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PART 1:

Journal Name:	British Microbiology Research Journal
Manuscript Number:	MS: 2012 BMRJ 2815
Title of the Manuscript:	Induced; thermal influence on morphology of <i>Aspergillus carbonarius</i> during raw starch digesting amylase production

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(<http://www.sciencedomain.org/page.php?id=sdi-general-editorial-policy#Peer-Review-Guideline>)

- This form has total 9 parts. Kindly note that you should use all the parts of this review form.



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PART 2: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
Compulsory REVISION comments	Recommendation and Comments This manuscript was described about "Induced; thermal influence on morphology of <i>Aspergillus carbonarius</i> during raw starch digesting amylase production". I think that this manuscript may be acceptable for British Microbiology Research Journal. But, I think that you should be improved the following points. 1. In Fig. 1: You should be added the explanation about a, b, and c. I cannot understand nothing about "a, b, c". 2. In Fig. 2: You should be added	In (Figure 1) the author tried to show that all forms of pellet morphology was expressed. Braun and Vecht-Lifshitz (1991) Wucherpfennig <i>et al</i>/ (2011) described the various pellet forms in filamentous production although the relationship between morphology and productivity is not clear. In figure 2 authors noted that compared to figure 1



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	the explanation about a, b, c, d, and e. I cannot understand nothing about “a, b, c, d and e”. 3. In Fig. 3: You should be added the explanation about a, b, c, d, and e. I cannot understand nothing about “a, b, c, d and e”.	above at an increased temperature (37°C) there appeared to be reduction in dense packing of hypha. Under this submerged cultivation of raw starch digesting amylase enzymes from <i>Aspergillus carbonarius</i>, pellets structures were fluffy with much loose outer zone as shown in Figure 2 (b and c) or fluffy with compact centre as shown in Fig. 2 (a, c and e). In this regard submerged cultivation temperature may have affected pellets structures Fig. 3 shows sheared off hairy parts of <i>Aspergillus carbonarius</i> pellets from a submerged raw starch digesting amylase fermentation at 37°C. It is a well known fact that agitation results in fragmentation of mycelia hypha, in addition to fragmentation induced by agitation, Figure 3 shows that mycelia fragmentation could also result from increased temperature, authors attributed this condition to increased vacuolation levels (Paul <i>et al</i> 1994, Papagianni <i>et al</i> 1999). Where hyphal vacuolation a natural process of aging may be induced by increase in temperature resulting in weakening of
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	In Fig. 4: You should be added the explanation about a, b, and c. I cannot understand nothing about “a, b, c”. 4. Why did change the morphology of <i>A. carbonarius</i> by the shifting the temperature from 27°C to 37°C? You had better add biochemical discussion about it.	the filaments and exposing them to damage. Also conditions such as glucose supply rate or oxygen limitation as well as pH may have induced fragmentation Havey <i>et al</i> (1998). Fig 4 (a,b,c)shows sheared off hairs from the surface of the pellets (at 37°C) coming together. The morphological properties of filamentous fungi play a significant role in metabolism during fermentation process (Nielson <i>et al</i> 1995, Johansen <i>et al</i> 1998, Paul <i>et al</i> 1999). However, the reason behind the coming together of the shaved off hairs is not clear at present. It is possible that agglomeration observed is likely to be as a result of swirling speed of (100 rpm) applied. The change that resulted in the morphology of <i>Aspergillus carbonarius</i> by the shift in temperature from 27°C to 37°C can be explained using the mechanism that occurs in dimorphic fungi. Dimorphic fungi are known to express phase transition due to numerous factors. External stimuli change intracellular signaling, metabolism and gene expression in dimorphic fungi
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	5. In Fig. 6: RSDA activity was not	triggering phase transition, other stimuli such as oxidative stress, changes in carbon dioxide tension and steroid hormones also regulate morphogenesis. In spite of all these temperature change is a major environmental stimulus that triggers (morphogenesis) the phase transition from mould (25°C) to yeast (37°C). Although these factors are associated with dimorphic fungi the same mechanism may apply for the resulting changes in pellet morphology under induced increase in temperature. Further more changes in membrane fluidity and lipid composition have been said to contribute to morphological transition i.e. as cells adapt to changes particularly temperature multiple changes occur in the lipid composition of the plasma membrane leading to remodeling and reorganization in the membrane (Maresca and Kabayashi 200, Gauthier and Klein 2008) lower temperatures decrease membrane fluidity, which is compensated by a decrease in the ratio of saturated fatty acid to unsaturated fatty acid the reverse being the case for higher temperature. This may eventually bring about change in cell shape and cell wall composition and may also account for the high compact nature of pellets at 27°C and fluffy nature of pellets at 37°C. The histidine kinase two component system have been shown to play a role in environmental sensing and cell development in eukaryote (Santos and Shiozaki 2001) Protein kinases mediate the phosphorylation that
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	altered in both 27°C and 37°C, but the morphology of these mycelia (in 27°C and 37°C cultured mycelia) is different. Why was <i>A. carbonarius</i> changed the morphology? You should be biochemical discussion from results of Fig. 6.	regulates protein function directly, or via signal transduction, in many areas of cell metabolism (McIntyre et al 2001). They are also essential in linking signal transduction cascade, protein modification and fungal morphogenesis. The variation in the morphology of <i>Aspergillus carbonarius</i> as a result of increase in temperature may be associated to the activity of the protein kinases. In filamentous fungi and in eukaryotes the protein kinases that phosphorylate either serine or threonine (Ser/Thr kinases) are mostly the cAMP-dependent kinases (PKA), protein kinases type C (PKC), mitogen-activated kinases (MAP), and p21-activated kinases (PAK) (Dickman and Yarden 1999). Of all these the PKAs are said to play important role in fungal development (McIntyre et al 2001). Since the PKAs play important role in fungal development they may be responsible for the change in the pellet morphology while still enabling the fungi retain their ability to secrete enzymes which brought about the unaltered enzyme activity in the different temperature (27°C to 37°C) conditions.
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Minor REVISION comments		
Optional/General comments		