a) Providing a pupa;

(b) Inoculating the pupa of step (a) with a recombinant baculovirus derived from *Autographa californica multicapsid nucleopolyhedrovirus* (AcMNPV);

(c) Incubating the inoculated pupa of step (b) for a period of time sufficient for the at least one recombinant protein to be expressed; and

(d) Obtaining the pupae comprising the at least one recombinant protein,

wherein the pupa belongs to the genus *Trichoplusia, Rachiplusia, Spodoptera, Heliothis,* or *Helicoverpa.*

10. The method according to claim 9, wherein the recombinant baculovirus comprises a nucleic acid sequence that allows for the expression above endogenous levels of the proteins IE-1, IE-0 and/or fragments thereof functioning as transcriptional regulators above endogenous levels obtained during baculovirus infection and a recombinant homologous region (*hr*) operably linked to any promoter that is suitable for driving the expression of a recombinant protein, wherein the nucleic acid sequence that allows for the expression above endogenous levels of the proteins IE-1, IE-0 and/or fragments thereof functioning as transcriptional regulators above endogenous levels obtained during baculovirus infection and the recombinant homologous region (*hr*) operably linked to any promoter that is suitable for driving the expression of a recombinant protein are as defined in claims 3 and/or 4.

11. The method according to claim 10, wherein the nucleic acid sequence that comprises combinations of recombinant promoters, sequences encoding transcriptional regulators and enhancer regions are selected from the group comprising SEQ ID NO: 15-20.

12. The method according to any one of claims 9 to 11, wherein the pupa further comprises a nucleic acid sequence encoding a recombinant protein.

13. A method for producing a silk-free pupa belonging to the genus *Trichoplusia*, *Rachiplusia*, *Spodoptera*, *Heliothis*, or *Helicoverpa*, comprising the steps of:

(a) Providing a pupa contained in a silk cocoon;

(b) Treating the silk cocoon containing a pupa with a solution of a salt of hypochlorous acid; and

(c) Obtaining a silk-free and externally disinfected pupa.

14. A method for producing a recombinant baculovirus comprising the steps of:

(a) Providing a silk-free pupa belonging to the genus *Trichoplusia, Rachiplusia, Spodoptera, Heliothis* or *Helicoverpa*;

(b) Transfecting the pupa of step (a) with a bacmid suitable for producing a recombinant baculovirus derived from *Autographa californica multicapsid nucleopolyhedrovirus* (AcMNPV);

(c) Incubating the inoculated pupa of step (b) for a period of time sufficient for the recombinant baculovirus is produced; and

(d) Obtaining the pupae comprising the recombinant baculovirus.

15. The method according to claim 14, wherein the bacmid suitable for producing a recombinant baculovirus derived from *Autographa californica multicapsid nucleopolyhedrovirus* (AcMNPV) comprises or alternatively, consists of, a nucleic acid sequence that allows for the expression above endogenous levels of the proteins IE-1, IE-0 and/or fragments thereof functioning as transcriptional regulators above endogenous levels obtained during baculovirus infection and a recombinant homologous region (*hr*) operably linked to any promoter that is suitable for driving the expression of a recombinant protein, wherein the nucleic acid sequence that allows for the expression above endogenous levels of the proteins IE-1, IE-0 and/or fragments thereof functioning as transcriptional regulators above endogenous levels obtained during baculovirus infection and the recombinant homologous region (*hr*) operably linked to any promoter that is suitable for driving the expression of a recombinant protein are as defined in claims 3 and/or 4.

16. The method according to claim 15, wherein the nucleic acid sequence that comprises combinations of recombinant promoters, sequences encoding transcriptional regulators and enhancer regions are selected from the group comprising SEQ ID NO: 15-20.

17. A device comprising a precision pump, a mobile mechanic arm and a needle suitable for injecting a fluid into a pupa belonging to the genus *Trichoplusia, Rachiplusia, Spodoptera, Heliothis,* or *Helicoverpa.*

18. The device according to claim 17, wherein the device further comprises a computer program for defining the position of the needle and/or for calculating the distance from the needle to the pupa and/or the distance of penetration of the needle into the pupa and/or the volume of liquid to be inoculated into the pupa.

19. A silk-eliminating device comprising at least one container which contains a silk-dissolving solution, wherein the device comprises a second container, which is a wash-container and comprises and/or disperses a solution suitable for removing traces of silk and silk-dissolving solution from the pupae belonging to the genus *Trichoplusia*, *Rachiplusia*, *Spodoptera*, *Heliothis*, or *Helicoverpa*.