Review Article

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A Study of the Factors Effective in Morphogenesis of Aspergillus terreus in order to Increase the Production of Lovastatin

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ABSTRACT

BACKGROUND AND OBJECTIVE: Lovastatin is one of the most important types of statin drugs, commonly produced by *Aspergillus terreus* in a liquid culture. Lovatatin use reduces blood cholesterol and prevents heart attacks and vascular stiffness. Since lovastatin is introduced as a valuable secondary metabolite, this study was conducted to analyze the factors effective in morphogenesis of this fungus to increase the industrial production of this drug.

METHODS: For data collection in this review article, the articles containing one of the words "cholesterol", "vascular stiffness", "morphogenesis", "lovastatin", "Aspergillus terreus", and " inducer" between the years 1960 and 2017 were searched and studied in Pubmed, Scopus, Science Direct and Islamic World Science (ISC) databases.

FINDINGS: Overall, 145 articles were found, among which 58 papers were considered appropriate for this study. According to the results of the studies, the slowly metabolized carbon source and combined culture medium, high stirrer speed at 600 rpm, an aeration equal to 70% saturation, an inoculation equal to 10^7 spores, and the use of inducers such as magnesium silicate hydrate, methionine, butyrolactone and linoleic acid cause a special morphogenesis called Pellet.

CONCLUSION: The results indicate that the type of carbon source has the greatest effect on morphogenesis of pellet. The formation of small pellets reduces the viscosity of the medium, increases the rate of oxygen transfer to microorganisms, and ultimately produces more lovastatin.

KEY WORDS: *Lovastatin, Aspergillus terreus, Cholesterol*.

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Introduction

Lovatatin (Mevacor) is a cholesterol – lowering agent that has two forms of acid and lactose in the culture medium (Figure 1), which inhibits the 3–hydroxyl– 3 – methylglutaryl – CoA reductase (HMG-CoA reductase) enzyme, thus preventing cholesterol production and cardiovascular disease (1). Fadaeipuor et al. showed that cholesterol is one of the risk factors for cancer (2). Setorki et al. also showed that the use of antioxidant substances has beneficial effects on atherosclerotic risk factors such as cholesterol (3). Asadi et al. showed that the hydroalcoholic extract of *Thymbra spicata* mimics the effects of lovastatin in reduction of fat levels (4). Mansoori et al. conducted a study on the optimization of monacolin production (a precursor in the production of lovastatin), and showed that maltose at a concentration of 10 g/l and $MgSO_4$ at a concentration of 0.78 g/l are effective in the production of lovastatin and biomass (5).

Nezami et al. showed that the use of statins such as lovastatin reduces the risk of vascular calcification and arterial stiffness (6). Generally, different types of culture methods for lovastatin production are classified into two classes of solid and liquid culture. Solid culture is a cost-effective method for producing lovastatin (7). In this type of culture, agricultural waste is used as a power supply and has simple extraction and downstream processing steps (8), however, this type of culture has disadvantages. The main problem with industrial solid-state culture is controlling the process parameters (such as oxygen, temperature, humidity) and changing the scale from the laboratory to the industrial level (7, 9).

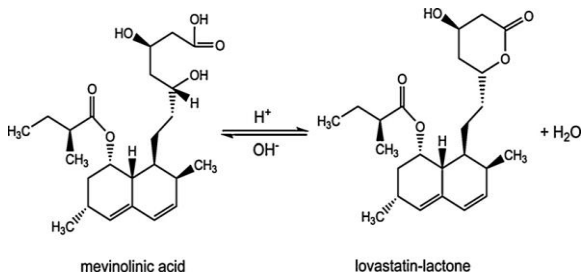

Figure 1. Forms of lovastatin

Therefore, only *Aspergillus terreus* is widely used today for lovastatin production through liquid culture (1, 10–15). Minooeian Haghighi et al. investigated the effect of cumin, ziziphora and nigella sativa essential oils on *Aspergillus* cells and showed that these substances negatively affect the growth of *Aspergillus* (16). Asadi et al. investigated the histochemical effects of consuming *Aspergillus*-contaminated food and showed that this fungus is a pathogen and produces mycotoxin (17). Today, several methods of liquid culture are used for the production of lovastatin, including batch culture, fed culture (12, 15,18) and two-stage culture (19, 20). Carbon sources for the production of lovastatin from *Aspergillus terreus* are usually composed of glucose, lactose, glycerol and complex carbon sources. Jaberi Ansari et al. investigated the factors influencing the production of lovastatin by *Aspergillus terreus* ATCC 20542 in liquid culture and showed that carbon sources, nitrogen sources, C/N ratio, pH and mineral elements play a significant role in the production of lovastatin (21).

One of the effective factors in the production of secondary metabolites in fungi is the morphogenesis of fungus. Gao et al. investigated the relationship between morphogenesis and the production of itaconic acid in the *Aspergillus terreus* fungus and showed that increase in phosphorus and temperature increases the diameter of the pellet and thus decreases the production of itaconic acid (22). Lu et al. showed that controlling morphogenesis results in increasing the production of secondary metabolites, such as itaconic acid, in liquid culture (23). Anuradha et al. showed that the pH 5.5, 28° C temperature and stirring at 200 rpm improved the formation of pellet and the production of enzymes from this fungus (24).

In 2017, Wang et al. introduced a new method for increasing the production of citric acid by *Aspergillus* fungus. Instead of culturing fungus spores, they used pellet morphogenesis for inoculation. This idea can be used as a promising way to increase production of other secondary metabolites are also effective in fermentation of fungi (25). Industrial production of lovastatin in liquid state can only be performed on very large scale such as 150,000 m3 cubic meters. But the problem that the industry encounters in the production of lovastatin through liquid culture in large fermenters is the favorable oxygen transfer to the fungi. So far, various methods have been used to overcome this problem, such as the use of oxygen carriers, the use of bubble columns and reducing the viscosity of the environment. Among these factors, it seems that reducing the viscosity of the environment has the greatest contribution to the effective transfer of oxygen at large scale. For this reason, this paper examines the factors affecting the morphogenesis of *Aspergillus terreus*, which plays a role in the viscosity of the environment

Methods

For data collection in this review article, the articles containing one of the words "cholesterol", "vascular stiffness", "morphogenesis", "lovastatin", "*Aspergillus terreus*", and "inducer" between the years 1960 and 2017 were searched and studied in Pubmed, Scopus, Science Direct and Islamic World Science (ISC) databases. Of the papers found in the various databases, 57 articles related to the role, relationship and effect of carbon, agitation, viscosity, aeration, inoculum and inducers on the morphogenesis of *Aspergillus terreus*, and the production of lovastatin and secondary metabolites were selected and others articles that did not relate to the subject of this study were excluded from the study.

Results

Morphogenesis growth of *Aspergillus terreus*: Morphogenesis depends on the factors related to microbiology, including the fungus strain, the onset of culture, the nature of the culture medium, as well as physicochemical factors such as breaking or cutting forces, detergents, pH, temperature, ion strength, aeration, etc. (26–28). There are various morphogenesis factors for fungi and actinomycetes in a liquid medium (29). In liquid culture, fungi can grow in the form of free mycelia or particular morphogenesis called pellets (29,30).

The accumulation of mycelia in the form of a dense mass creates a particular morphogenesis called pellets. Razeghi Yadek et al. reported that the combination of culture medium, pH and temperature is effective on the growth rate of shiitake mushroom, while low pH 4.5 and a temperature of 25 °C results in the highest growth rate of the mushroom (31). The effect of the variety of organism morphogenesis on the fermentation process has been extensively investigated (26, 32,33).

König et al. promoted the comparative performance of pellets and mycelia in the production of penicillin in the air column (34). Takahashi et al. reported a significant difference in viscosity between mycelia and pellets in penicillium chrysogenum (35). Kumar et al. reported that the growth of compact pellets in *Aspergillus terreus* produces more lovastatin compared to mycelial and filamentous growth (12).

Concentration of spore: Gbewonyo et al. reported that the initial concentration of spore and aeration rate determine pellet formation (29). Since the number of spores is effective on the biomass, the number of spores should be carefully kept to a certain level so that the results of the experiments are repeatable, and an average of about 10^7 spores/ml should be inoculated into the culture medium (36). At low concentration of spores, small quantities of mycelium accumulate and small diameter pellets are obtained. At high concentrations of spores, large quantities of mycelium accumulate and the diameter of the pellets is large. At very high concentrations, the size of mycelia is small and accumulation does not take place (37).

Carbon source: There is a correlation between the carbon source of lovastatin production and morphogenesis changes (38). The target culture media for the production of lovastatin are classified into two types of synthetic medium and complex medium. The carbon sources are classified into two categories of readily metabolized carbon sources and slowly utilized carbon sources. The use of fast metabolizable carbon sources leads to an uncontrolled mycelium growth (12, 39 and 40). Moreover, these types of carbon sources cause catabolic suppression of the secondary metabolites and, therefore, reduce the production of lovastatin (38).

When using slowly utilized carbon sources, morphogenesis is structurally more compact, regular, and smaller, and it is beneficial for the production of secondary metabolites, resulting in more lovastatin production with slowly utilized carbon sources (38). The second type of culture media for the production of lovastatin are compound culture media. Jaberi Ansari et al showed that the production of lovastatin in compound media is more than three times than the synthetic culture medium containing glucose, and the pellets formed in this medium are smaller than the pellets formed in the synthesis medium (41).

Jaberi Ansari et al. in 2015 used the date syrup for the first time as a carbon source for the production of recombinant β-NGF protein and showed that the growth of microorganisms and the production of recombinant β-NGF protein increases during the use of date syrup (42).

According to Tavana et al., maltose sugar leads to the largest biomass production and extracellular polysaccharide by the *ganoderma lucidum* fungus (43). Azizi et al. also showed that the highest rate of sporulation efficiency was obtained when using hornbeam bran (44).

Stirrer's speed: The stirrer's speed is one of the factors affecting the diameter of the pellet. The stirrer's speed is considered as an oxygen supply for growth of the fungus and results in higher levels of lovastatin.

According to Flickinger et al., one of the reasons for reducing the production of lovastatin at high velocities compared to lower speeds is the collapse of the pellets structure (45). In a stirred-tank bioreactor, at 300 rpm aeration, pellets with a diameter more than 2300 µm are stable. However, at 600 rpm aeration, pellets with a diameter of less than 900 µm are stable. The high shearing force of the stirred-tank bioreactor (usually more than 600 rpm) reduces the length of the pellets to less than 900 µm. With increasing speed from 300 rpm to 800 rpm, the pellet diameter decreases (18) (Fig 2).

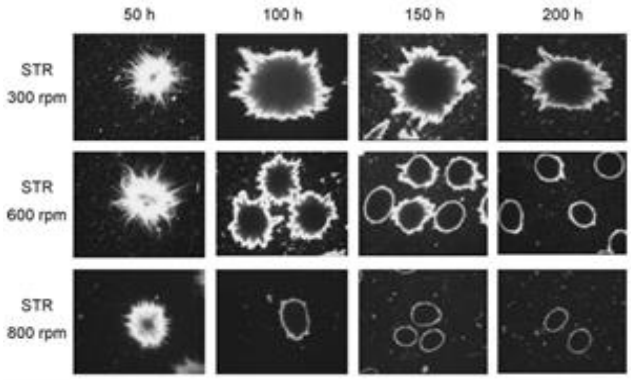

Figure 2. Represents various pellet morphogenesis in the synthetic bioreactor at different speeds and hours

Aeration: Oxygen is important for the formation of metabolites that are dependent on oxidoreductase. In the production of lovastatin, there is one oxidation step performed by Cytokrom 450p (46,47). The highest levels of lovastatin production are achieved by inoculating spherical pellets and 70% oxygen dissolution in the bioreactor. When the oxygen content of the solution reaches about 80% saturation in the bioreactor, the opportunity for shearing and breakdown of mycelium is provided by high aeration rates, and when the oxygen content of the solution is lower than 20-35% saturation, oxygen is limited to the biosynthesis of lovastatin (15).

In addition, the diameter of the pellets and the concentration of the pellets are both influenced by aeration rate (37). The use of pure oxygen causes the pellets to change. The softness, compressibility and diameter of the pellets are also affected by aeration and stirring speed (18). The pellets are small at first, but they get thick when they receive the pure oxygen (48). The results show that the rate of absorption of oxygen in the fermentation of the pellet is not sensitive to the stirring speed and is equal to 20 mM/l.h. However, in the fermentation of mycelia, oxygen absorption rate is sensitive to the stirring speed. The concentration of dissolved oxygen in the pellet fermentation is sensitive to the change in stirring speed, but on the contrary, the dissolved oxygen concentration in the mycelium fermentation is about 0% (29) (Fig 3).

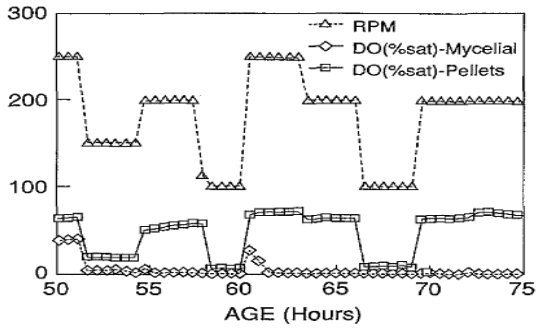

Figure 3. The response of dissolved oxygen to RPM

Inducers: Inducers are materials that affect the enzymes and increase the desired product (49). Gonciarz et al. reported that adding vine to the *Aspergillus terreus* medium produces more compact pellets and produces more lovastatin (50). Quorum sensing materials are also a type of inducer that is relevant to the population of microorganisms. Quorum sensing molecules include Acyl homoserine lactone, gamma-Butyrolactone, and small peptides in bacteria and oxylipins, farnesol and butyrolactone I in fungi (51-53). *Aspergillus terreus* has the ability to produce butyrolactone I (54). Butyrolactone I acts as an induction of morphogenetic changes and, as a result, the growth of spores and lovastatin. The use of slowly utilized carbon sources further secretes this substance (55, 56). Adding 100 nM butyrolactone I doubles the production when cell density is high (stationary phase) (54). Linoleic acid also acts as a signaling molecule in cell-cell communication, and increases the production of lovastatin in *Aspergillus terreus* (57).

Discussion

An important issue in the pharmaceutical industry is how giant bioreactors can hold the concentration of secondary metabolites, including lovastatin, at the same level as laboratory scale production. Because on a laboratory scale, tests are usually carried out at the level of small Erlenmeyer and 2 – 30 L bioreactors, which eases the oxygen transfer to microorganisms and the concentrations of production are high. However, when the scale rises and reaches larger generators in the sizes of 1,000 – 150,000 cubic

meters, the production concentration decreases in proportion to the increase in scale. Since the concentration of oxygen in the bioreactor is gradually reduced, it makes it difficult to supply the microorganism with oxygen. In this review article, the factors affecting the viscosity of the medium, which play the most important role in the delivery of oxygen to microorganisms, have been investigated.

The most important factor in the viscosity of the medium is the morphogenesis of microorganism growth. As mentioned above, the *Aspergillus terreus* is a filamentous fungi and therefore, has various morphogenesis in the liquid medium. According to the studies by Jaberi Ansari et al., the viscosity of the media in pellet morphogenesis is much more reduced compared to mycelial morphogenesis (41).

This is entirely consistent with other studies, such as the results of König et al. (34), or Kumar et al. (12) on different morphogenesis in the filamentous fungi to increase the production of secondary metabolites. As mentioned earlier, spore concentration is one of the effective factors in the morphogenesis of pellet. Jaberi Ansari et al., in 2017 showed that the use of older spores (25 and 175 days old) produced about two times more lovastatin than inoculated spores (10 days old) (58). Today, instead of *Aspergillus terreus* spores, newly formed pellets are used for inoculation, thereby reducing the duration of the fermentation process, since formation of spores and becoming a vegetative form takes several days. Moreover, the formation of a vegetative form as pellet morphogenesis is assured, since only the inoculated pellets grow, but at the time of spore inoculation, a spectrum of morphogenesis from mycelium to compressed pellets is obtained (25). Studies of Jia et al. showed that the type of carbon source plays an important role in the morphogenesis of mycelium and pellet (38).

In addition, Jaberi Ansari et al. also found that the type of carbon source such as date syrup, could also affect pellet size, in addition to its formation. Small pellets grow in date syrup medium, while in synthetic medium containing glucose, pellets are produced in larger sizes. The morphogenesis of small pellets reduces viscosity of the liquid phase and provides better oxygen transfer.

On the contrary, the mycelial network structures create high-viscosity newtonian solutions that prevent the oxygen transfer. Therefore, this study suggests that, in addition to the formation of pellets, pellet size also plays a significant role in the production of lovastatin, resulting in a threefold production of this drug using small pellets compared to larger pellets (41). In addition to the aforementioned issues, the stirring speed and aeration are also effective in the production of lovastatin. The highest levels of lovastatin production were obtained in a date syrup medium at 150 rpm, according to the studies by Jaberi Ansari et al., which is a relatively low speed (41). This is contrary to most research results, which indicates that the relatively high stirring speed or aeration increases the production of lovastatin (18).

The reason for this is not that the stirring speed and aeration do not affect the production of lovastatin, but rather this is because the type of carbon source has a greater effect on morphogenesis, and the effect of morphogenesis on viscosity and oxygen transfer is greater than that of oxygen transfer by stirring speed and aeration. This team also investigated the effect of linoleic acid as an inducer on the production of lovastatin and the morphogenesis of *Aspergillus terreus* in a date syrup medium and found that the production of lovastatin increased by 1.5 fold (58), which is similar to the study of Sorrentino et al. on the positive effect of inducers the production of lovastatin. However, the growth of the pellets did not change significantly, which suggests that carbon sources affect the morphogenesis of fungus more than inducers (57).

In addition to the factors affecting morphogenesis and viscosity of the medium, other factors such as nitrogen sources, the C/N ratio, pH and mineral elements also affect the production of lovastatin, which was studied by Jaberi Ansari et al. (21). Finally, it can be said that the most important factor for the morphogenesis of pellet is carbon source.

After the carbon source, the concentration of spores at 10^7 spores per milliliter, high stirring speed at around 600 rpm, aeration of 70% saturation and the use of inducers such as magnesium silicate hydrate, methionine, butyrolactone and linoleic acid affect the formation of pellets, reduce the viscosity of the medium and increase the oxygen transfer to the *Aspergillus terreus* and thus increase the production of lovastatin.

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