BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, June 2022. Vol. **19**(2), p. 387-394

Prebiotic Profiling of Indigenous Selected Dioscorea Spp. Using *In-vitro* Techniques

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http://dx.doi.org/10.13005/bbra/2993

(Received: 18 January 2022; accepted: 30 March 2022)

The current study used an in-vitro technique to evaluate the functional potential of Dioscorea alata L. and D. bulbifera L. extracts as prebiotics. Prebiotics are nondigestible carbohydrates that undergo a selective fermentation process in the gut to benefit the host, according to Gibson and Roberfroid in 1995. Many wild edible plants are high in carbohydrates and are utilised as both a staple food and medicine for a variety of stomach-related disorders. This study employed sweet tuber (ST), bitter tuber (BT), sweet bulbils (SB), and bitter bulbils (BB) from D. bulbifera, as well as tuber (AT) from D. alata and extracted prebiotics using standard method.The AT plant sample seemed to have the least reducing sugars, with a concentration of 2.83 mg/mL. The prebiotic activity of ST, BT, SB, BB, and AT samples was examined as the sole carbon source for microorganisms; among these, AT exhibited a considerable increase in the growth of recognised probiotics Lactobacillus plantarum, Saccharomyces cerevisiae, S. boulardii, and Pichia spp. in-vitro when compared to fructooligosaccharides (FOS). This preliminary investigation indicates that AT has the potential to be used as a promising prebiotic.

Keywords: Dioscorea spp.,; Gut Microbiome; Prebiotic; Probiotics.

Humans have trillions of complex communities of microorganisms in their gastrointestinal tract, known as the gut microbiome. The preservation of the structure and function of the gut microbiome is critical for host homeostasis and immunity. This gut microbiome is impeded by a variety of factors such as nutrition, modern lifestyle, antibiotic usage, and so on, leading in dysbiosis. Dysbiosis is characterised as an excess of pathogens in the gut, which has the potential to induce inflammatory bowel disease (IBD), colon cancer, and other diseases. The use of functional foods such as prebiotics and probiotics, on the other hand, can assist to return a dysbiotic condition to a healthy one[1–4]. The use of functional foods such as prebiotics and probiotics, on the other hand, can assist to return a dysbiotic condition to a healthy one. Nowadays, there is an increasing trend of consumer

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awareness toward the demand for functional foods that are claimed to improve the consumer's health. Apart from other food ingredients, prebiotics is among those which have attracted much attention recently[4,5]. Gibson and Roberfroid introduced the concept of prebiotics in 1995, defining them as "a non-digestible and selectively fermented ingredient that allows specific changes in composition and/or activity in the gastrointestinal microbiota that confers health benefits to hosts"[5–7].Non-digestible substrates such as inulin, fructooligosaccharides (FOS), galactooligosaccharides (GOS), and lactulose have been identified as prebiotics since they serve as a medium for saccharolytic bacteria. Prebiotics selectively increase/decrease specific gut bacteria, that can result in health advantages such as *Lactobacillus* growth advantage over *E. coli* and *Clostridium* spp., which cannot utilize these substances, resulting in the *Lactobacillus* population domination.

Prebiotics can influence the makeup and function of gut bacteria by providing energy sources. Specific probiotic species can utilize a given prebiotic such as fermentation of inulin and FOS by *Lactobacillus* spp. Prebiotics can also change the environment of the gastrointestinal tract. The fermentation products of prebiotics are mainly acids, which lower the gut pH[8–10]. The pH shift can affect the population of acid-sensitive organisms like Proteobacteria and encourage Firmicutes. The role of these short-chain fatty acids is diverse that provokes antimicrobial, anti-inflammatory, immunomodulatory activities[11–13].Furthermore, the molecular effect of prebiotics is mostly given through the stimulation of probiotics that generate short-chain fatty acids (SCFAs), which indirectly reduces pathogen development while changing the activity and composition of gut microbiota[14–16]. The incorporation of prebiotics into the diet is garnering global interest for human health purposes. Due to the obvious rising demand for prebiotics, there is a need to investigate new commercially feasible and sustainable sources of prebiotics. Prebiotics are mostly extracted from well-established Jerusalem plants such as artichoke, chicory root, onion, and leeks[17–19].

However, the usage of wild edible plants in Maharashtra has not been intensively investigated. As a result, the purpose of this research is to investigate the prebiotic potential of *Dioscorea alata* and *D. bulbifera*. *Dioscorea* is a wild medicinal plant that has been used for food, immunomodulation, and the treatment of gastrointestinal diseases[20,21]. The genus has gained much importance, not just as a source of sustenance, but also to sustain tribal livelihoods. The genus has been associated with a plethora of phytochemicals such as diosgenin, dioscin, and others that have a promising potential in the pharmaceutical business.Since prebiotics are widely recognised from natural sources such as chicory root and Jerusalem artichokes, there is still room for improvement in finding better prebiotics that may have antimicrobial activities when coupled with probiotics.

Aside from being abundant in nutrients, Dioscorea tubers and bulbils are used to cure diarrhoea, constipation, stomach ulcers, and other ailments[22]. Their proximate investigation indicated the presence of significant amounts of polysaccharides and crude fibres, indicating the possibility of extracting a significant quantity of prebiotic from these tubers. Prebiotics like inulin and fructooligosaccharides have been used to treat gastrointestinal issues like dysentery, constipation, and ulcers. As a result, our research have shown another possible application for *Dioscorea bulbifera* and *D. alata* tubers and bulbils as a source of prebiotics. Hence, the objective of the study are: (i) to quantify the amount of water-soluble extract from *D. bulbifera* and *D. alata* tubers and bulbils; (ii) to characterize extract; and (iii) to elucidate the function of extract in supporting the growth of *L. plantarum*, *S. boulardii*, *S. cerevisiae*, and *Pichia* spp. using the *in-vitro* approach.

MATERIAL AND METHODS

Sample Collection

Wild edible plant parts of *Dioscorea bulbifera* sweet and bitter tubers (ST, BT respectively), sweet and bitter bulbils (SB, BB respectively); and *D. alata* tubers (AT) were collected from the village, Chavani, Tal. Khalapur, Dist.- Raigad, Maharashtra State, India and were authenticated by Dr. Suresh Jagtap (Plant Taxonomist). Required prior approval of the biodiversity board was obtained (MSBB/Research/576/2021-22). These samples were washed with distilled water to remove dirt particles

and were dried in a hot air oven at 60 °C till constant weight was achieved. Further, the dried samples were grinded to a fine powder and stored in airtight poly bags at room temperature for further use.

While probiotic cultures were isolated and identified as *Lactobacillus plantarum* (LB-VII) (NCBI Accession no. MK608674.1), *Pichia kudriavzevii* (S-I) (NCBI Accession no. LC528140.1), *Saccharomyces cerevisiae* (MD) (NCBI Accession no. LC528142.1), and *Saccharomyces boulardii* (SB) were procured from Pathology lab, Rajiv Gandhi Institute of I.T. and Biotechnology, Bharati Vidyapeeth (Deemed to be University), Pune [23–26].

***In-vitro* Gastrointestinal Environment Simulation for Enrichment of Prebiotic Content**

Simulated gastrointestinal treatment on ST, BT, SB, BB and AT powders were performed as described by Yadav S et al., 2014 [27]. Accordingly, dried powders at a concentration of 10% w/v were added to gastric juice containing 0.6 mL of pepsin (pH 2.0) and the mixture was incubated for 2 hours at 120 revolutions per minute (rpm) to simulate the gastric environment; whereas for the intestinal environment, pH was adjusted to 7.5 along with the addition of bile pancreatin mixture (4 mL) which was incubated on a shaker for 2 hours at 37°C. The enzymatic reaction was nullified by incubating the resultant residue in cold distilled water for 1 hour. Finally, the residue was filtrated through a cheesecloth and the undigested residue was oven-dried at 55°C and used for further experiment.

Determination of Total Reducing Sugar

The reducing sugar content of undigested and digested residues was determined using the 3,5-dinitrosalicylic acid (DNS) assay[28]. In brief, 4 mg/mL concentrations of undigested and digested samples were treated with 1 mL of DNS reagent for 5 minutes at 100ºC. Following that, the tubes were diluted to a level of 10 mL with distilled water and spectrophotometric analysis was done at 520 nm. By plotting a standard graph with a known quantity of maltose, the concentrations of reducing sugars in these samples were estimated.

Assessment of Prebiotic Potential

The growth response of probiotic cultures was measured in presence of undigested, digested samples and standard prebiotic fructooligosaccharides (FOS). Prebiotics at a concentration of 4 mg/mL were added in Yeast extract Peptone Dextrose(YPD) medium[26] (yeast extract- 10 g/L; peptone- 20 g/L) for yeasts while for *Lactobacillus* modified de Man Rogosa Sharpe (MRS) medium (Peptone- 10 g/L; yeast extract- 5 g/L; tween 80- 1 g/L; sodium acetate- 5 g/L; magnesium sulphate- 0.1 g/L; manganese sulphate- 0.05 g/L; dipotassium hydrogen phosphate- 2 g/L). These growth media were devoid of any other carbon source but prebiotics was autoclaved at 121°C for 15 min. Cultures S-I, MD, SB, and LB-VII were grown under respective broth conditions and were centrifuged for 5 min at 10,000 rpm; re-suspended in sterile saline before inoculation of 100 ìl individual culture in prebiotic containing media and incubated at 37°C. Culture response to this prebiotic were assessed spectrophotometrically at 600 nm with time intervals of 0, 24 and 48 hours. Growth media devoid of cultures was used as negative control while for standard control FOS was used. Medium YPD and MRS along with glucose were used as a positive control[29].

Statistical analysis

Each experiment was performed in triplicate and data were analysed using one-way analysis of variance (ANOVA) software and results expressed as Mean ± SD. Differences were considered statistically significant when $p < 0.05$ ($p > 0.05$ = ns, $p < 0.05$ = *, $p < 0.01$ = **, $p < 0.001$ = ***).

RESULTS AND DISCUSSION

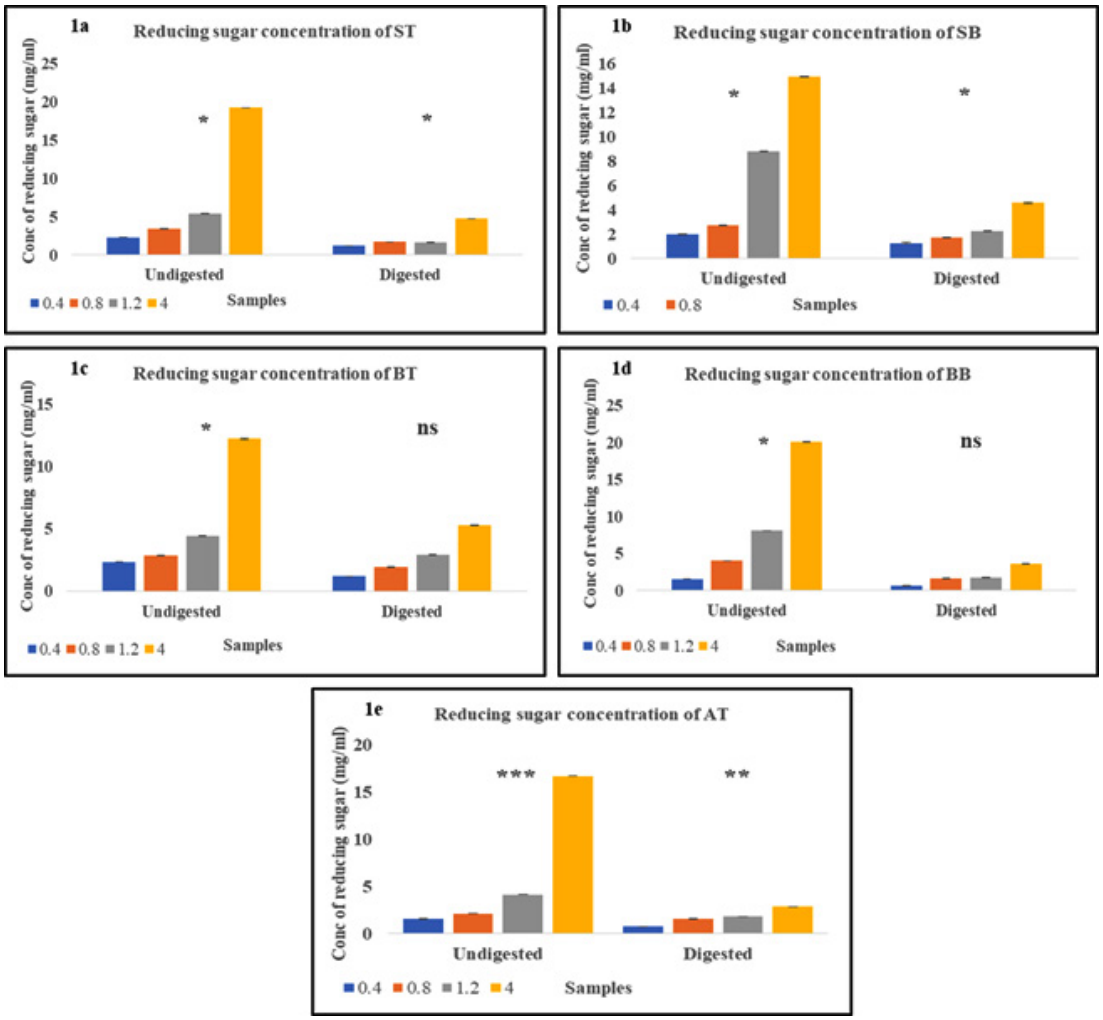
Gastrointestinal Tolerance of Prebiotics

Prebiotics are short-chain carbohydrates that are resistant to the cleavage action of human digestive enzymes. As a result, for prebiotics to be optimum and efficient, they must enter the intestine[30]. These short-chain carbohydrates have 3 to 10 sugar moieties with a degree of polymerization (DP) ranging from 2 to 60. It has been proposed that resistance to digestion can be caused by either the arrangement of glycosidic linkages between monomeric sugar units or the substrate specificity of human digestive enzymes. However, to provide prebiotic effects, they must be resistant to the action of digestive enzymes as well as extreme pH conditions[31]. In this study, AT sample was found to possess high amounts of non-digestible matter with 7.71%; followed by SB (6.2%); ST (5.24%); BT (4.32%); and BB (4.08%)

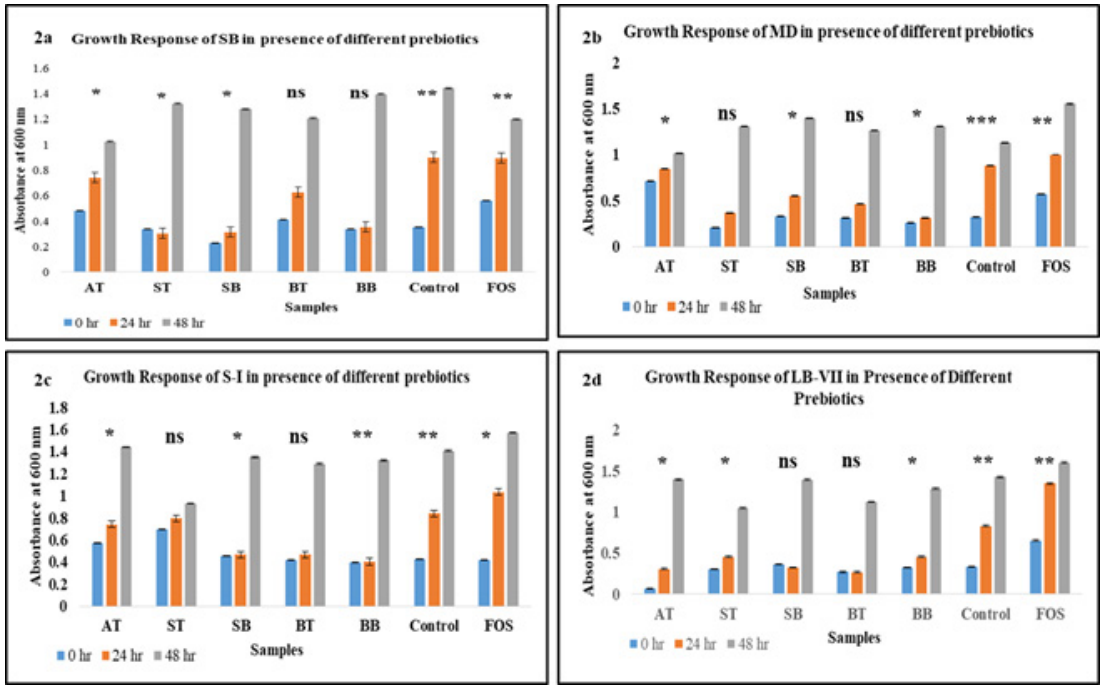
on a dry weight basis. Similarly, the proximate analysis of *Dioscorea bulbifera* and *Dioscorea alata* revealed fiber contents to be in a range of 4.1-11.0% respectively, on a dry weight basis[32]. Similar findings were found in this study when dried powders of ST, BT, SB, BB, and AT were subjected to harsh conditions of the gastrointestinal environment. Gastric juice composed of pepsin at lower pH degrades proteins into amino acids, while intestinal juice contains a mixture of digestive enzymes such as amylase, protease and lipase along with bile are responsible to catabolize carbohydrates, proteins and fats respectively into their monomeric form.

Estimation of reducing sugar

Prebiotics, which are non-digestible compounds, are made up of sugar units connected in such a way that they form non-reducing ends and so resist treatment with DNS reagent[33]. In this study, the sugar contents of undigested and digested samples were measured. The undigested BB sample had the most reducing sugars (20.09 mg/mL), while the digested BT sample contained the most (5.29 mg/mL) (Graph 1c and 1d respectively). There was, however, a considerable decline in digested samples, with the AT sample having the lowest quantity (2.83 mg/mL) (Graph 1e) Chard (9.5 mg/mL), Fennel leaves (11.7 mg/mL), and



Graph 1. (a), (b), (c), (d), and (e):Comparison of reducing the sugar by DNS method for undigested and digested residues of respective samples



Graph 2. (a), (b), (c), and (d):Growth stimulatory effect of prebiotics from AT, ST, SB, BT, and BB in presence of known probiotic strains

Mushroom buttons (11 mg/mL) all had comparable sugar profiles, according to Nowak R et al., 2017[34]. Hence, the preliminary data reveals the presence of non-reducing and non-digestible compounds in digested samples.

Growth Stimulatory Effect

Carbohydrates that reach the intestine can indeed be fermented by the gastrointestinal microbiota. To meet the prebiotic criterion, it must be fermentable by gut microorganisms selectively, resulting in host benefit. Several studies have demonstrated that prebiotics like FOS and inulin stimulate the growth of probiotics like *Lactobacillus* and *Bifidobacteria*. Probiotics stimulate the production of short-chain fatty acids (SCFAs), which serve a variety of roles in the host. The method by which prebiotics influence microbial diversity in the colon is currently under investigation. Prebiotics have the advantage of promoting the growth of target microorganisms, which then compete with species that are specific to energy sources and exclude them by protecting or promoting the production of beneficial fermentation substances, such as SCFAs, which have immunomodulatory properties, influencing toll-like receptor signaling and the production of pro-inflammatory cytokines[35].

In this study, the prebiotic potential of ST, BT, SB, BB, and AT samples were assessed with digestive treatment along with standard control FOS. Known probiotic cultures such as SB, SC, S-I, and LB-VII growth response to different digested samples were estimated spectrophotometrically. It was found that overall, AT sample was comparative with FOS. AT sample could stimulate SB, MD, and S-I with an optical density of 0.74, 0.84, 0.74 respectively after 24 hours of incubation. While FOS induced growth of 0.89, 0.99, 1.03 for SB, MD, and S-I respectively. The cultures further showed good growth after 48-hour incubation reaching an optical density of 1.442 at 600 nm (Graph 2a – 2d). A similar study reported by Sawangwan T et al., 2018 showed prebiotics extracted from mushroom supports the growth of probiotics such as *Lactobacillus acidophilus* and *L. plantarum* which is comparable to FOS[28].

The control of the gut microbiome is critical since many illnesses are connected

to microbial profiles; hence, prebiotics can be utilized as a therapeutic agent to prevent and reverse dysbiotic conditions. Prebiotics are widely established from plants such as Jerusalem artichoke, chicory, onions, and so on; nevertheless, the usage of wild edible plants of Maharashtra for prebiotic potential is yet unexplored. Exploring wild plants for prebiotics with higher prebiotic potential can also serve as a new commercial source of prebiotic extraction, as can introducing wild plants into agricultural fields.

CONCLUSION

Traditional medicine is used to cure ailments in the majority of the globe. However, the emergence of novel infectious illnesses, as well as the increased usage of traditionally used pharmaceutics, has prompted a quest for new biotherapeutics. The gastrointestinal system is home to diverse microbial communities known as the gut microbiome, which perform critical tasks. The number of probiotics in the gut is decreasing as a result of an urbanized lifestyle and the usage of medications. Prebiotics, on the other hand, are well defined as a growth-stimulating ingredient for probiotics. Hence, prebiotics as a source of biotherapeutics can accomplish the modernized need. As defined prebiotics pass through the digestive tract unchanged and modulate gut microbiota. Prebiotics were extracted from *Dioscorea bulbifera* bulbils (sweet and bitter), tubers (sweet and bitter), and *D. alata* tubers in this investigation, with AT exhibiting the least presence of reducing sugar after treatment with digestive enzymes. The growth of known probiotic cultures *Lactobacillus plantarum*, *Saccharomyces cerevisiae*, *S. boulardii*, and *Pichia* spp. was stimulated by all extracted prebiotics ST, BT, SB, BB, and AT; however, AT demonstrated superior growth potential than known prebiotic FOS. This provided a strong indication that AT can be used further as a prebiotic with additional therapeutic properties. In conclusion, *Dioscorea alata* tubers represent a promising source of natural prebiotics. However, more experimentation on pre-clinical trials is needed to validate the efficacy of prebiotics.

ACKNOWLEDGEMENT

The authors would like to thank authorities of Bharati Vidyapeeth (Deemed to be University), Pune, Maharashtra for supporting this study.

Conflict of Interest

All authors declare no conflict of interest.

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