学位論文内容の要旨

博士の専攻分野名称：博士（農学）　　氏　名：Helena de Fátima Silva Lopes

学位論文題名

Bacterial profile of different indigo fluids and the effect of the addition of *Indigofera tinctoria* leaf powder on *sukumo* preparations

（原料の異なる藍染め発酵液の微生物叢の解析およびスクモを用いた藍染め発酵液へのインド藍粉の効果）

There are many methods for preservation and extraction of natural indigo, and they can be basically divided into 2 distinct categories: microbial composting and direct extraction. In the composting method, the leaves of the indigo bearing plants undergo a long fermentation period ranging from weeks to months. This methodology is exemplified by the production of woad (*Isatis tinctoria*) in Europe and *sukumo* (*Polygonum tinctorium*) in Japan. In the case of direct extraction, leaves are fermented briefly (up to hours) to release the precursors, and then aerated in a beating vat to react with oxygen. The newly formed indigo is decanted, boiled, filtered, and pressed to form an indigo cake, which is the case of Indian indigo. In the method used in Okinawa, Japan, the leaves are fermented for days and the indigo is decanted by the reaction with $Ca(OH)_2$ to obtain an indigo paste.

Indigo is insoluble in water, and to be used as a dye it needs to be transformed into leuco-indigo, which is its reduced form. There are many ways to reduce indigo. In the microbial method, the indigo source (woad, sukumo, Indian indigo and others) is fermented in an alkaline environment and specific bacteria from the community reduce indigo to its leuco form. Indigo sources originated from composting, such as woad and *sukumo*, already have the necessary microorganisms to start the fermentation, whereas Indian indigo and other extracted indigo may need the addition of a seed culture.

While fermentation fluids made with *sukumo* have already been characterized, the bacterial community of different preparations lack definition. Therefore, one of the focus of this study is the comparison between *sukumo* fluids from different origins and fluids made from other sources of indigo. Furthermore, the composting process that produce *sukumo* is not always stable and the quality of the final product can fluctuate. This characteristic also affects the onset of indigo reduction which sometimes can take more than 2 weeks to start. To address this problem, we also evaluated the effect of the addition of *Indigofera tinctoria* leaf powder (LP) in different *sukumo* fermentations.

1. Analysis of bacterial flora of indigo fermentation fluids utilizing composted indigo leaves (*sukumo*) and indigo extracted from plants (Ryukyu-ai and Indian indigo)

To clarify the factors behind the bacterial diversity and the sustainability of indigo fermentations, the bacterial community of preparations using different sources of indigo, composted (*sukumo*) and extracted (Ryukyu-ai paste and Indian indigo cake), were studied. Next Generation Sequencing (NGS) technology was used to access the bacterial community

profile. The common feature of all preparations was the strong presence of obligate anaerobic bacteria, especially the families *Proteinivoraceae* and *Tissierellaceae*, which suggests their high affinity to the fermentation environment (hyperalkaline and low redox potential). Communities from *sukumo* fermentations were more diverse when compared to preparations using extracted indigo. This characteristic is related to the presence of seed microorganisms and various nutrients in *sukumo*. In addition, the debris in *sukumo* fermentations allows the formation of low pH niches which promotes the diversity by reducing pH stress. On the other hand, fermentation using extracted indigo presented a simpler community and was highly influenced by the seed culture added. Nutrients and the strong microbial network inside these fermentations were responsible for their stability. Ryukyu-ai fermentation, for instance, had a very stable community regardless of the fermentation age.

2. Indigofera tinctoria leaf powder as a promising additive to improve indigo fermentation prepared with sukumo (composted *Polygonum tinctorium* leaves)

The onset of the indigo reduction in *sukumo* fermentations can vary greatly (from 3-4 days to weeks). Since certain additives can improve indigo fermentation, the effects of *Indigofera tinctoria* LP on the reduction initiation and bacterial community were studied. To observe the effects on the reduction, pieces of cloth were dyed using the fermentation fluid. The intensity of the dye was then compared between control and LP treatment. The redox potential (ORP) was also measured and the bacterial community in the initial phase and in older fluids were analyzed using NGS. The addition of LP improved the speed of the dyeing in most experiments, and a markedly decrease of ORP was observed since day one. The observed effect on ORP was likely a result of the phytochemicals present in *I. tinctoria* LP acting as oxygen scavengers. Regarding the effects in the microbial community, there were no significant changes in the identity directly related to the bacteria present in LP. The decrease in *Bacillaceae* and increase in *Proteinivoraceae* at the onset of fermentation and the ratio of facultative to obligate anaerobes are vital to the initiation and maintenance of the indigo reduction. Both characteristics appeared earlier in fluids treated with LP.

3. Conclusions and perspectives

The bacterial community is deeply influenced by the materials used to make the fermentation. The community of fluids originated from extracted indigo will be influenced by the microbial seed added, while *sukumo* fermentation will have a more diverse community influenced by the aging of the fermentation and the complex nature of *sukumo* (seed microorganisms, nutrients, and debris). Possibly because of *sukumo* complexity, the addition of I. *tinctoria* LP did not directly influence the community identity, it promoted the selection of the necessary bacterial community. This effect was probably due to the acceleration of necessary transitional changes for indigo reduction caused by the rapid decrease of ORP. These findings can contribute to the development of new fermentation procedures, the creation of stable artificial communities for reduction, and the manipulation of environmental characteristics to accomplish a more efficient fermentation.