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Addressing the mycotoxin deoxynivalenol contamination with soil-derived bacterial and enzymatic transformations targeting the C3 carbon

Y.I. Hassan and T. Zhou*

Guelph Research and Development Centre, Agriculture and Agri-Food Canada, 93 Stone Road West, Guelph, Ontario N1G 5C9, Canada; ting.zhou@agr.gc.ca

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REVIEW ARTICLE

Abstract

The search for feasible biological means of detoxifying mycotoxins has attained successful accomplishments in the past twenty years due to the involvement of many teams coming from diverse backgrounds and research expertise. The recently witnessed breakthroughs in the field of bacterial genomics (including next-generation sequencing), proteomics, and computational biology helped all in shaping the current understanding of how microorganisms/mycotoxins/environmental factors intertwined and interact together, hence paving the road for some substantial discoveries. This perspective review summarises the advances that were observed in the past two decades within the deoxynivalenol (DON) bio-detoxification field. It highlights the research efforts and progresses that were made in the arena of the aerobic oxidation and epimerization of this mycotoxin at the C3 carbon carried out by multiple *Devosia* species. Moreover, it sets practical examples and discusses how the recent standing-knowledge of bacterial detoxifications of this mycotoxin has evolved into a fascinating potential of empirical bacterial and enzymatic solutions aiming at addressing DON contamination. The obtained results argue for determining the involved enzyme's co-factors and defining the chemistry behind the established catalytic activity at an early stage of investigation to maximise the chances of isolating the responsible enzymes.

Keywords: fungal toxins, bio-detoxification, vomitoxin, *Devosia*, enzymes

1. Introduction

Mycotoxins are among the most challenging contaminants to address in food and feed matrixes (Doll and Danicke, 2011; Overberg, 2009). Most of these compounds are stable under average food/feed processing conditions (temperature and pH) (Kaushik, 2015; Park, 2002) and all exert unwanted harmful influences that range from acute to chronic toxicities (Payros *et al.*, 2016; Pestka, 2010) to even carcinogenic ones (aflatoxins and patulin as examples) (Iwahashi *et al.*, 2006). The recently witnessed changes in the global climate are being increasingly associated with elevated levels of mycotoxins in raw food and feed commodities worldwide. Despite that fact that the past three decades noticed a plethora of research efforts related to the characterisation and the detection of these fungal secondary-metabolites; the need for reliable, cost-efficient, and robust strategies of mycotoxin control/mitigation still exists at large (Zhu *et al.*, 2016b, 2017).

Among the visited approaches of mitigation are the biological ones where certain microorganisms degrade the secreted mycotoxins and use them as energy sources. Such a theme is very common among non-fastidious soil and environmentally-derived microorganisms that utilise many organic and non-organic compounds as energy mines (Douglass *et al.*, 2017; Horemans *et al.*, 2016; Zhu *et al.*, 2016a).

The search of mycotoxin-degrading/metabolising microorganisms dates back to the late 1960's and early 1970's (Ciegler *et al.*, 1966; Lillehoj *et al.*, 1967, 1971) and throughout the years many microorganisms were reported to interact with different classes of mycotoxins with functionalities ranging from the catabolic degradation of

mycotoxins (Hassan *et al.*, 2017; He *et al.*, 2010; Karlovsky, 2011; Shima *et al.*, 1997; Volkl *et al.*, 2004) to the cellular adsorption/binding of the screened toxins (Hassan and Bullerman, 2013; Hassan *et al.*, 2015b; Niderkorn *et al.*, 2006; Zoghi *et al.*, 2014). In many cases and due to the absence of a confirmatory data, such as the detection of newly appearing metabolites within the test matrix/media, one would expect that most of these reported degradations can be categorised under the cellular adsorption phenomenon (El-Nezami *et al.*, 2002; Franco *et al.*, 2011). In fact, some rigorously conducted studies have called to include reagents of surface-tension reduction, such as sodium dodecyl sulphate, to minimise the interference of bacterial cell-wall binding with the successful screening of bacterial isolates for their mycotoxin inactivation/detoxification capabilities (Volkl *et al.*, 2004).

The success rate of identifying microorganisms that are capable of degrading or detoxifying mycotoxins is usually dependent upon: (a) the chemical nature of targeted mycotoxin(s) and (b) how urgent such a biological transformation is needed (compound's carcinogenicity as the case of aflatoxins for example puts higher emphasis) and (c) if other alternative strategies of control exist (such as the presence of natural clays or synthetic polymers for the specific binding/adsorption/inactivation) of the targeted mycotoxin. Many promising bacterial and enzymatic solutions were reported targeting aflatoxins, fumonisins, and ochratoxins as a result of past research efforts (Abrunhosa *et al.*, 2010; Burgess *et al.*, 2016; Chang *et al.*, 2015; Dobritzsch *et al.*, 2014; Hartinger *et al.*, 2011; Heinl *et al.*, 2010; Rawal *et al.*, 2010; Taylor *et al.*, 2010; Varga *et al.*, 2005; Wang *et al.*, 2011; Wu *et al.*, 2009). The engagement with deoxynivalenol (DON) from an early stage was inevitable due to its wide prevalence (the most detected agricultural mycotoxin worldwide) and its chemical nature (small polar moiety) that makes it difficult to identify or develop adsorption reagents which can irreversibly bind this mycotoxin. The above two factors made DON a challenging yet an interesting target for many innovative research explorations aiming at finding feasible and sustainable biological solutions to address DON accidental contamination in food and feed chains.

2. A growing number of scientific studies detailing the microbial transformation of deoxynivalenol

The field of biological detoxifications of DON was established by the early observations that tracked DON toxicity in farm animals (ruminants and poultry more specifically). Earlier studies reported the existence of a defence mechanism(s) within rumen fluids that protected these hosts from the adverse effects of low DON doses (Kiessling *et al.*, 1984). Research efforts were later intensified to highlight the involved detoxifying mechanism(s) and eventually led to the isolation of BBSH 797, a bacterial strain that degrades DON anaerobically to its de-epoxy form [DOM(-1)] (Fuchs *et al.*, 2000, 2002). More microorganisms with DON to DOM(-1) capabilities were reported later from chicken digesta and other sources (Li *et al.*, 2011; Yu *et al.*, 2010). The anaerobic nature of DON deepoxydation confined its usage to certain applications and stipulated the rational and motivation for investigating alternative bio-detoxifications that proceed under aerobic conditions.

To date, several DON transformation mechanisms under aerobic conditions have been reported (Figure 1) including:

a. The aerobic deepoxydation of DON to DOM(-1) where two mixed cultures capable of achieving this type of detoxification in the presence of oxygen were reported (He *et al*., 2016b; Islam *et al*., 2012).
b. DON hydroxylation at the C16 position to form 16-HDON (Ito *et al.*, 2013).
c. DON simultaneous epimerization and deepoxydation resulting in 3-*epi*-DOM(-1) formation claimed to take place under aerobic conditions (Vanhoutte *et al*., 2017).
d. DON aerobic oxidation at the C-3 carbon to form 3-keto-DON (Shima *et al.*, 1997; Volkl *et al.,* 2004).
e. DON epimerization at the C-3 position to form 3-*epi*-DON (He *et al*., 2015a, 2016a; Zhou and He, 2009, 2010).

While all of the above transformations are considered promising for the development of mitigation strategies, each reported mechanism comes with unique advantages and challenges. For example, the aerobic deepoxydation of DON to DOM(-1) is still under scrutiny as no pure single bacterial strain capable of achieving this detoxification was obtained. The studies that reported the above activity indicated the presence of multiple bacterial genera in each mixed culture and attributed the deepoxydation activity to either facultative anaerobes, such as *Citrobacter* and *Clostridium* (Islam *et al.*, 2012), or to strictly anaerobic species (Villemur *et al.*, 2006), such as *Desulfitobacterium* (He *et al.*, 2016b). Our prediction is that a bacterial synergy is a must for the aerobic deepoxydation of DON to take place where one or more species possibly influences oxygen availability, while the other(s) completes the reductive deepoxydation of DON.

Recently, there have been a few excellent reviews covering mycotoxin bio-detoxifications at large scope (Karlovsky, 2011; McCormick, 2013; Tian *et al.*, 2016; Zhu *et al.*, 2016b, 2017), the review presented here was purposefully focused to highlight research efforts (and progresses) that were made in the arena of the aerobic oxidation and epimerization of DON at the C3 group carried out by multiple soil microorganisms, mainly belonging to the Gram-negative genus *Devosia*.

Shima *et al.* (1997) presented the earliest report of DON oxidation to 3-keto-DON by a soil bacterial strain E3-39,

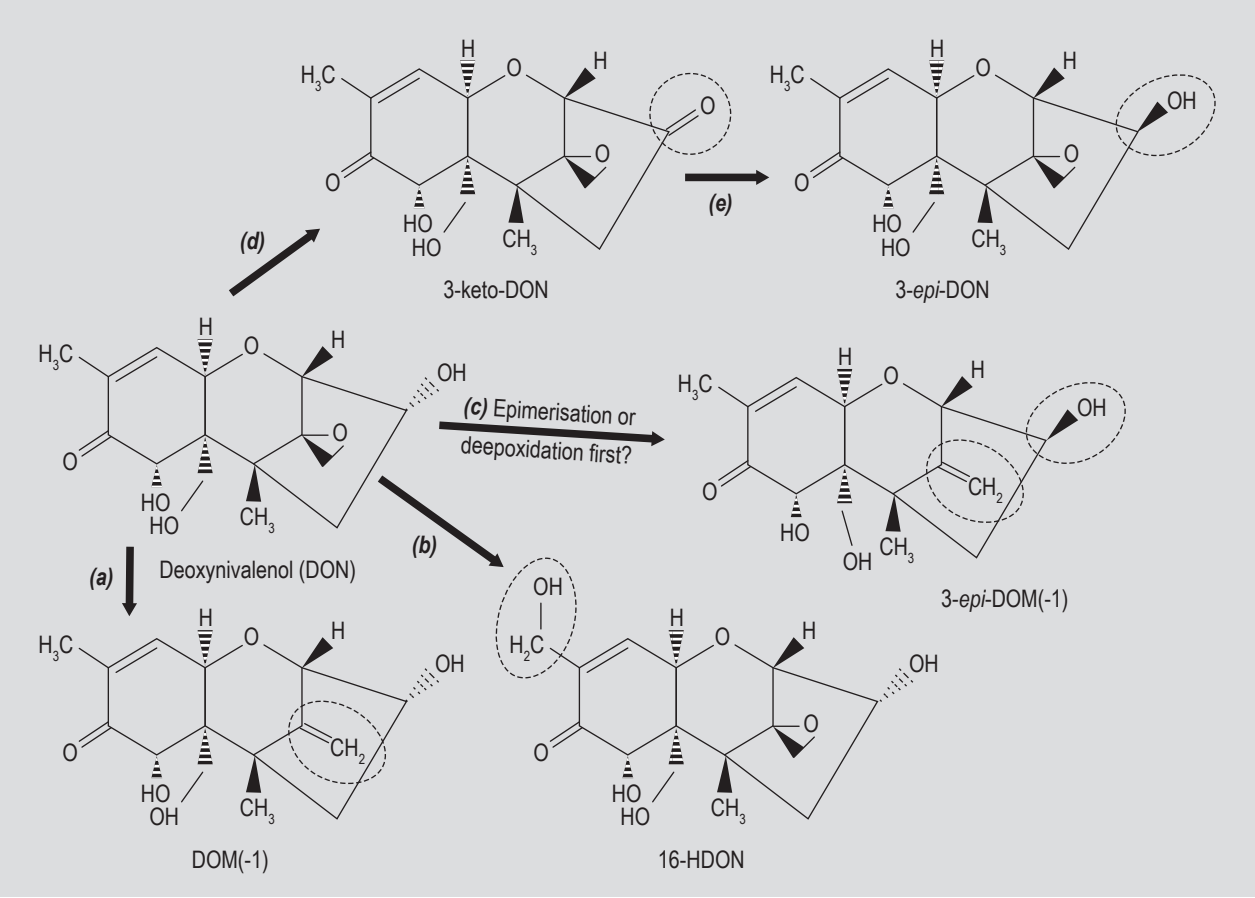

Figure 1. The bacterial detoxification of deoxynivalenol. The reported bacterial transformations of deoxynivalenol (DON) including: (a) DON anaerobic and aerobic deepoxydation, (b) the formation of 16-hydroxy-DON, (c) the simultaneous deepoxydation/epimerization of DON to 3-*epi*-DOM(-1), (d) the oxidation of DON to 3-keto-DON coupled with (e) a subsequent selective reduction to 3-*epi*-DON.

thought to belong to the *Agrobacterium-Rhizobium* group. The report also demonstrated a reduced toxicity profile of the resulting DON derivative (3-keto-DON) when tested using mitogen-induced and mitogen-free proliferations of mouse spleen lymphocytes (Shima *et al.*, 1997). The identity of the aforementioned bacterium was elusive until a more recent study confirmed through rigorous 16S rRNA comparisons that this isolate might indeed belong to the *Devosia* genus (Sato *et al.*, 2012). A mixed bacterial culture, designated as D107, originating from a soil source and capable of oxidizing DON to 3-keto-DON was reported years later in Germany (Volkl *et al.*, 2004). Unfortunately no definite answer about the identity of the responsible bacteria within that mixed culture was obtained at the time of the study even though an affiliation to the *Devosia* genus was suggested in a later date (Karlovsky, 2011).

The first *Devosia* species capable of completely epimerizing DON to 3-*epi*-DON was isolated by our research team from a soil sample collected from an alfalfa field (He *et al.*, 2016a; Zhou and He, 2009, 2010). The extensive research efforts that pioneered screening numerous soil samples obtained from different agricultural locations across Ontario (Canada) in a search for an aerobic transformation mechanism(s) of DON, successfully led to the isolation of the 17-2-E-8 strain, which was later identified as *D. mutans* 17-2-E-8 (He *et al.*, 2016a). Simply through applying a selective pressure on the naturally present microflora by growing in the presence of artificially high levels of DON, similar to what was pursued in earlier studies (Islam *et al.*, 2012; Yu *et al.*, 2010), the most efficient DON-detoxifiers were isolated.

The C3 epimerization was assumed for a while to be a two-step enzymatic detoxification that encompasses a first-step oxidation of DON to 3-keto-DON, shared with the two isolates reported earlier (Shima *et al.*, 1997; Volkl *et al.*, 2004), and a second-step of selective reduction of 3-keto-DON to 3-*epi*-DON (Karlovsky, 2011). The aforementioned hypothesis circulated for years before it was proven most recently through chemically synthesising 3-keto-DON and tracking DON epimerization and 3-keto-DON reduction activities in *D. mutans* 17-2-E-8 (Hassan *et al.*, 2017). Hassan *et al.* (2017) further provided the evidence that 3-keto-DON was the main intermediate for 3-*epi*-DON

accumulation in many other *Devosia* species/strains (Hassan *et al.*, 2017).

The industrial interest in any reported bacterial detoxification of mycotoxins ameliorates unless the final metabolites are defined, toxicity-wise. The characterisation of bio-detoxification metabolite(s) is pivotal in order to sort a genuine detoxification process from a bacterial transformation that does not affect the toxicity of the targeted toxin hence in-depth toxicological studies are always necessary. The example of the early reports of promising bacterial transformations of zearalenone to zearalenol (El-Sharkaway *et al.*, 1991), which was reported to be more oestrogenic in much later times, comes into mind within this context.

Due to the aforementioned fact, the years that followed the dissemination of DON epimerization as an established bacterial transformation (He *et al.*, 2016a; Zhou and He, 2009, 2010) witnessed a vibrant and focused work to purify and obtain enough quantities of the involved intermediates/final metabolites to embark on *in vitro* and *in vivo* toxicity testing. A robust high-speed counter-current chromatography (HSCCC) method was developed by He *et al.* (2015b) for 3-keto-DON and 3-*epi*-DON purifications, respectively. The yields of these critically-needed metabolites were promising (He *et al.*, 2015b) and led to the initiation of mammalian cell lines and mouse models toxicity-studies (He *et al.*, 2015a) aiming at elucidating changes in the toxicological profiles of the above DON-metabolites. The collected data confirmed the attenuation of the cellular and animal toxicity of 3-keto-DON (up to 5-fold reduction in toxicity) and the diminished toxicity of 3-*epi*-DON (50-fold reduction of toxicity in comparison to DON) (He *et al.*, 2015a). The above studies also confirmed the earlier conclusions of Shima *et al.* (1997) in regard to 3-keto-DON reduced toxicity.

Furthermore and based on the knowledge that the mycotoxin research community accumulated in regard to masked mycotoxins/DON-conjugates (such as 3-acetyl-DON), a careful approach to mechanistically understand the reduced toxicity of 3-*epi*-DON at both the cellular and molecular levels was pursued. The carried out experiments demonstrated that the epimerization of DON alters its ability to tightly bind the ribosomal peptidyl transferase centre (as the case of DON) hence diminishing its toxicity (Pierron *et al.*, 2016). Other studies reported changes in 3-*epi*-DON polarity and molecular interactions (Figure 2) with enzymes/cellular targets (Hassan *et al.*, 2016) adding to the emerging understanding of this stereoisomer overall toxicity (Payros *et al.*, 2016).

Throughout the years, more bacterial species/isolates originating from soil sources have been shown to contain the full DON epimerization pathway, including Gram-positives. Ikunaga *et al.* (2011) was able to successfully purify a bacterial culture from a soil sample that was collected from wheat fields and capable of aerobically epimerizing DON (Ikunaga *et al.*, 2011). The isolated bacterium, designated as strain WSN05-2, was classified as a Gram-positive bacterium belonging to the genus *Nocardioides* (Ikunaga *et al.*, 2011). Collectively, all the aforementioned studies with others (Sato *et al.*, 2012) indicated that DON oxidation/epimerization is possibly more common in soil microorganisms (Gram-negatives and positives alike) than it was previously perceived (Karlovsky, 2011; Sato *et al.*, 2012).

3. The *Devosia* genus and DON, heredity or a brief environmental encounter

Since the genome assembly of *Devosia mutans* 17-2-E-8 (Hassan *et al.*, 2014) was announced, the efforts aiming at understanding the involvement of the *Devosia* genus in DON detoxifications were growing. This was evident by the increasing number of novel *Devosia* isolates reported to detoxify DON (to known or unknown metabolites) (Onyango *et al.*, 2014; Yin *et al.*, 2016; Zhao *et al.*, 2016) in addition to the booming numbers of whole-genome sequencing projects related to such *Devosia* strains (Onyango *et al.*, 2014; Yin *et al.*, 2016). Likewise, the number of *Devosia*-related whole-genome sequencing assemblies has been increasing exponentially at the same time period. The National Center for Biotechnology Information (NCBI) genome-assembly database was harbouring close to 32 assemblies of various *Devosia* isolates by the time of writing this review (September 2017) which were all deposited between 2014-2017.

With the increasing number of scientific reports showing the involvement of many *Devosia* species originating from different geographic locations (Canada, Japan, Germany, and China among other places) in DON transformation, the question that became relevant is how common this oxidation/epimerization functionally is spread among members of this bacterial genus and what's unique about the transforming isolates in comparison to the non-transforming ones.

Hassan *et al.* (2017) investigated multiple *Devosia* type-strains for their abilities to support the full-epimerization of DON, which encompasses an oxidation step coupled with a reduction step as mentioned earlier, in addition to investigating the selective reduction of 3-keto-DON to 3-*epi*-DON separately. The presented findings argue that the epimerization functionality is much more limited to certain selected species compared to the selective reduction of 3-keto-DON to 3-*epi*-DON. This in return highlights the fact that the oxidation of DON to 3-keto-DON being the bottle-neck step for DON epimerization. Yet these findings do not explain why both E3-39 and D107 isolates

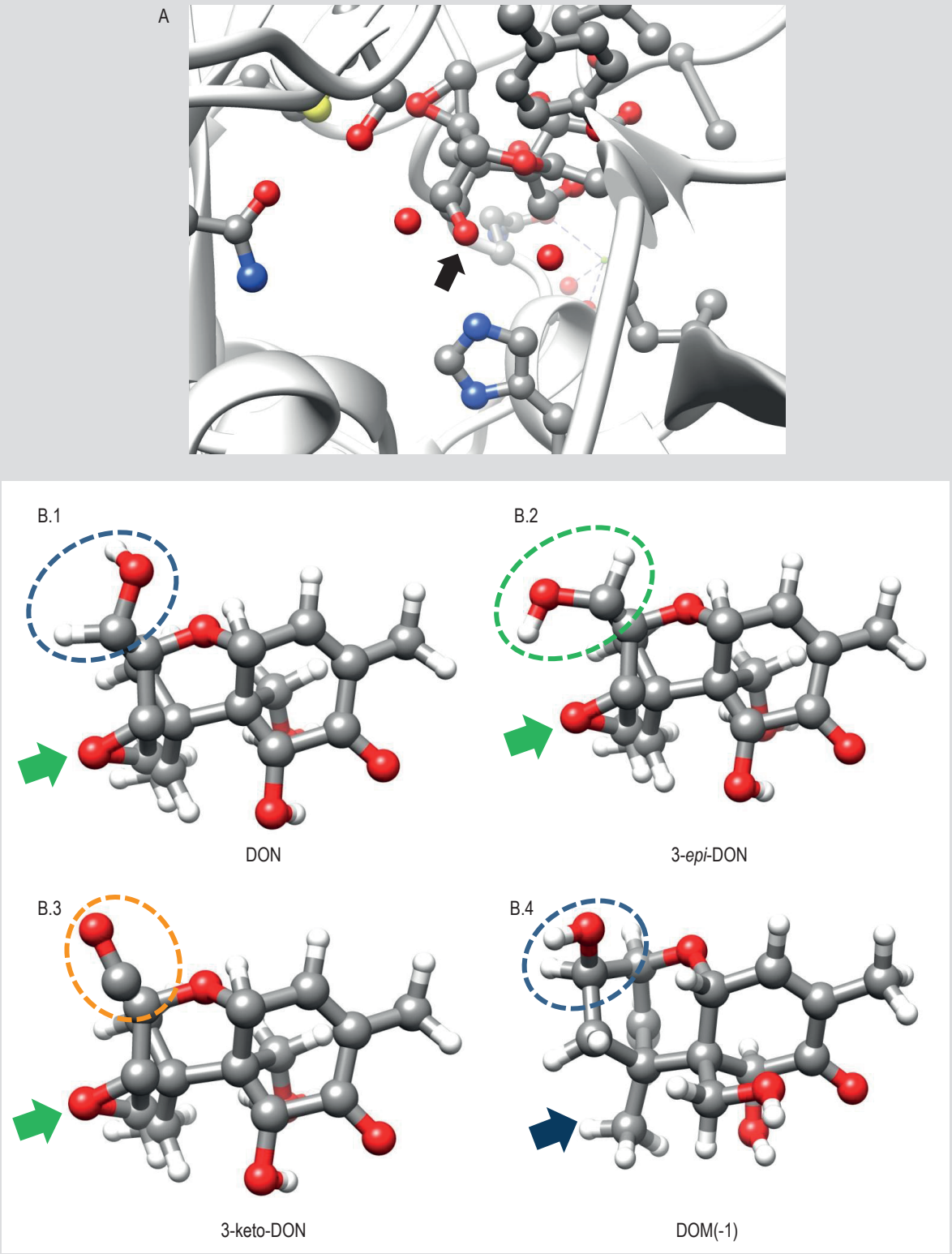

Figure 2. Deoxynivalenol molecular interactions and the chemical and structural changes that affect such interactions. (A) The orientation of C3-OH group (black arrow) significantly influences DON interactions with 3-O-acetyltransferase/Tri101 (Garvey *et al.*, 2008; Hassan *et al.*, 2016) in addition to affecting its binding within eukaryotic ribosomes (Pierron *et al.*, 2016). (B) Many chemical and structural changes (He *et al.*, 2016a) of DON including the C-3 group oxidation/epimerization (orange and green circles) or ring de-epoxydation (blue arrow) have been carefully scrutinised and connected with reduced cellular and molecular toxicity.

cannot perform the full epimerization of DON (Shima *et al.*, 1997; Volkl *et al.*, 2004).

Furthermore, the above results in coordination with the physical separation of the geographical locations of reported DON active transformers among the studied *Devosia* isolates indicate that the epimerization process is not a genetically inherited trait throughout a commonly shared ancestor but rather an environmentally acquired functionality that was obtained either through a horizontal gene-transfer (Philippe and Douady, 2003) or an induced genetic evolution (Michael, 2017). The fact that most of the reported *Devosia* isolates that can epimerize (or oxidise) DON originated from agricultural soils with possible earlier exposures to DON (through soil, roots, or fusarium-infected plants), affirms this assumption. Figure (3) shows the phylogenetic distribution of many of the recently reported *Devosia* isolates that transform DON, some of which cluster within the same phylogenetic clade, such as *D. mutans* (Canada) and *D.* SS5 and *D.* RS1 (Japan) despite originating from two different continents, yet surprisingly a closer isolate such as *D. yakushimensis* clusters in a different branch.

Finally, the *Devosia* genus did not only emerge as an outstanding DON bio-detoxifier (Hassan *et al.*, 2014; Onyango *et al.*, 2014; Yin *et al.*, 2016; Zhao *et al.*, 2016) in the recent years, but also attracted the attention as a reservoir for genes/enzymes that can potentially degrade organic pollutants, such as polychlorinated biphenyls (Papale *et al.*, 2017) or pass the resistance to salinity (Ramos *et al.*, 2015), hydrocarbons (such as hexachlorocyclohexane) (Dua *et al.*, 2013; Hassan *et al.*, 2015a; Kumar *et al.*, 2008; Verma *et al.*, 2009), and sulfadiazine (Ding *et al.*, 2014) in addition to its involvement in nitrogen-fixation (Bautista *et al.*, 2010; Rivas *et al.*, 2002; Vanparys *et al.*, 2005; Wolinska *et al.*, 2017) and phosphorus absorption (Zuo *et al.*, 2016). The last two traits make this soil-associated microorganism an attractive target for investigating the possibility of developing green biocontrol agents that can benefit the growing plants through colonising their roots/leaves and providing the protection from the damaging role which DON plays during *Fusarium* pathogenesis yet the same time aid in plant growth/soil-establishment through converting atmospheric N_2 into ammonia.

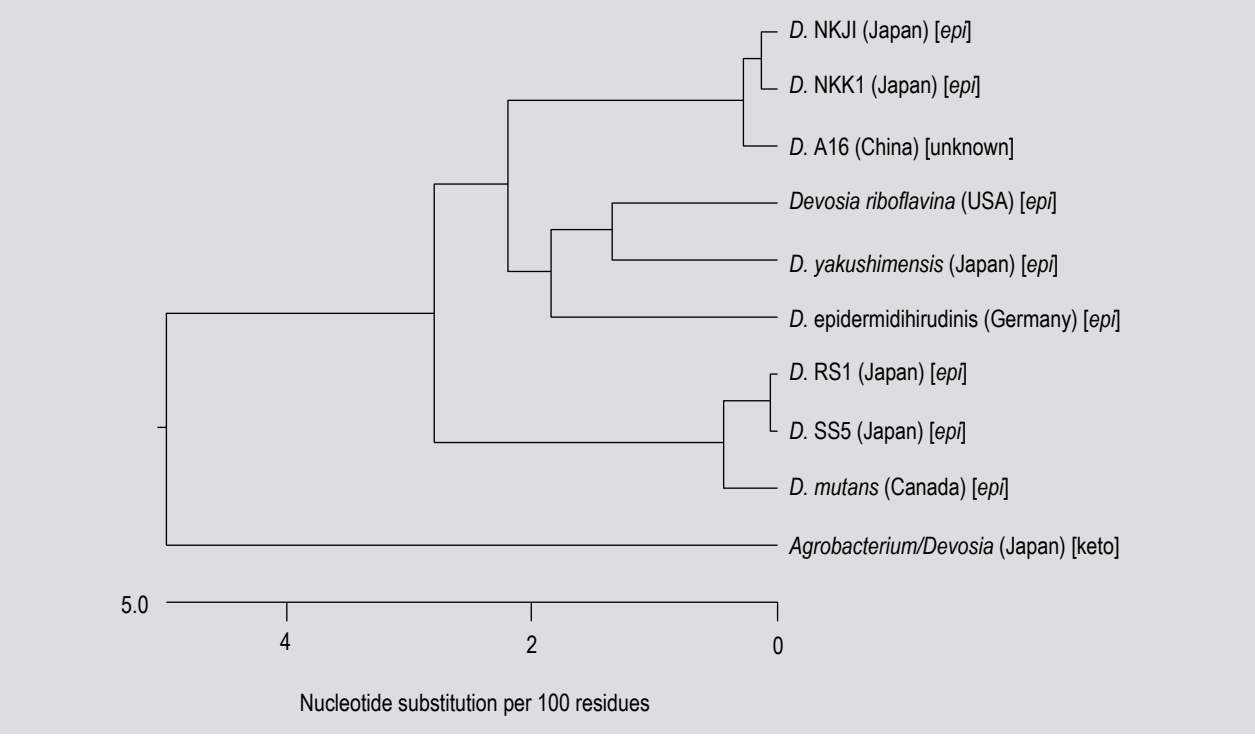

Figure 3. The phylogenetic analysis of different *Devosia* isolates reported for their deoxynivalenol detoxification functionalities (C3 oxidation/epimerization). The shown tree was established by accessing 16S rRNA sequences deposited in NCBI (https://www.ncbi.nlm.nih.gov) and using DNASTAR-MegAlign software package. The presented data argues that the observed DON detoxification functionality is likely to be an environmental adaptation of the reported bacterium to utilise DON as an energy source due to the bacterium existence within a bio-niche that contained DON rather than being an inherited trait from a common ancestor. This is evident by the sporadic clustering of isolates that were obtained from nearby geographical locations such as the *Devosia* NKK1/NKJI/RS1/SS5/*yakushimensis*.

4. Rushing the isolation of responsible enzymes

In addition to the promising applications that can be developed based on the utilisation of living-microorganisms in various agricultural fields, soil-derived bacteria with confirmed detoxification capabilities can be used also for the purification and identification of the responsible enzymes to develop recombinant-enzymes applications in a later stage.

Increasing evidence collected by hundreds of published manuscripts that scrutinised the functionality of enzyme(s) purified from soil bacteria suggests that a biochemically well-defined enzymatic reaction in terms of activity localisation (cellular, secreted, or membrane-bound) and cofactor [NAD(H), NADP(H), FAD(H), CoA, ATP, SAM, or PQQ] involvement is essential for successful purification outcomes.

The realisation that the enzymatic epimerization of DON carried out by *D. mutans* 17-2-E-8 was proceeding through a two-step fashion with the involvement of 3-keto-DON as intermediate (Hassan *et al.*, 2017) took some time to shape. Similar to the fact that the pyrroloquinoline quinone (PQQ) redox cofactor was essential for the first-step oxidation of DON to 3-keto-DON (Carere *et al.*, 2017). As soon as these two influential parameters were recognised, the purifications of the first- and second-step enzymes were instrumental (Carere *et al.*, 2017; Zhou *et al.*, 2017). Both of the identified enzymes reside within the soluble fraction of bacterial cell lysates with a confirmed dependency on PQQ (Carere *et al.*, 2017) and NADP(H) (Hassan *et al.*, 2017; Zhou *et al.*, 2017), respectively.

Using established molecular and biochemical approaches, a homologous enzyme was recently purified from a soil bacterium belonging to the *Sphingomonas* genus, where the bacterium is reported to accumulate 3-keto-DON as the major metabolite of DON in depleted cultures in addition to traces of 3-*epi*-DON (He *et al.*, 2017). While the purified first-step enzyme supposedly converts DON to 3-keto-DON using $NADP^+$ as a cofactor (rather than PQQ), the enzymatic efficiency of that homolog judged by the presented data was perceived low (He *et al.*, 2017).

5. The accumulated knowledge and the anticipated future challenges

The process of how a unique detoxification approach of DON using soil-derived bacteria had evolved and presented in this review (Figure 4) provides a template for some practical recommendations and technical observations

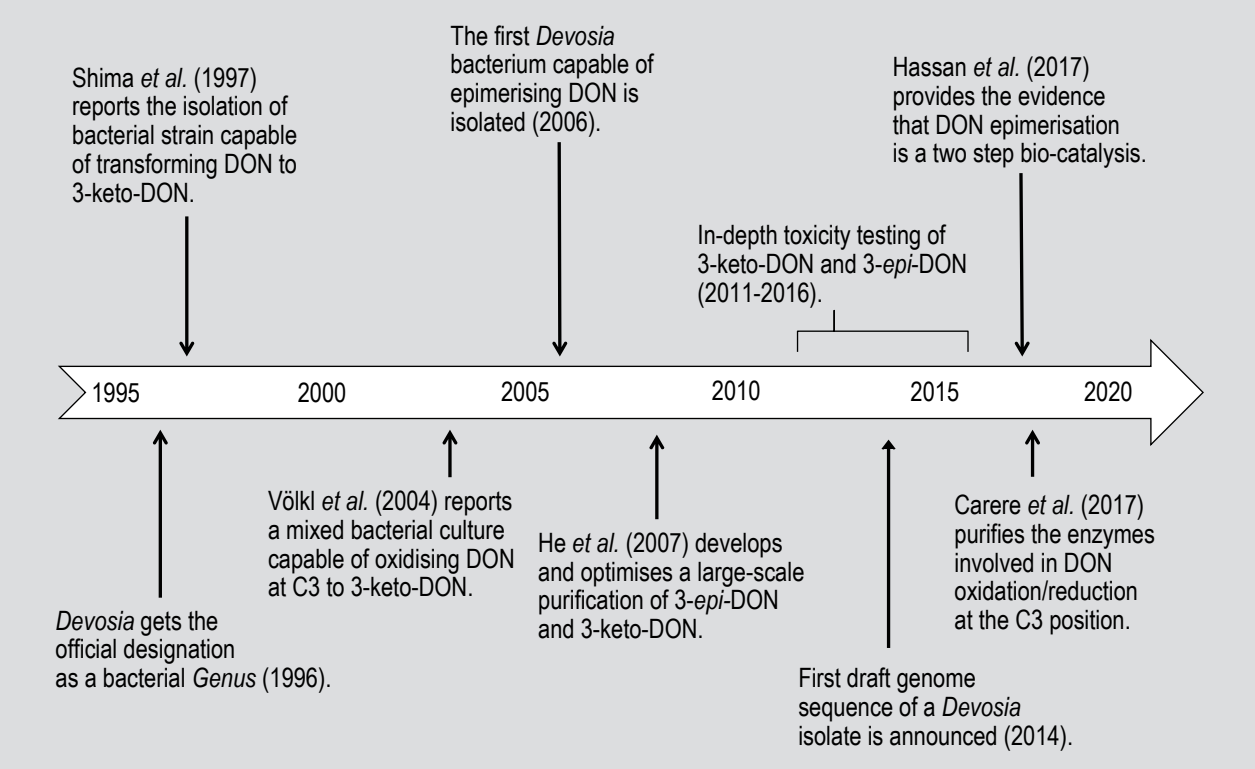

Figure 4. An outline for deoxynivalenol (DON)-C3 carbon oxidation/epimerization research as it progressed in the past two decades. The development of powerful microbiological/molecular/enzymatic tools set the field for interesting hallmarks in the arena of DON oxidation/epimerization spanning the isolation of first bacterium capable of oxidising DON (Shima *et al.*, 1997), the purification of 3-*epi*-DON and other metabolites (He *et al.*, 2015b; Zhou and He, 2009; 2010), the establishment of two-step reactions (Hassan *et al.*, 2017), to the discovery of the entire enzymatic systems responsible for DON epimerization (Carere *et al.*, 2017).

that were collected throughout the described research journey and shared by many talented research teams to shape the foundations of our current scientific knowledge within this arena.

Among the points to consider are:

1. Each successful bacterial screening to purify DON-detoxifiers almost exclusively started with an enrichment step of the involved soil samples with DON beyond the naturally found levels or alternatively used DON as the sole carbon source for bacterial growth. This in turn and as mentioned earlier selected for either microorganisms that tolerate DON (possibly through a detoxification mechanism) or microorganisms that possibly encountered DON in an earlier stage (pond, plant roots, and heavily *Fusarium*-infested maize/wheat fields).
2. Attention should be paid as early as possible to the involvement of enzyme cofactor(s). This is pivotal for each and every enzyme purification attempt especially for enzymes originating from soil sources. The soil by nature is a very rich environmental-niche and many metals and cofactors are recycled and reused throughout a dynamic state of exchange. In the case of DON first-step enzyme(s) responsible of its oxidation to 3-keto-DON, the enzyme was found to be a PQQ-dependent (Carere *et al.*, 2017). While this cofactor is commonly used by many soil bacteria, it's not readily available in *Escherichia coli,* which is considered as the standard/preferred screening host for many microbiology/molecular biology laboratories engaged in enzyme/protein overexpression. Enzymes demanding unique co-factors (such as PQQ in the above case) can be easily missed if a genomic-library screening approach was to be performed with *E. coli* as a host without a prior knowledge of the PQQ-dependency of such enzymes. Other teams shared similar experience/concerns (Ito *et al.*, 2013).
3. State-of-the-art techniques and research approaches, such as comparative genomics, genomic-libraries screening, and RNA_Seq transcriptomics are definitely attractive tools and the first to cross the mind in enzyme-mining projects, but investing into establishing a good understanding of the basic enzymatic mechanism(s) under investigation through conventional biochemical methods (heat stability, pH range, substrate specificity, inhibitors availability, etc.) pays off in a big time on the long run. These basic parameters can direct the entire purification procedure and give guided insights about the influence of such factors on the involved enzymatic functionalities and highlight how simple or complicated the entire purification process can be (one- or two-step detoxifications for example).
4. Time has its unique way of addressing issues and explaining ambiguities (including the scientific ones) and no matter of how an observation might look irrelevant or difficult to explain at a time, it can become a very vital piece of information in a later date. This feels particularly true when we look back to the work that we established to address substrate specificity, influence of inhibitors/competitors, and co-factor dependence of the first-step enzyme responsible for DON oxidation to 3-keto-DON.
5. While all teams, generally speaking, rush to highlight the industrial applicability of any reported bacterium or discovered enzyme(s), a critical scrutiny is surely needed to highlight the strength of the presented solution in addition to any possibly-needed downstream troubleshooting. As mentioned in the above point, an immediate verdict might not be feasible (or in fact needed) and more time might be required to see a commercially-feasible outcome. The recent identification and purification of the two enzymes responsible for DON oxidation and reduction does not mean that this exciting research quest is over. It rather means that another exciting phase of optimisation and enhancement at the enzymes/proteins levels is going to start.

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