Scholars Academic Journal of Biosciences
Abbreviated Key Title: Sch Acad J Biosci
ISSN 2347-9515 (Print) | ISSN 2321-6883 (Online)
Journal homepage: https://saspublishers.com

OPEN ACCESS

Biology

Effect of Methyl Jasmonate on Biomass and Saponin Content in Javanese Ginseng (*Talinum paniculatum* (Jacq.) Gaertn.) Callus Culture

Ratih Restiani[1*], Novianti Barlin[1], Dwi Aditiyarini[1]

[1]Department of Biology, Faculty of Biotechnology, Duta Wacana Christian University, Yogyakarta, Indonesia

DOI: 10.36347/sajb.2022.v10i07.003 | **Received:** 27.06.2022 | **Accepted:** 21.07.2022 | **Published:** 22.07.2022

***Corresponding author:** Ratih Restiani
Department of Biology, Faculty of Biotechnology, Duta Wacana Christian University, Yogyakarta, Indonesia

Abstract **Original Research Article**

Talinum paniculatum (Jacq.) Gaertn, or known as Javanese Ginseng, refers to one of the medicinal plants that is often used as a raw material for herbal medicine. Its potential is inseparable from the bioactive compounds contained in every part of the plant, especially the saponin. One of the efforts that can be made to increase the saponin content is through the elicitation of callus culture using methyl jasmonate. Therefore, this study aimed to identify the effect of concentration and elicitation time of methyl jasmonate on biomass and saponin production in callus culture. The results showed that variations in concentration and elicitation time had no effect on the decrease of callus biomass when compared to control. The highest callus biomass was produced in the callus which was elicited in MS medium with the addition of a methyl jasmonate concentration of 0.05 mM for 5 days and 0.2 mM for 15 days. In addition, the largest saponin content by stain area (0.563 cm^2) and the intensity of the TLC colour intensity (5 out of 5) on the callus were elicited at a methyl jasmonate concentration of 0.15 mM for 15 days. The results of this study indicated that the addition of methyl jasmonate to the callus culture medium could affect the saponin production. In addition, optimal concentration and elicitation time can increase biomass and saponin production in Javanese ginseng callus (T. *paniculatum*).

Keywords: *Talinum paniculatum*, callus culture, elicitation, methyl jasmonate, saponin.

INTRODUCTION

Talinum paniculatum (Jacq.) Gaertn., also known as Javanese Ginseng, is a herbaceous plant that has a root morphology similar to Korean ginseng (*Panax ginseng*) [1]. In Indonesia, *Talinum paniculatum* leaf are often consumed as a vegetable because it contains quite high levels of antioxidant [2]. In addition, this plant is also used as a medicinal plant because its bioactivity as an antidiarrheal, antibacterial, ulcer treating, stamina enhancer, aphrodisiac, and breast milk production enhancer [3].

All parts of Javanese ginseng plant contain bioactive compounds such as saponins, flavonoids, terpenoids, alkaloids, and tannins [3-5]. Saponins are bioactive compounds that are predominantly produced in Javanese ginseng plants and have the potential to be used as active ingredients in the manufacture of medicine. This makes the plant need to be available in large quantities so its availability in the production process can be established. However, the limitation in the extraction of bioactive compounds directly from plants in the field are the quality and quantity of bioactive compounds that are not the same because they are influenced by various factors such as climate, geographical conditions, disease pests, and harvesting age. Therefore, a solution is needed to overcome these limitation.

One effective method in Biotechnology to increase the production of secondary metabolites through *in vitro* culture is by the addition of elicitor [6]. Methyl jasmonate is a type of abiotic elicitor that can be used to increase saponin content in *in vitro* cultures [7-9]. To date, various studies have been carried out to improve saponin through the addition of methyl jasmonate using root cultures of *T.paniculatum*. Ahmad & Anggita (2019) succeeded in increasing the saponin content in *T. paniculatum* root culture by 30 mg/g of dry weight with the addition of MJ of 0.2 mM into the MS medium for 15 days of elicitation. However, there have been no studies that use callus culture to increase saponins in *Talinum paniculatum*. Therefore, this study aimed to identify the effect of methyl jasmonate addition on biomass and saponin production in the callus culture of *Talinum paniculatum* (Jacq.) Gaertn.

MATERIALS AND METHODS

Research Materials

Talinum paniculatum was obtained from CV. Merapi Farma Herbal in Yogyakarta, Indonesia. The characteristic of mother plant to be the source of explant were healthy plants, had fresh green color, and did not wither.

Callus Initiation

Explant was isolated from the leaves of *T. paniculatum* on the position of 2-3 from the shoots. The leaf explants were further washed with liquid detergent and tween 20 and rinsed under running water until clean. The cleaned explants were taken to *Laminar Air Flow* for the sterilization stage. The explant was then sterilized using 50% alcohol (v/v) for 3 minutes and rinsed with sterile aquade 3 times. The explants were cut into pieces of 1x1 cm^2 and inoculated into Murashige and Skoog (MS) medium with the addition of sucrose 30 g/L, agar 8 g/L, 2.4-D 2 mg/L, and kinetin 3 mg/L. Cultures were incubated at a temperature of 25°C, under light condition for 24 hours, and with a light intensity of 1000 lux until the callus reached the stationary phase.

Callus Elicitation

The 66-day-old callus was sub-cultured in MS media containing various concentrations of methyl jasmonate (0.025; 0.05; 0.1; 0.15; and 0.2) mM. The culture was incubated at 25°C, under light conditions for 24 hours with a light intensity of 1000 lux. Each treatment was repeated 3 times. Callus was harvested after the elicitation of the 0^{th}, 5^{th}, 10^{th}, and 15^{th} days. After that, a measurement of the dry weight of the callus and an analysis of its saponin content were conducted.

Saponin Analysis

The extraction of the callus of *T. paniculatum* was carried out based on a modification of the research method by [7]. Callus was oven-dried at 50°C until the dry weight was constant. Dry callus was mashed using a mortar. A total of 0.1 g of callus powder was extracted using 10 ml of 96% *analytical grade* ethanol and then heated using a water bath at a temperature of 80°C for 45 minutes. The sample was filtered, and the remaining solvent was evaporated until a volume of 0.2 ml remained. A total of 3 µL extracts and a standard solution of 5% saponins were plotted in plat silica gel GF_{254} with a size of 10 x 8 cm, then they were diluted with eluent propanol: water (14:3). After that, the plate was wind-dried, sprayed with anisaldehyde reagent containing 0.5 ml of anisaldehyde, 10 ml of acetic acid, 5 ml of concentrated sulfuric acid, and 85 ml of ethanol. Next, the plate was heated in an oven at a temperature of 105°C for 7–10 minutes. Results were observed under 254 nm UV light. Identification of saponin content on the extract was carried out by calculating the value of *Retention Factor* (Rf). Semi-quantitative analysis of saponins used a modified method, namely calculating the stain area and color intensity assessment on the GF_{254} *gel silica* plate.

RESULTS AND DISCUSSION

Callus Growth

T. paniculatum callus grew on leaf explants starting from 1^{st} week to 9^{th} week. Callus had a friable texture (Figure 1). Friable callus had a fragile texture and was easily separated. During its growth, the callus absorbed water and nutrients from the medium, so that the callus width was increased. The friable texture of the callus underwent rapid cell division. It was influenced by the effective combination of the hormone 2,4-D (auxin) and Kinetin (cytokinin), which stimulated the cells to continue to divide and enlarge. The higher the concentration of 2,4-D, the faster the cell ability to divide, leading to the formation of a friable callus [11]. The results of this study were in line with the study [12] where the use of 2 mg/L 2,4-D combined with BAP (1 and 2 mg/L) resulted in callus with a friable texture and better visual.

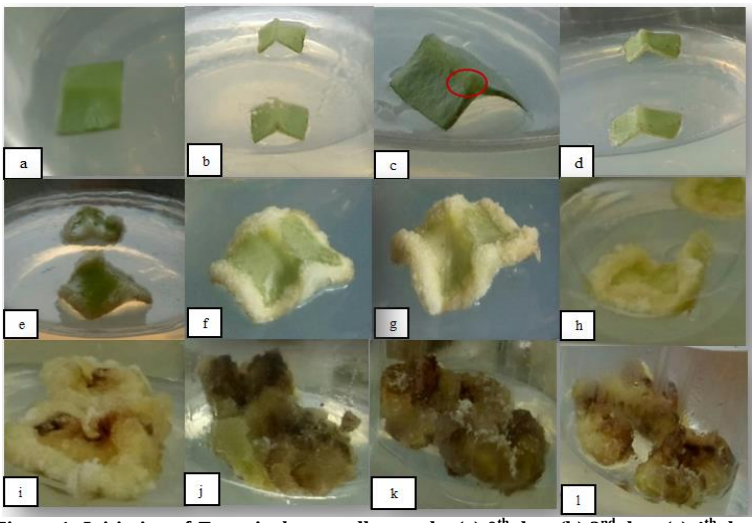

Figure 1: Initiation of *T. paniculatum* callus on the (a) 0^{th} day, (b) 2^{nd} day, (c) 4^{th} day, (d) 8^{th} day, (e) 10^{th} day, (f) 13^{th} day, (g) 17^{th} day, (h) 24^{th} day, (i) 35^{th} day, (j) 42^{nd} day, (k) 58^{th} day, (l) 66^{th} day

The Effect of Concentration and Time of Elicitation on Callus Biomass

The effect of elicitation on the growth of *T. paniculatum* callus was measured based on callus biomass. Based on the callus biomass in Figure 2, growth of callus in the methyl jasmonate treatment showed an increase in biomass (dry weight) of (0.052 – 0.063) g compared to the control (0.050 g). The highest biomass was produced at the treatment of MJ_2H_1 (concentration of 0.05 mM, elicitation time of 5 days) and MJ_5H_3 (concentration of 0.2 mM, elicitation time of 15 days).

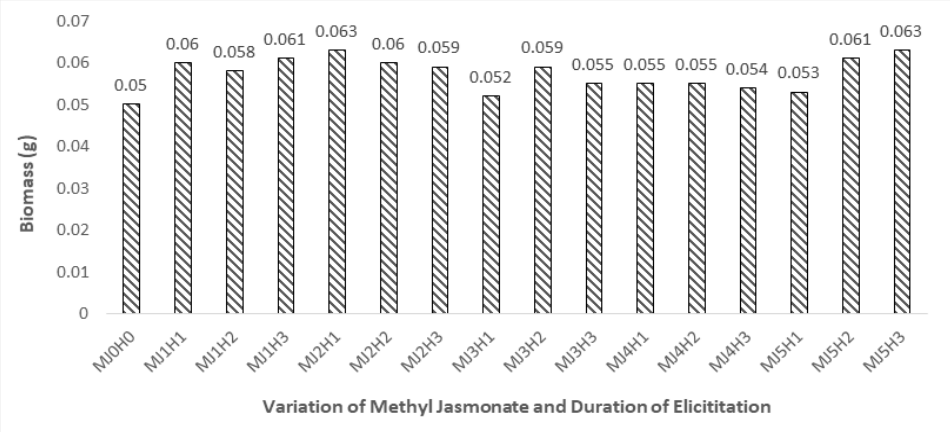

Figure 2: The effect of concentration and time of methyl jasmonate elicitation on the biomass of Javanese ginseng (*Talinum paniculatum* Gaertn) callus. Description: MJ_0 = 0 mM, MJ_1 = 0,025 mM; MJ_2 = 0,05 mM; MJ_3 = 0,1 mM; MJ_4 = 0,15 mM; MJ_5 = 0,2 mM; H_0 = 0 day; H_1 = 5 days; H_2 = 10 days; H_3 = 15 days

The results of this study showed that the concentration of methyl jasmonate and the time of elicitation had an effect on increasing the biomass of *T. paniculatum* callus. The increase in callus biomass indicated that the addition of methyl jasmonate at low concentrations (0.05–0.2) mM had not been able to stimulate stress for callus so that growth continued, and biomass did not decrease when compared to the control. The results of this study were in line with [13], which showed an increase in palm oil callus growth after adding jasmonic acid at a concentration of (0.25, 50, 75) µM. Methyl jasmonate is a type of growth hormone that causes plants to withstand stressful conditions and, at the proper concentration, plays a role in supporting growth and defense against depression (Asgher *et al*., 2015). It can be seen based on the results in Figure 2. That callus biomass did not show any decline until the 15th day of elicitation. In addition, another factor that could be the cause of an increase in callus biomass was the effect of the combination of the hormones 2.4 D, kinetin, and methyl jasmonate on the elicitation medium. At low concentrations, the three types of growth hormone interacted with each other to increase callus biomass.

Effect of Concentration and Time of Elicitation on Saponin Production

The content of saponin in the callus was measured based on stain area and color intensity of stains formed on KLT plates (Figure 3 and Table 1).

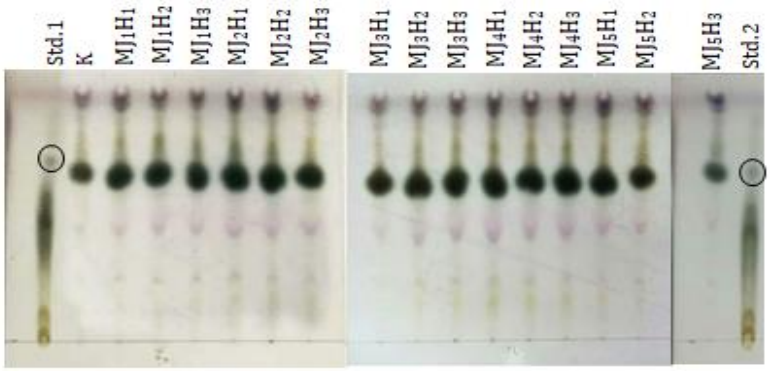

Figure 3: Effect of concentration and elicitation time of methyl jasmonate on the saponin contents of *T. paniculatum* callus. Description: Std.1 : saponin standards in ethanol (5%); Std.2 : saponin standard in water (5%); K : 0 mM; MJ_1 : 0,025 mM; MJ_2 : 0,05 mM; MJ_3 : 0,1 mM; MJ_4 : 0,15 mM; MJ_5 : 0,2 mM; H_0 : 0 day; H_1 : 5 days; H_2 : 10 days; H_3 : 15 days

The saponin content of *T. paniculatum* callus extract was detected by comparing the Rf value of the stain with a standard saponin solution after being sprayed with anisaldehyde-H_2SO_4. Based on Figure 3, it

is known that the control and extract of the *T. paniculatum* callus produced intense green stains. These stains formed saponins. This is in accordance with the statement [7] that the saponin stain will be solid green after being sprayed by an anisaldehyde reagent.

Table 1: The effect of concentration and elicitation time of methyl jasmonate on the color intensity of saponins

Treatment	Rf	Area of Saponin Stains (cm^2)	Color intensity
Saponin Standard 1 (5%)	0.65	0.094	1
Saponin Standard 2 (5%)	0.613	0.094	1
Control	0.613	0.330	3
MJ_1H_1	0.600	0.385	4
MJ_1H_2	0.613	0.440	4
MJ_1H_3	0.600	0.424	4
MJ_2H_1	0.600	0.440	5
MJ_2H_2	0.588	0.440	5
MJ_2H_3	0.600	0.502	4
MJ_3H_1	0.600	0.440	4
MJ_3H_2	0.588	0.495	5
MJ_3H_3	0.600	0.495	5
MJ_4H_1	0.588	0.495	5
MJ_4H_2	0.600	0.502	5
MJ_4H_3	0.600	0.563	5
MJ_5H_1	0.600	0.495	5
MJ_5H_2	0.613	0.440	4
MJ_5H_3	0.613	0.330	4

Description: Numbers 1–5 on color grading are from the most faded to the most intense colors (containing saponins in small-to-large quantities).

Saponins on callus were indicated by dense green patches with varying Rf values (Figure 3 and Table 1) on KLT plates. Table 1 shows the standard Rf values of saponins 1 and 2, which were 0.65 and 0.613. The control treatment was 0.613, and the stain from the elicitor treatment was 0.588–0.613. Those Rf values showed not very different results. This indicated that the stains in the control sample and the elicitation treatment stains were saponin compounds because the Rf value was close to the standard Rf value of the saponin. Table 1 showed that the largest saponin stain area produced by the methyl jasmonate elicitor treatment MJ_4H_3 (concentration of 0.15 mM and elicitation time of 15 days) was 0.563 cm^2 with an Rf value of 0.600. The lowest saponin stain area was in the methyl jasmonate elicitor treatment (0.2 mM incubation time of 15 days) and the control treatment was 0.330 cm^2 with an Rf value of 0.613. Various concentration of methyl jasmonate were successfully increased saponin levels compared to control (Table 1).

The determination of the quantity of saponins was also measured based on color scoring by correlating colors 1–5 from the faded to concentrated. The elicitor treatment of methyl jasmonate 0.15 mM with an incubation time of 5, 10, and 15 days (MJ_4H_1, MJ_4H_2, and MJ_4H_3) showed the intensity of the intense color of the saponin stain with a scoring value of 5 out of 5. Elicitation with MJ showed that the highest saponin production was found in the treatment of methyl jasmonate elicitor with a concentration of 0.15 mM. This suggested that the methyl jasmonate used in *T. paniculatum* callus cultures required higher concentrations to increase the content of saponin. Elicitation carried out by the addition of methyl jasmonate could stimulate the production of saponins in callus cultures of the T. *paniculatum* leaf explant. The results of this study were linear with previous studies by [9], which succeeded in proving that the MJ at higher concentrations (0.2 mM) and a longer elicitation time (15 days) in *T. paniculatum* root cultures could increase saponin production by 1.5 times compared to the controls.

Based on the results in Figure 3 and Table 1, it shows that the longer the time of elicitation, the higher the saponin content. It is in accordance with the research conducted [9] that the most optimal incubation time for saponin production is 15 days after being given elicitation treatment. The highest production of saponin compounds elicited for 15 days at a concentration of 0.15 mM (MJ_4H_3) was evidenced by the highest saponin stain area (0.563 cm^2) with the most intense color intensity of 5 out of 5 and followed by a decrease in callus biomass. This study is in line with the previous study conducted by [9] that successfully showed an increase in saponin compounds at an elicitor concentration of methyl jasmonate of 0.2 mM with an incubation time of 15 days in adventitious root cultures of Javanese ginseng (*Talinum paniculatum* Gaertn.) followed by a decrease in callus biomass.

Concentration of methyl jasmonate of 0.2 mM with an elicitation time for 15 days tend to increase callus biomass followed by a decrease in saponin levels. This is might be because methyl jasmonate at a concentration of 0.2 mM acts more as a growth regulator

that results in callus growth than provides a stress condition for callus. This was shown by an increase in callus biomass followed by a decrease in saponin levels by 0.330 cm^2. In contrast, a concentration of 0.15 mM methyl jasmonate with the same elicitation time for 15 days was able to increase saponin content by 0.563 cm^2 and decreased callus growth shown from callus biomass data. This is in accordance with the theory that methyl jasmonate at certain concentrations can decrease callus growth because methyl jasmonate provides a stress condition on the callus which results in the production of secondary metabolites. According to [14], methyl jasmonate activates the metabolism pathway of saponins in *Panax ginseng*. Methyl jasmonate stimulates terpenoid biosynthesis by inducing the genes that regulate it [15] also states that the addition of exogenous methyl jasmonate into the *in vitro* culture medium may induce jasmonic acid (JA) biosynthesis, which further stimulates the expression of genes involved in saponin biosynthesis in ginseng plants.

CONCLUSION

The highest callus biomass of 0.063 g was produced by MS media added with 0.05 mM methyl jasmonate and elicitation time for 5 days (MJ_2H_1) and 0.2 mM methyl jasmonate concentrations and elicitation time for 15 days (MJ_5H_3). Meanwhile, the highest saponin content was produced by callus at the treatment of methyl jasmonate concentration of 0.15 mM and elicitation time for 10 days ($MJ_4H_{3)}$ with a stain area of 0.563 cm^2 and a color intensity of 5 out of 5. These results suggest that optimal concentration and elicitation time could increase saponin production through *T. paniculatum* callus culture.

ACKNOWLEDGEMENT

The author would like to thank the Faculty of Biotechnology, Duta Wacana Christian University of Yogyakarta for funding this research.

REFERENCES

1. Lakitan, B., Kartika, K., Widuri, L. I., Siaga, E., & Fadilah, L. N. (2021). Lesser-known ethnic leafy vegetables Talinum paniculatum grown at tropical ecosystem: Morphological traits and non-destructive estimation of total leaf area per branch. *Biodiversitas Journal of Biological Diversity*, *22*(10), 4487–4495. doi: 10.13057/biodiv/d221042.
2. Lestario, L. N., Christian, A. E., & Martono, Y. (2009). Aktivitas antioksidan daun ginseng jawa (Talinum paniculatum Gaertn). *Agritech*, *29*(2), 71–78.
3. Yeni, L. F. (2022). Aktivitas Antibakteri Ekstrak Etanol Talinum paniculatum Lokal Kalimantan Barat terhadap Shigella sonnei. *Quagga: Jurnal Pendidikan dan Biologi*, *14*(1), 51-58. doi: 10.25134/quagga.v14i1.50801
4. Wijaya, R., Restiani, R., & Aditiyarini, D. (2020, September). Pengaruh kitosan terhadap produksi saponin kultur kalus daun ginseng jawa (Talinum paniculatum (Jacq.) Gaertn.). In *Prosiding Seminar Nasional Biologi* (Vol. 6, No. 1, pp. 253-262).
5. Seswita, D. (2010). Som Jawa (Talinum paniculatum) Ginseng Indonesia Penyembuh Berbagai Penyakit. *Warta Penelitian dan Pengembangan Tanaman*, *16*(2), 21-23.
6. Wawrosch, C., & Zotchev, S. B. (2021). Production of bioactive plant secondary metabolites through in vitro technologies—status and outlook. *Applied Microbiology and Biotechnology*, *105*(18), 6649-6668. doi: 10.1007/s00253-021-11539-w
7. Manuhara, Y. S. W., Kristanti, A. N., & Utami, E. S. W. (2015). Optimization of cukture conditions of Talinum paniculatum Gaertn. adventitious roots in ballon-type bubble bioreactor using aeration rate and unitial inoculum density. *Asian Journal of Biological Sciences*, *2015*(8), 83-92. doi: 10.3923/ajbs.2015.83.92
8. Suan See, K., Bhatt, A., & Lai Keng, C. (2011). Effect of sucrose and methyl jasmonate on biomass and anthocyanin production in cell suspension culture of Melastoma malabathricum (Melastomaceae). *Revista de biologia tropical*, *59*(2), 597-606.
9. Ahmad, F., & Anggita, V. S. (2019). Enhancement of saponin accumulation in adventitious root culture of Javanese ginseng (Talinum paniculatum Gaertn.) through methyl jasmonate and salicylic acid elicitation. *African journal of biotechnology*, *18*(6), 130-135. doi: 10.5897/ajb2018.16736.
10. Manuhara, Y. S. W., Kristanti, A. N., Utami, E. S. W., & Yachya, A. (2015). Effect of sucrose and potassium nitrate on biomass and saponin content of Talinum paniculatum Gaertn. hairy root in balloon-type bubble bioreactor. *Asian Pacific Journal of Tropical Biomedicine*, *5*(12), 1027-1032. doi: 10.1016/j.apjtb.2015.09.009.
11. Noli, Z. A. (2015). Induksi kalus Artemisia vulgaris L. dengan Pemberian Beberapa Konsentrasi 2, 4-Dichlorophenoxyacetic Acid (2, 4-D). *Jurnal Biologi UNAND*, *4*(4), 216-222.
12. Mahadi, I., Syafi'i, W., & Sari, Y. (2016). Pengaruh Pemberian Hormon 2, 4-D Dan BAP Terhadap Pertumbuhan Kalus Jeruk Kasturi [Citrus microcarpa]. *Jurnal Biogenesis*, *12*(2), 99-104.
13. Al-Qatrani, M. K., Al Khalifa, A. A., & Obaid, N. A. (2021, November). Effect of Jasmonic Acid on Stimulating The Growth and Development of Date Palm Callus (Phoenix Dactylifera L.) Cultivar Shukar in Vitro Under Salt Stress Conditions. In *IOP Conference Series: Earth and Environmental Science* (Vol. 923, No. 1, p. 012017). IOP Publishing. doi: 10.1088/1755-1315/923/1/012017.
14. Wang, J., Li, J., Li, J., Liu, S., Wu, X., Li, J., & Gao, W. (2016). Transcriptome profiling shows gene regulation patterns in ginsenoside pathway in response to methyl jasmonate in Panax Quinquefolium adventitious root. *Scientific reports*, *6*(1), 1-11. doi: 10.1038/srep37263.
15. Zhao, J., Davis, L. C., & Verpoorte, R. (2005). Elicitor signal transduction leading to production of plant secondary metabolites. *Biotechnology advances*, *23*(4), 283-333. doi: 10.1016/j.biotechadv.2005.01.003.