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# Environmental factors impacting the ecotoxicology of azo dyes on gut microbial communities

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FACULTY OF SCIENCE AND TECHNOLOGY NANCY  
Master MICROBIOLOGY 2nd year – EHM (environmental and  
health microbiology)  
University year 2022/2023

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Environmental factors impacting the ecotoxicology of azo dyes  
on gut microbial communities

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Submission by

REZER Emilie

Internship from 9 January to 30 June 2023

At the Micalis Institute

Supervised by LEPAGE Patricia

Head of the PhylHom team

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## Abbreviation

ADHD: Attention deficit hyperactivity disorder

BHI: Brain Heart Infusion

IBD: Inflammatory Bowel Disease

mGAM: modified Gifu Anaerobic Broth

PCR: Polymerase Chain Reaction

OD: optical density

## I. General Introduction

Azo dyes are molecules widely found in the food (lollipops, soda, cakes, etc.), pharmaceutical and textile industries. The general public, and children in particular, are exposed to these type of dyes, that have been associated with occurrence of irritable bowel disease (IBD) and impacting human immune system (Kus and Eroglu, 2015). Since azo dyes are commonly found in food, the bacteria in the intestines (gut microbiota) are exposed to them. It has been also shown that the gut microbiota can degrade these dyes (Zahran *et al.* 2021) (Zahran *et al.* 2019 ; Rafii *et al.* 1990)(Fig. 1), however the influence of abiotic factors in the gut on bacteria azo dyes interactions are unknown.

As part of my research project, I aimed to answer three major questions: (i) Which members of the gut microbiota metabolize azo dyes? (ii) How environmental factors present in the gut influence microbial azo dyes metabolism? (iii) What effect will bacterial metabolism in the presence of dyes have on the viability of colon and liver cells? To answer these questions, the project was divided into four main parts. First, I isolated and then identified eighty-two bacteria capable of degrading two kinds of azo dyes (allura red AC and tartrazine) by Sanger sequencing of the 16S rRNA gene. Then, I characterised kinetics of 30 isolates to metabolize the azo dyes (allura red and tartrazine) by comparing the rate of degradation measured at 0, 3, 6 and 24 hours of incubation. With this approach, I defined various degrader profiles and then selected five isolates. The third part aimed to characterize the impact of various abiotic factors (pH, vitamins supplementations, presence or absence of oxygen) on the bacterial metabolism of the azo dyes. Finally, the last part of the internship project involved studying the toxicity of bacterial azo dye metabolism on colon and liver cells.

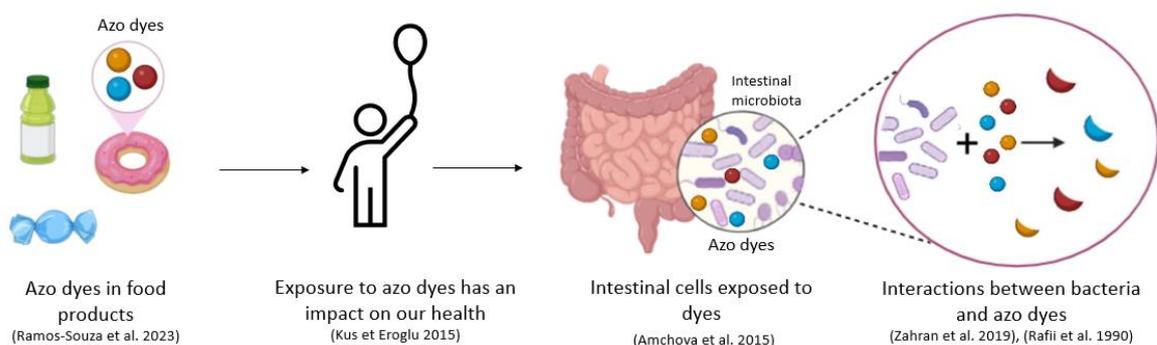


Figure 1. The interaction between intestinal microbiota and azo dyes

## II. The host structure

The Micalis Institute is a research institute associated with INRAE and is located in Jouy-en-Josas in the Ile-de-France region. The institute's area of research is "food microbiology for health". Micalis brings together INRAE, AgroParisTech and is associated with the University Paris-Saclay. This joint research unit, founded on 1 January 2010, is made up of about twenty research teams working on three research axis:

- Bacterial Adaptation and Pathogenesis (BAP)
- Systems and Synthetic Microbiology" (MSS)
- **Food and Digestive Ecosystems (FDE)**

The research pole FDE is composed of seven teams including PhylHom which works on Phylogeny and Physiology of the Human Microbiome. Patricia Lepage, research director, is the leader of PhylHom team. Ms. Lepage and Zehra Esra Ilhan (Scientist) were my internship tutors. One of the research focus of the team is to characterize the human gut microbiome to predict and impact the human response to xenobiotics such as drugs or diet.

### III. Literature review

#### III.1 The azo dyes

##### A. The discovery of azo dyes and their widespread use

In 1856, the first synthetic dye was discovered by the scientist William Perkin (Science History Institute, 2017). Since then, dyes have taken an increasing share of the world market and have become indispensable. It appears that the consumption of dyes in the US has increased since the 1950s, with a 5-fold increase in consumption in just 60 years (Stevens *et al.* 2014). The use of these synthetic dyes has become widespread because, unlike natural dyes, they offer a wider range of colours and are much more affordable. In addition, these synthetic dyes have a longer shelf life and are more readily available than natural dyes, which justifies their more attractive price, and extensive use in the food and pharmaceutical industries (Vojdani and Vojdani, 2015).

Azo dyes have a two-benzene ring connected by an azo group (Zollinger, 2003; Lafta *et al.* 2016)(Fig. 2). These molecules are widespread in food (Ramos-Souza *et al.* 2023) and textile industries (Pérez-Díaz and McFeeters 2009) and have different colours, including allura red AC (red), tartrazine (yellow), brilliant black BN (black) and brilliant blue FCF (blue). Tartrazine is often present in food products such as chewing gum, yoghurt, mustard (Rovina *et al.* 2017). The dye allura red AC(E129) is present in dairy products, soft drinks, and baked goods (Waliul Khan, 2022).

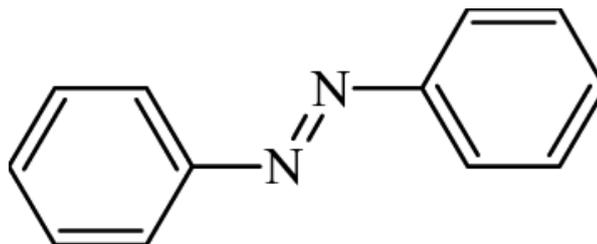


Figure 2. Structure of azo dyes (Lafta *et al.* 2016)

##### B. Exposure and impact on health

Humans are continuously exposed to azo dyes. It was only in the 1950s that the first studies began to highlight their dangerous potential. The risk is mainly due to the ingestion of

products containing these dyes (Ramos-Souza *et al.* 2023) and concerns our digestive tract and our overall health (Amchova *et al.* 2015). Long-term exposure to the azo dyes in postmenopausal women may also increase the risk of developing primary biliary cirrhosis (Amchova *et al.* 2015). A study has shown that the azo dye allura red AC promotes inflammation and promotes also the allergic reaction such as asthma or urticaria (Amchova *et al.* 2015).

The azo dyes impact the skin as well due to exposure from pharmaceuticals and cosmetics (cream, makeup, etc.) (Vojdani and Vojdani, 2015). These molecules will encounter our immune system, which will have difficulty in coping with them because of their small size. Azo dyes disrupt our immune system and are responsible for inflammatory reactions and autoimmune reactions.(Vojdani and Vojdani, 2015).

Children are more affected by the harmful effects of azo dyes due to the high consumption of sweets, soft drinks and syrups containing numerous dyes. In 1997, a study linked the consumption of these dyes to cases of hyperactivity in young children (aged between seven and thirteen years old). This study highlighted that in 60% of these 486 hyperactive children with Attention deficit hyperactivity disorder (ADHD) , the consumption of products such as azo dyes or chemical detergents was responsible for an increase in behavioural problems (WARD, 1997). Those health issues included the development of eczema and ear and/or chest infections. The consumption of two dyes (tartrazine, sunshine yellow) in a quantity of 50 mg in the group of hyperactive children resulted in an increase in the production of zinc in the urine and a decrease in the amount of zinc in the blood serum. It has been shown that zinc indirectly regulates the activation of dopamine. Melatonin activates dopamine and melatonin production is regulated by zinc. (Ghoreishy *et al.* 2021). Dyes that have a negative impact on zinc levels in the blood may play a direct role in attention disorders (WARD, 1997). However, for the control group of children without ADHD, the consumption of tartrazine or sunshine yellow did not impact the zinc levels.

Before the dyes can be approved for the use in food products, numerous tests are carried out to investigate the toxicity of the dye, such as mutagenicity, reproductive toxicity and accumulation in the body. Therefore, The European Parliament and the Council have drafted Regulation (EC) No 1333/2008 on colours used in the food industry. This regulation requires the European Food Safety Authority (EFSA) to re-evaluate the toxicity of additives by January

2009 (Amchova *et al.* 2015). What's more, with the increasing consumption of dyes in food products, it is crucial to find a solution to reduce their impact on human health.

## III.2 The human microbiota

### A. The intestinal microbiota, a hidden organ

The gut microbiota is made up of more than a trillion of bacteria that can be found all along our intestinal tract (Corthier, 2011). Within the gut microbiota, more than thousand species could be identified and about 160 different species are found per individual (Landman and Quévrain, 2016). Mainly composed of bacteria, the microbiota also includes fungi, viruses and archaea. There are four major phyla : Firmicutes, Bacteroidetes, Actinobacteria and Proteobacteria (Landman and Quévrain, 2016). Its composition varies between individuals, geographical locations, and age (Corthier, 2011). Although the abundance of major phyla varies between individuals, the functions of the gut microbiota remain similar due to functional redundancy in healthy individuals (Landman and Quévrain, 2016).

These microorganisms are essential in various functions such as the digestion of food that cannot be assimilated by our body, a barrier effect against pathogens and other roles (Lecerf, 2021). The bacteria in the microbiota have degradation, synthesis or metabolic functions such as digestion of dietary fibres into simple sugars (Landman and Quévrain, 2016). The microbiota plays a role in the metabolism of fats and carbohydrates and in their absorption and the latter also promotes fat storage and therefore weight gain (Turnbaugh *et al.* 2006). A recent study showed a difference in the development of the immune system between normal mice and axenic mice (without microbiota) (Macpherson and Harris, 2004). It has also been shown that the production of bacteriocins and microbial peptides is controlled by our intestinal microbiota (Hooper, 2004).

The function of the gut microbiota can be manipulated for the development of treatments such as probiotics/prebiotics or faecal transplants to improve the health of the humans. For example , a study published in 2015 showed the beneficial impact of faecal transplantation in the case of *Clostridioides difficile* infection and resolution of diarrhea (Rossen *et al.* 2015). The intestinal microbiota plays a key role and has a direct impact on our state of health.

## B. The role of microbiota in the first years of life

At birth, the infant is colonized by contact with mother's vaginal and anal microbiota in the case of a vaginal delivery (Lecerf, 2021) or with the surrounding microbiota (Lecerf, 2021) if the baby is delivered by caesarean section. The gut microbiota develops mainly in the first three years of the child's life and evolves throughout the life (Nagpal *et al.* 2018)(Fig. 3). The microbiota of the newborn is mainly composed of Proteobacteria. Then the number of species belonging to the phyla of Proteobacteria will decrease during childhood. The microbiota of young children will be mainly colonized by bacteria belonging to the phyla Actinobacteria, and specifically bacterial genus of Bifidobacterium (Figure 3). Microbiota diversity is the lowest in childhood compared to other life stages. (Nagpal *et al.* 2018). The use of antibiotics, the presence of pets and the diet will influence its composition (Vandenplas *et al.* 2020) (Fig. 3).

The intestinal microbiota that develops during our first years of life will play an important role in our future state of health. For example, a greater risk of allergies or diabetes would be an outcome of an imbalanced microbiota during early years of life (Nagpal *et al.* 2018).

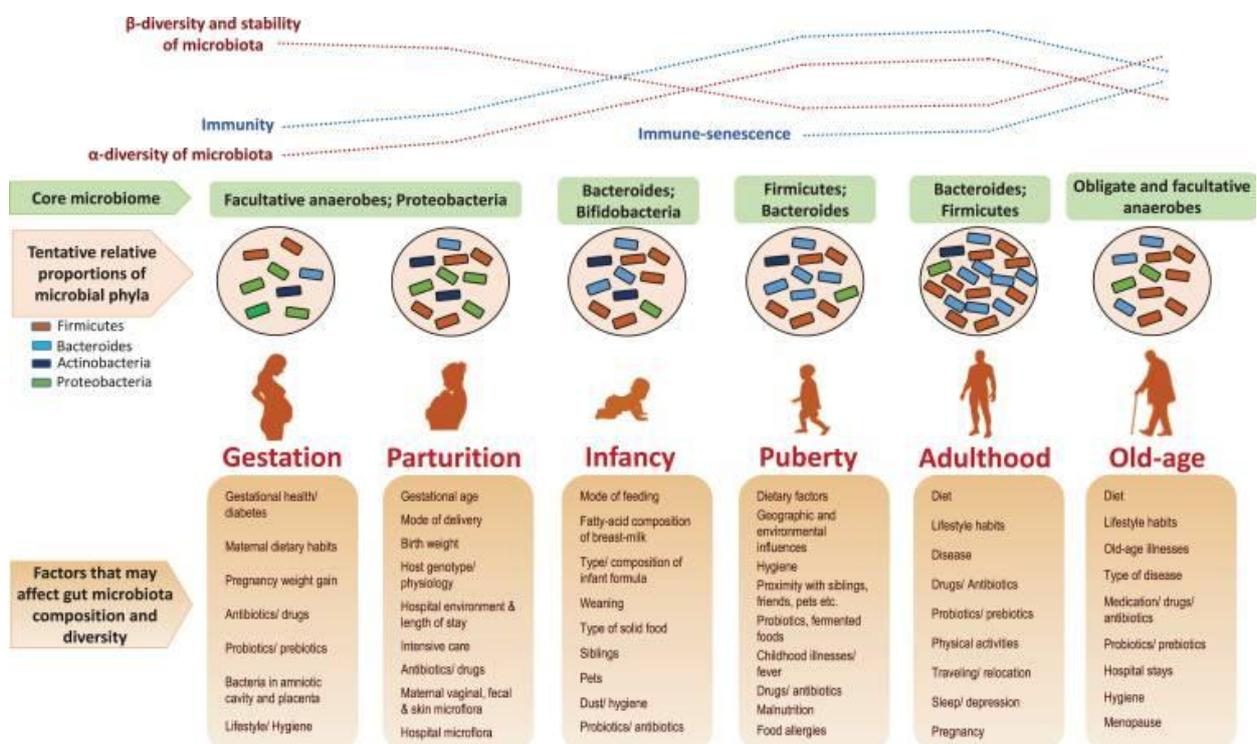


Figure 3. Age-related changes in the human gut microbial ecosystem, and potential factors that could affect microbiota composition at different stages of life.  $\alpha$ -diversity: species (taxa) richness within a single host/microbial environment;  $\beta$ -diversity: diversity in microbial community (taxonomic abundance profiles) between different environments/samples). (Nagpal *et al.* 2018).

### III.3 Exposure of our intestinal microbiota to xenobiotics such as azo dyes

A study carried out in 2010 showed that xenobiotics such as antibiotics have an impact on the composition of the intestinal microbiota in a mouse model (Robinson and Young, 2010). In the study they focused on the impact of vancomycin. Vancomycin was shown to affect the growth of Gram-positive bacteria, but not Gram-negative bacteria such as *Enterobacteriaceae* (Robinson and Young, 2010). However, Proteobacteria, gram positive bacteria were not affected by vancomycin (Fig. 4).

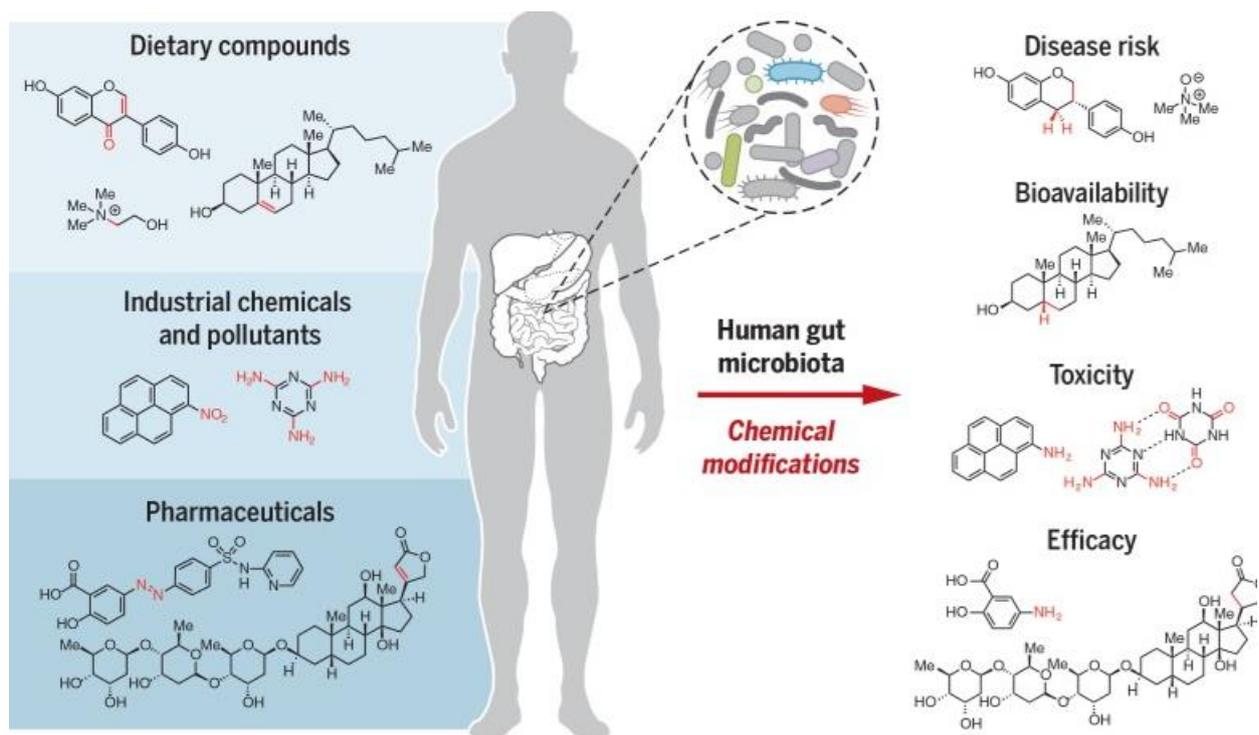


Figure 4. Human gut microbes metabolize xenobiotics. The microorganisms that inhabit the human gut alter the chemical structures of ingested compounds, including dietary components, industrial chemicals, and drugs. These changes affect xenobiotic toxicity, biological activity, and bioavailability. The gut microbiota enzymes responsible for many of these transformations are poorly understood. (Koppel *et al.* 2018).

Another study focusing on the impact of azo dyes showed that OATP2B1, one of the main intestinal transporters, is inhibited (Zou *et al.* 2020). These excipients (azo dyes) are inactivated by the production of metabolites by bacteria. Bacterial metabolism thus makes it possible to inactivate these excipients and thus enables the intestinal transporter involved in drug absorption to function (Zou *et al.* 2020).

### III.4 Strategies used by microorganisms to degrade azo dyes

#### A. Azoreductase activity in human gut microbiota

Azoreductases are enzymes that are capable of reducing the azo bonds present in azo dyes, thereby enabling their detoxification (Misal and Gawai, 2018). Among these metabolic processes are found azoreductase involved in the metabolism of azo dyes in the members bacteria of intestinal microbiota, including *Escherichia coli*, *Enterococcus faecalis* and many other species (Zahran *et al.* 2019). These bacteria can use the azo dyes as growth substrate and therefore metabolizes them (Rafii and Cerniglia, 1995).

A 2021 study was conducted to isolate and identify bacteria from the intestinal microbiota with azoreductase activity in 16 adult individuals from their faecal extract (Zahran *et al.* 2021). Among these bacteria, two phyla have been identified: Firmicutes and Bacteroidetes (Zahran *et al.* 2021). The bacteria belonging to the phylum Firmicutes had high azo-reductive activity and conversely that belonging to the genus Bacteroidetes would have a low activity. This study demonstrated that a microbiota rich in Firmicutes but poor in Bacteroidetes would have high azo dyes reductase activity (Zahran *et al.* 2021). Azoreductase have also been identified from *Enterococcus avium* (AZO-Ef) or *E. coli* (Azo-Ec)(Zahran *et al.* 2019). These two studies carried out by Zahran's team highlighted the difference in azo dye degradation rates depending on the bacterial phyla (Zahran *et al.* 2021).

Other complementary studies showed the presence of azoreductases in several bacteria (Zou *et al.* 2020). *Bifidobacterium pseudocatenulatum* from Actinobacteria, *Bacteroides fragilis* and *Bacteroides uniformis* from Bacteroidetes and also bacteria of Firmicutes phylum possess this enzymes likes *Faecalicoccus pleomorphus* (Zou *et al.* 2020)(Fig. 5).

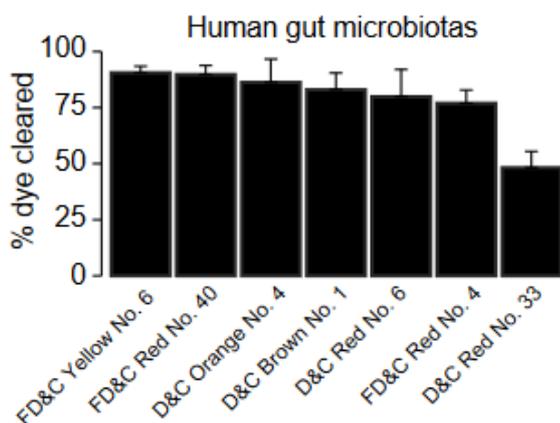


Figure 5. Human gut bacteria metabolize azo dye excipients. (A) Excipient azo dyes are cleared following the *ex vivo* incubation with human faecal samples. Dilutions of human faecal suspensions from three healthy unrelated individuals were incubated anaerobically for 24h in BHI+ media supplemented with various excipient azo dyes and conditioned media were sampled and analysed for residual dye concentration spectrophotometrically. Data are normalized to uninoculated controls. Values represent mean  $\pm$  SEM. (Zou *et al.* 2020)

## B. Modulation of azoreductase activity in bacteria

The activity of azoreductase can be modulated by various abiotic factors. The microbial azoreductase activity exists in both anaerobic and aerobic microorganisms (Zimmermann *et al.* 1984). They do not have the same tolerance to oxygen, very often require different cofactors, such as flavin or NADH and their function is dependent on environmental pH. Many azoreductases are stable when the pH is between 5 and 9. Although a physiological pH would allow an optimal activity of these enzymes (Misal and Gawai, 2018). Minor differences in the gut pH can still modulate azoreductase activity. Nevertheless, the optimum pH for degradation varies between each dye and each azoreductase. For example, for the azoreductase identified in *E. faecalis*, the majority of the dyes tested in this study were reduced at higher rates when the pH was 6, whereas other dyes such as tartrazine will be reduced equally by the azoreductase of *E. faecalis* whatever the pH (Zahran *et al.* 2019).

The activity of these enzymes may be affected by other factors such as the amount of oxygen. In the study conducted by Zahran and coll., azo dye degradation was tested under aerobic and microaerophilic conditions (Zahran *et al.* 2019). It appears that azoreductase produced by *E. coli* is more effective in aerobic than microaerophilic conditions. While for the azoreductase identified in *E. faecalis* the amount of oxygen present in its environment does not seem to impact its degradation activity. Finally, it has been shown that azoreductase of *E. avium*, degradation takes longer to start in microaerophilic condition. Conversely, the rate of initial degradation is faster in aerobic condition, although in the end the rate of degradation of Amaranth in both conditions is almost identical (Zahran *et al.* 2019).

## IV. Material and methods

### IV.1 Faecal extract

To isolate potential azo dyes bacterial degraders, I cultivated bacteria from faecal suspensions (80 % of glycerol) collected from five individuals. The sample IDs were as follows: F8, F9, F10, F16, F19. The individuals were all young children between 11 months and 48 months and included two girls and three boys. (Annexe 1).

### IV.2 Azo dyes

My goal was to evaluate the impact of four azo dye treatments, allura red (Sigma-Aldrich), tartrazine (Sigma-Aldrich), brilliant blue FCF (Sigma-Aldrich) and brilliant black BN (Sigma-Aldrich).

### IV.3 Media

I used two media for the isolation of azo dyes degraders from the human faecal cultures, the first one was Gifu Anaerobic Broth medium (mGAM) which is recommend for the cultivation and isolation of bacteria from clinical samples but also for susceptibility testing (« Gifu Anaerobic Broth, HiMedia » s. d.). Brain Heart Infusion (BHI) medium is the second medium I used, it was previously used for the isolation azo dyes degraders (Zahran *et al.* 2019).

## IV.4 Isolation and identification of isolates

To identified bacteria with azo dyes degradation activity, I inoculated 150 µl of frozen faecal stock on mGAM medium with a concentration of 10 mM (500 µl/L from a stock solution prepared at 100 mM) for each dye. I optimized the number of colonies that can be counted on the plates; I used the dilution  $10^{-3}$  to dilution  $10^{-6}$  to obtain the best concentration of bacteria to isolate the degraders on the plates. After the inoculation using the glass beads to evenly spread the inoculum under sterile conditions, I incubated the plates at 37°C for 24 hours under anaerobic conditions (Bactron 600 anaerobic chamber) to simulate the conditions of the human gut.

After 24 hours of incubation, I observed clear zones around some colonies that indicated the degradation of the azo dyes. I visually selected various colonies based on morphology (colour, appearance of the colony and size) (Fig. 6).

I picked up the selected colonies with sterile inoculation loops and inoculated them on an mGAM agar with the corresponding azo dyes (allura red or tartrazine and Brilliant blue FCF for the F9 faecal extract on mGAM medium only). I incubated the plates under previously described conditions (37 °C, anaerobic condition, 24 h).

Once the isolates grew in the fresh solid media, I selected individual colonies and streak on fresh mGAM plates and made a bacterial loam to maximize biomass production for (i) DNA extraction and (ii) glycerol stocks to be stored at -80 °C. Briefly, I resuspended the biomass collected with inoculation loops in 1 mL of liquid mGAM media. These steps were carried out in the anaerobic chamber (BACTRONS 600).

To prepare the frozen stocks, 500 µl of culture were transferred to a sterile 1,5 mL cryotube that contains 150 µL of 80% glycerol. I mixed the solution by pipetting upside down for 5 times. For the DNA extraction, I centrifuged 500 µl of culture at 21,300 rpm for 3 minutes, removed the supernatant, and kept the pellets in the freezer (-20 °C) until the DNA extraction. Frozen stocks were kept in the -80 °C freezer, for further experiments.

I extracted the microbial DNA of the isolates with PowerFecal Pro DNA (QiAamp). Then, I verified the quantity of DNA extract with the nanodrop (Thermo Scientific). I performed polymerase chain reaction (PCR) to amplify the 16S rRNA gene. This ribosomal gene is essential for all bacteria because it encodes the synthesis of 16S ribosomal RNA which is

involved in one of the subunits of ribosome. I used two universal primers, the primer 27f (5'-AGAGTTTGATCCTGGCTCAG-3')(Zahran et al. 2019) and the primer 1492r (5'-GGTTACCTTGTTACGACTT-3') (Zahran et al. 2019) and the Phusion enzyme (Thermo Fischer Scientific). For each PCR reaction I added 48  $\mu$ L of PCR mix (100  $\mu$ L of Phusion HF buffer (10x), 10  $\mu$ L of dNTP(10mM), 12,5  $\mu$ L of primers 1492r (20mM) and primers 27f (20mM), 340  $\mu$ L of H<sub>2</sub>O sterile and 5  $\mu$ L Phusion) and 2  $\mu$ L of DNA from the isolates. The thermocycler program for the PCR reaction was as follows: 1 minute at 98°C →30 seconds at 98°C (denaturation)→ 30 seconds at 52°C (hybridation)→ 45 seconds at 72°C (elongation)→10 minutes at 72°C. This program followed for 35 cycles. I verified the presence of the target gene (about 1550 bp) using agarose gel electrophoresis and visualized the target bands in comparison to the DNA ladder.

To identify the isolates, I sent the PCR products to Eurofins (Germany) for Sanger sequencing. I entered the FASTA sequences into the NCBI and, using the BLAST tool against the Nucleotide collection (nr/nt) database, I was able to identify the bacterial species of each isolate.

## IV.5 Degradation kinetics of azo dyes

We classified the isolated strains into three categories based on azo dyes degradation rate: fast degraders, moderate degraders, and slow degraders. To do this, it was necessary to measure the kinetic degradation of the dyes over a defined time. In order to measure the degradation of the dyes over time, I used a spectrophotometer (TECAN, Infinite® 200 PRO) to read the absorbance of each dye added to the liquid mGAM. For each of the dyes, I made a set of measurements for different dye concentrations (from 0.05 mM to 1.6 mM) at different wavelengths (from 380 to 580 nm with a gap of 20 between each measurement). I then made the calibration curves for each wavelength and selected the most linear curve based on R<sup>2</sup> value of the linear fit. For the allura red AC dye, I chose the calibration curve with a wavelength at 420 nm and for the tartrazine dye the one at 500 nm (Annexe 3). All the measurements were made at these wavelengths. For monitoring bacterial growth, I measured optical density at 600 nm after shaking the plate for 15 seconds using the spectrophotometer (TECAN). The experimental approach is illustrated in Figure 6.

In total, I selected 30 isolates for the degradation kinetics experiment. For the choice of isolates I selected one strain of each species for each of the faecal extracts for the two dyes. These strains had different azo dye dissipation activities. For each isolate I measured the degradation of the two chosen dyes: allura red AC and tartrazine at the same time. I inoculated 10 $\mu$ l of isolates from the frozen stocks into 1ml of liquid mGAM medium placed in a 96 deep-well plates and incubated them under anaerobic conditions at 37°C overnight. Then I prepared deep-well plates with 1.2 mM tartrazine and or allura AC red dye in 1 mL mGAM per well. For each deep-well, I tested eleven isolates and one negative control without bacteria. For each condition I had three replicates. I prepared a 96-well plate to measure growth in mGAM media without any dyes in triplicates. I added 200 $\mu$ L of liquid mGAM medium without dye as the objective was only to control the growth of each strain. I kept all plates in the anaerobic chamber overnight to reduce the oxygen concentration in the medium prior to experimentation.

I measured the OD<sub>600</sub> of each isolate and then calculated the amount of bacterial culture to be added with 1X PBS buffer in order to obtain a similar OD equal to 0.8 in a final volume of 1 mL. After that, I inoculated 50  $\mu$ L of bacterial culture diluted in 1X PBS buffer into each of the three replicates for each isolate with the same OD (0,8). I also inoculated 10 $\mu$ L of bacterial culture into a microplate (COSTAR, Assay plates, 96 well Flat Bottom, 3370) as a growth control. I then proceeded to the first measurement at time 0 (0 hours). For the growth control, absorbance measurements are directly performed on the microplate. For the deep well, 200 $\mu$ l of the culture in the medium with the dye was taken at each measurement time and transferred to a microplate. An additional centrifugation step (10 minutes at 5000g) was added for times 4, 6 and 24 hours. This step allowed the removal of bacterial biomass that would interfere with the absorbance measurements of the dyes otherwise. The bacterial culture was transferred to a centrifuge plate. After the centrifugation step, I transferred 180  $\mu$ L of supernatant to a microplate without touching the bacterial pellet. (Fig. 6)

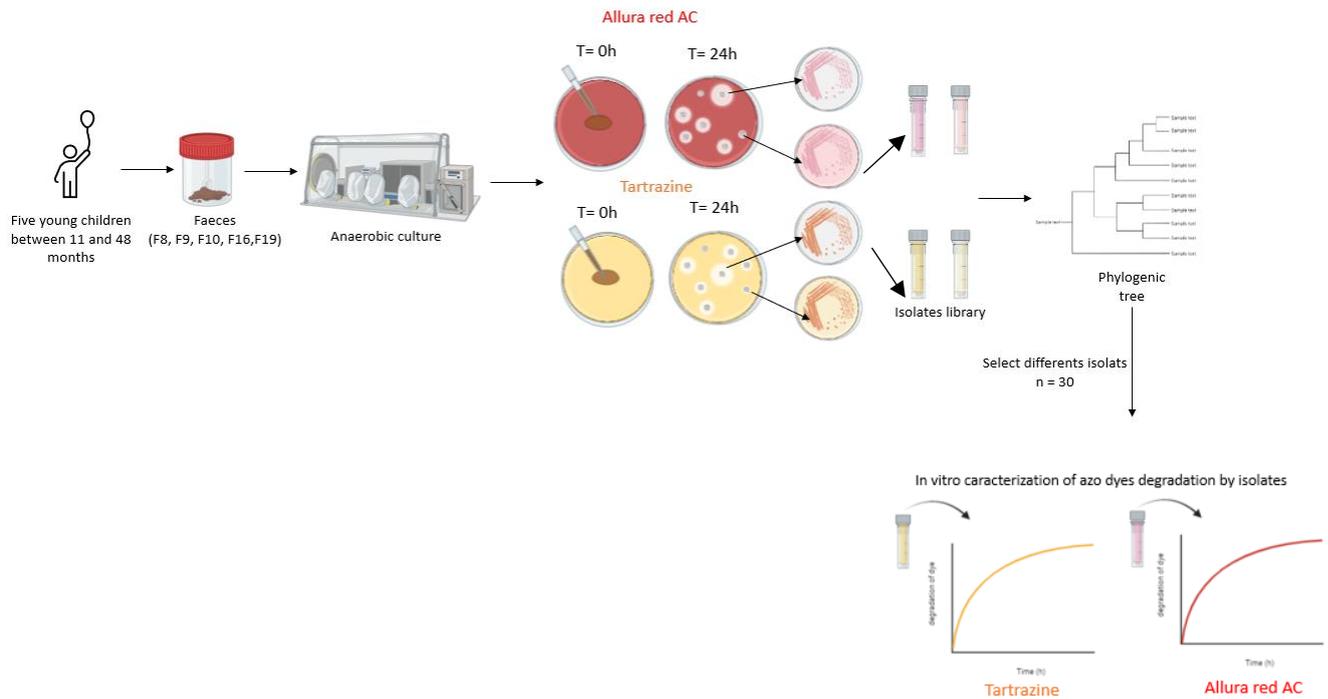


Figure 6. Protocol for the isolation and identification of isolates able to degrade azo dyes (allura red AC and tartrazine).

I optimized this protocol to reduce interference of fermentation gases on the azo dye stability. First, the centrifugation time was doubled (from 5 to 10 minutes) and the isolates were spaced out on the plate and optimized the dye concentration while respecting the absorbance measurement limits to get closer to the recommended doses.

#### IV.6 Impact of abiotic factors on dyes degradation

To assess the impact of various abiotic factors on the interaction between the bacteria and azo dye allura red AC, we selected three abiotic factors: i) the presence or absence of oxygen, ii) pH and iii) vitamins supplementation. For pH, we selected pH 5.5, 6.5, 7 and 7.5 which correspond to the different pH found in the human digestive tract. I adjusted the pH of the culture medium using a base (sodium hydroxide solution) and an acid (hydrochloric acid solution). To test the impact of the presence or absence of oxygen, I performed the experiments at ambient atmosphere and anaerobic chamber (5 % H<sub>2</sub>) environment. Finally, I tested the impact of micronutrients that can act as coenzymes for azoreductase or potential

oxidoreductases, selecting four vitamins: B1, B2, B12 and K1. Each vitamin was added to these media at the same concentration (50 µg/mL).

I selected five isolates for this experiment, two fast degraders (*E. faecalis* F16-Y2 and *P. mirabilis* F10-R1), one moderate degrader (*E. coli* F8-R1) and two slow degraders (*E. gilvus* F19-YC and *E. coli* F19-R3). To culture the isolates, I repeated the same steps as before (part 2: degradation kinetics) (Fig. 7).

We proceeded in the same way as for monitoring dye degradation kinetics. I prepared the deep well plates with azo dye (specific condition for vitamins and pH such as describe previously) and inoculated the wells as previously described. I repeated the same steps with a centrifugation (10 minutes at 5000 g) for times 3, 6 and 24 hours and took the OD measures at the following times: 0, 3, 6 and 24 hours.

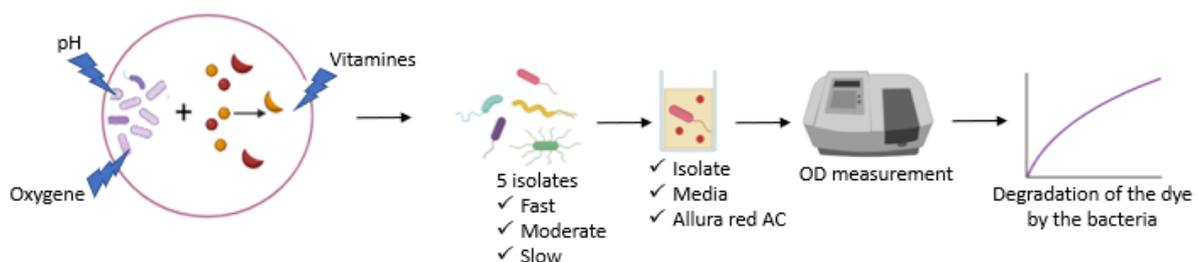


Figure 7. Protocol for testing the impact of different abiotic factors on dyes degradation

#### IV.7 Effect of microbial azo dye metabolism on viability of Hep62 and HT29 cells.

To evaluate the impact of microbial azo dye metabolism on host epithelium, we assessed cell viability using two cell models: Liver cells (HepG2) and colon (HT29) cells. I grew the selected five bacteria (same isolates used in abiotic factors experiment) in mGAM media under anaerobic conditions over night, then grew in the media containing 1,2 mM allura red AC for 24 hours. 1 mL of bacterial culture from each isolate was centrifuged for 5 minutes at 21,300 rpm and bacterial supernatant was filter sterilized using 0,22 µm filters.

The cell culture was done by Dr Esra Ilhan (CRCN in the lab) based on a protocol from a previous research (Ilhan *et al.* 2022). Both cell types (HT29 and HepG2) were cultured in a medium as previously described (Marinelli *et al.* 2019). The cells were then inoculated into a

96-well plate with the same quantity, i.e.,  $3.5 \times 10^4$  cells per well. The plate was incubated overnight. Cell proliferation was monitored using CellTiter 96 Aqueous One (Promega). This was based on the ability of the selected cell lines to reduce tetrazolium compounds. We measured this reduction by spectroscopy with absorbance set at 490 nm (Fig. 8)

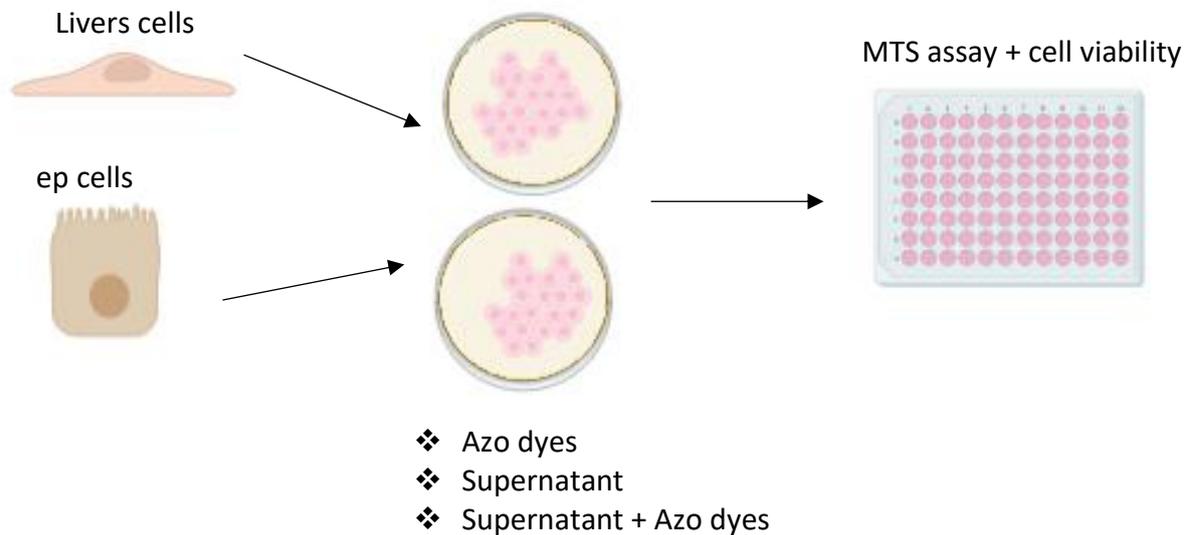


Figure 8. Protocol for to test the toxicity microbial azo dyes metabolism on viability of Hep62 and HT29 cells

## IV.8 Statistics

The data were analysed using Prism software (version 9, GraphPad, Dotmatics). The software was used to carry out statistical tests and heat maps. For the phylogenetic trees, I used Geneious Prime Tool (2023.1 version, Dotmatics co). The BLAST sequences of each isolate were aligned (Multiple alignment, MUSCLE Alignment). To create a phylogenetic tree, I selected HKY genetic distance model and Neighbor-Joining for tree build method. I chose the HKY model because it is a widespread model that gives robust results.

## V. Results

### V.1 Isolation and Identification of azo dyes degrading strains

For this part, I used four azo dyes but the brilliant Black BN was excluded from the study due to rapid abiotic reduction of this dye under anaerobic conditions. Bacteria grew on the media with the brilliant blue FCF but they were not able to degrade it. Therefore, I continued the experiment with only allura red AC and tartrazine. On the BHI and mGAM media, I obtained 82 isolates: 67 on the mGAM medium and 15 on the BHI medium. For the azo dye allura red AC, I obtained 42 isolates and for the azo dye tartrazine, I isolated 40 strains. These isolates belong to four phyla: Firmicutes (n = 38), Bacteroidetes (n = 12), Proteobacteria (n = 29) and Actinobacteria (n = 3), which are the four main phyla of the intestinal microbiota (Lecerf 2021) as visualized on Figure 9. The isolates on the mGAM medium belonged to only Firmicutes and Proteobacteria phyla (Fig. 9A). On the BHI medium, isolates belonged to two other phyla, Bacteroidetes and Actinobacteria (Fig. 9B). The number of isolates was lower for the BHI medium because isolations were carried out for only three faecal samples (F8, F9 and F10).

On Figure 9C, I represented the isolation results on a phylogenetic tree. For Firmicutes we found 37 bacterial isolates belonging to the *Enterococcus* gender, but also species such as *Clostridium perfringens* and *Streptococcus pasteurianus*. Four species of Proteobacteria have been identified: *Escherichia coli*, *Proteus mirabilis*, *Shigella sonnei* and *Escherichia fergusonii*. Within the Actinobacteria phyla, we identified only *Bifidobacterium bifidum*. Finally, within the Proteobacteria phylum, we isolated species such as *Phocaeicola dorei* and *Bacteroides fragilis*. The two species, *E. coli* and *Enterococcus faecalis*, together accounted for half of the isolates obtained, with 22 isolates affiliated to *E. coli* and 19 isolates were *E. faecalis*. The composition of the degraders obtained for each of the faecal samples (F8, F9, F10, F16 and F19) was different. Some species such as *E. coli* or *E. faecalis* were common in all faecal inoculum and *S. pasteurianus* or *Enterococcus gilvus* were specific to F19 faecal inoculum (Fig. 9C).

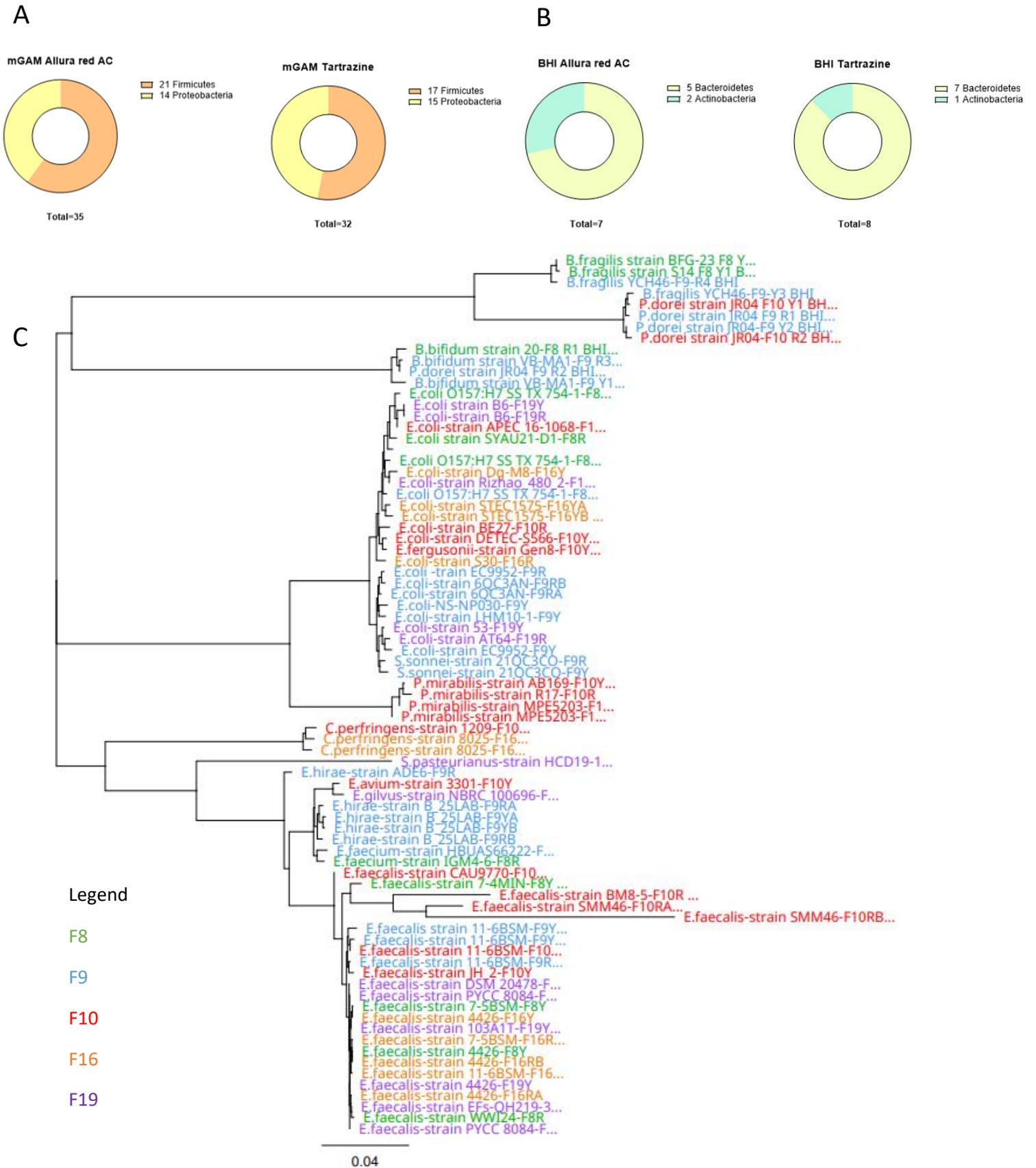


Figure 9. Isolation and identification of isolates obtained on mGAM and BHI media in the presence of allura red AC and tartrazine. (A) distribution of bacterial phyla of isolates obtained on mGAM medium.

(B) distribution of bacterial phyla of isolates obtained on BHI medium. (C) phylogenetic based on HKY substitution model tree of isolates obtained on mGAM and BHI media.

## V.2 Degradation kinetics of azo dyes

I classified isolates into different groups according to their ability to degrade azo dyes. To meet this objective, we measured the degradation of two dyes (allura red AC and tartrazine) over 24 hours for the 30 isolates selected and obtained the following results shown in Figure 10. *E. faecalis* was more efficient in metabolising the dyes compared to other isolates. On the contrary, *E. coli* was the least efficient at metabolising these two dyes. Between these fast metabolisers (*E. faecalis*) and the slow metabolisers (*E. coli*), we observed moderate metabolisers such as *E. hirae* (F9 Y2). For the allura red AC dye, after 24 hours incubation, the *E. faecalis* isolate (F16 Y2), *E. hirae* isolate (F9 Y2) and *E. coli* (F19 R1) achieved 100%, 64% and 22% degradation of the azo dye, respectively (Fig. 10).

All metabolizers isolated from the allura red AC were also capable of metabolizing tartrazine. For the majority of isolated strains (around 64%), allura red AC was degraded more quickly than tartrazine after 24 hours of incubation. However, 10 strains such as *E. faecalis* strain F19 Y2 degrades tartrazine dye (88% degradation after 24 hours of incubation) faster than allura red AC (22% after 24 hours of incubation) (Fig.10).



degradation of allura red AC by these isolates. Degradation capacity of *P. mirabilis* (F10 R1) was not impacted by the presence of oxygen.

### A. Impact of pH

The second selected abiotic factor was the pH. We tested four different pH ranging from an acidic pH of 5.5 to a basic pH of 7.5. A Student's t test was performed for each condition (pH 5.5 ; 6.5 ; 7 and 7.5). There was no significant difference in growth for the isolates *E. coli* (F8 R1) and *E. gilvus* (F19 YC) between each pH tested (5.5, 6.5 and 7.5) and control pH of 7 (Annexe 5). The more acidic the pH, the slower the bacterial metabolism of the dyes in the early hour of the incubation (3 hours). For example, for the *E. gilvus* (F19 YC) isolate after 3 h incubation, the allura red AC was degraded by 9 % at pH 5.5, 12 % at pH 6.5 and 17 % at pH 7.5. The growth of the isolates for different pH conditions were not significantly different (Student's t-Test) and the P values are shown in annexe 5. However, for all isolates except *E. coli* (F19 R3), 100 % degradation was reached more quickly when the pH of the mGAM medium was neutral. The degradation of the allura red AC by the strain *E. faecalis* (F16 Y2) is the same whatever the pH, unlike the other four bacterial strains.

### B. Impact of vitamin supplementation

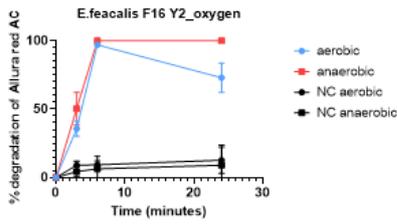
The last abiotic factor tested was vitamins supplementation. I selected four vitamins: B1, B2, B12 and K1. I chose these vitamins because they can act as coenzymes for azoreductases or potential oxidoreductases. I performed a Student's t-test to compare the growth of bacteria in the presence of vitamins and without vitamin supplementation. There was no significant difference between the vitamin-supplemented conditions and the control conditions with the *E. coli* F19 R3 vitamin B12 (Annexe 6). Vitamin B2 supplementation had a positive impact on the metabolic activity of the bacteria in the presence of the allura red AC , as the percentage of degradation was 100 % for the four isolates but not for the strain *P. mirabilis* (F10 R1). The addition of vitamin K1 after 24 hours of incubation gave the following results, the isolate *E. coli* (F19 R3) had a lower degradation 23 % unlike the other four isolates which had degradation rates between 99 % and 100 %. The presence of vitamin K1 was therefore

deleterious for only two of the five isolates. Vitamin K1 had a negative impact on the overall metabolism of the five strains, with degradation rates of between 38 % and 77 % after 24 hours incubation. Finally, vitamin B12 had a negative impact on the metabolism of the *E. coli* F19 Y3 strain, which achieved only 8 % degradation of the allura red AC after 24 hours incubation. For the other four strains, the rate of degradation in the presence of vitamin B12 was between 89 % and 100 %.

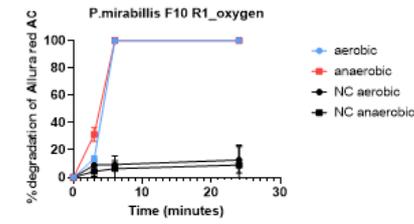
### C. Comparison of abiotic conditions on azo dye degradation

I compared the degradation of allura red AC dye under abiotic conditions (presence of oxygen, supplementation of vitamin and pH) after 24 hours. Under anaerobic conditions, the addition of vitamins K1 and B1 is deleterious to the metabolic activity of bacteria; unlike a condition with the addition of vitamin B2 or the absence of oxygen. Strain *E. faecalis* (F16 Y2), which was identified as a fast dissipater, was able to degrade allura red AC dye in all tested conditions. Strain *E. coli* (F8 R1), which was a medium dissipater, showed intermediate activity when I add vitamin B12 or vitamin B1. In aerobic condition this isolate had a poor metabolism in presence of allura red AC and best metabolism for the rest of the conditions. The absence of oxygen encourages the metabolic activity of bacteria to break down the dye. For conditions with the addition of different vitamins or different pH levels, we can rank from the most favourable condition for metabolic activity to the most unfavourable. Thus, we have the following results: pH 6.5 then pH 7.5 and finally pH 5.5 or 7 which are the two least favourable pH conditions for the degradation of the allura red AC dye. Finally, for the vitamins, we have vitamin B2 first, then K1, then B12 and finally vitamin B1, which is the one with which we obtain the lowest percentages of degradation (Fig. 11).

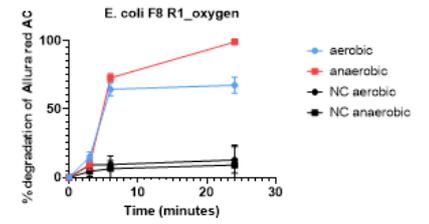
**A**



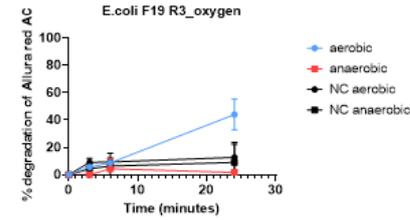
	<i>E. faecalis</i> F16Y2			
	T0	T3	T6	T24
anaerobic	0,08	0,21	0,51	0,48
aerobic	0,08	0,19	0,58	0,5



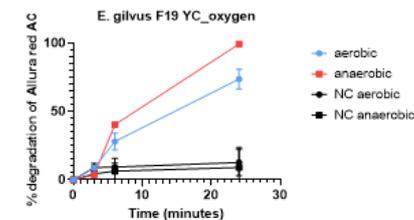
	<i>P. mirabilis</i> F10 R1			
	T0	T3	T6	T24
anaerobic	0,11	0,15	0,65	0,69
aerobic	0,08	0,18	0,68	0,86



	<i>E. coli</i> F8 R1			
	T0	T3	T6	T24
anaerobic	0,08	0,26	0,56	0,55
aerobic	0,08	0,24	0,59	0,67

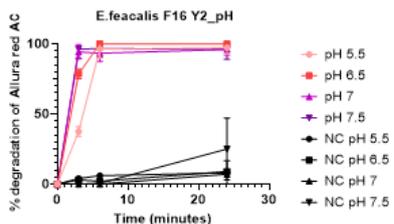


	<i>E. coli</i> F19 R3			
	T0	T3	T6	T24
anaerobic	0,08	0,21	0,39	0,51
aerobic	0,08	0,18	0,4	0,68

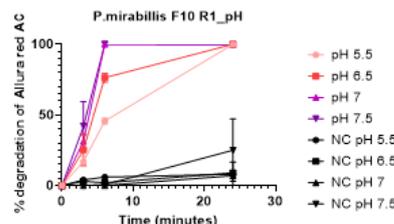


	<i>E. gilvus</i> F19 YC			
	T0	T3	T6	T24
anaerobic	0,08	0,18	0,54	0,65
aerobic	0,08	0,15	0,51	0,7

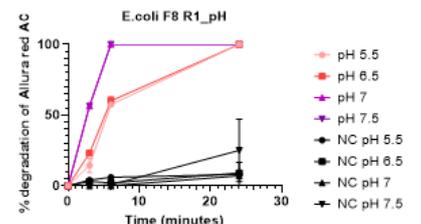
**B**



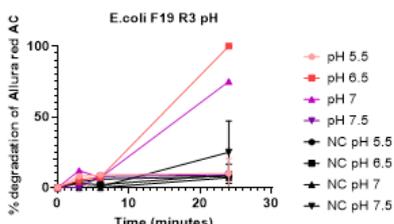
	<i>E. faecalis</i> F16 Y2			
	T0	T3	T6	T24
pH 5.5	0,07	0,1	0,38	0,35
pH 6.5	0,07	0,18	0,54	0,62
pH 7	0,12	0,28	0,57	0,62
pH 7.5	0,08	0,21	0,52	0,53



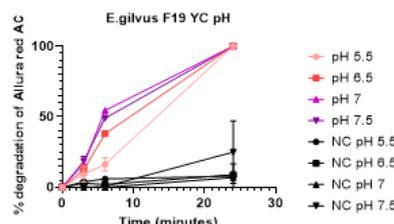
	<i>P. mirabilis</i> F10 R1			
	T0	T3	T6	T24
pH 5.5	0,2	0,14	0,61	0,73
pH 6.5	0,24	0,14	0,42	0,42
pH 7	0,11	0,15	0,65	0,69
pH 7.5	0,14	0,17	0,65	0,66



	<i>E. coli</i> F8 R1			
	T0	T3	T6	T24
pH 5.5	0,07	0,09	0,29	0,51
pH 6.5	0,07	0,21	0,59	0,55
pH 7	0,12	0,33	0,56	0,59
pH 7.5	0,07	0,27	0,55	0,58



	<i>E. coli</i> F19 R3			
	T0	T3	T6	T24
pH 5.5	0,07	0,08	0,11	0,24
pH 6.5	0,07	0,14	0,33	0,36
pH 7	0,12	0,22	0,41	0,46
pH 7.5	0,07	0,19	0,43	0,43



	<i>E. gilvus</i> F19 YC			
	T0	T3	T6	T24
pH 5.5	0,07	0,08	0,21	0,54
pH 6.5	0,07	0,12	0,43	0,63
pH 7	0,11	0,25	0,53	0,66
pH 7.5	0,07	0,13	0,5	0,67

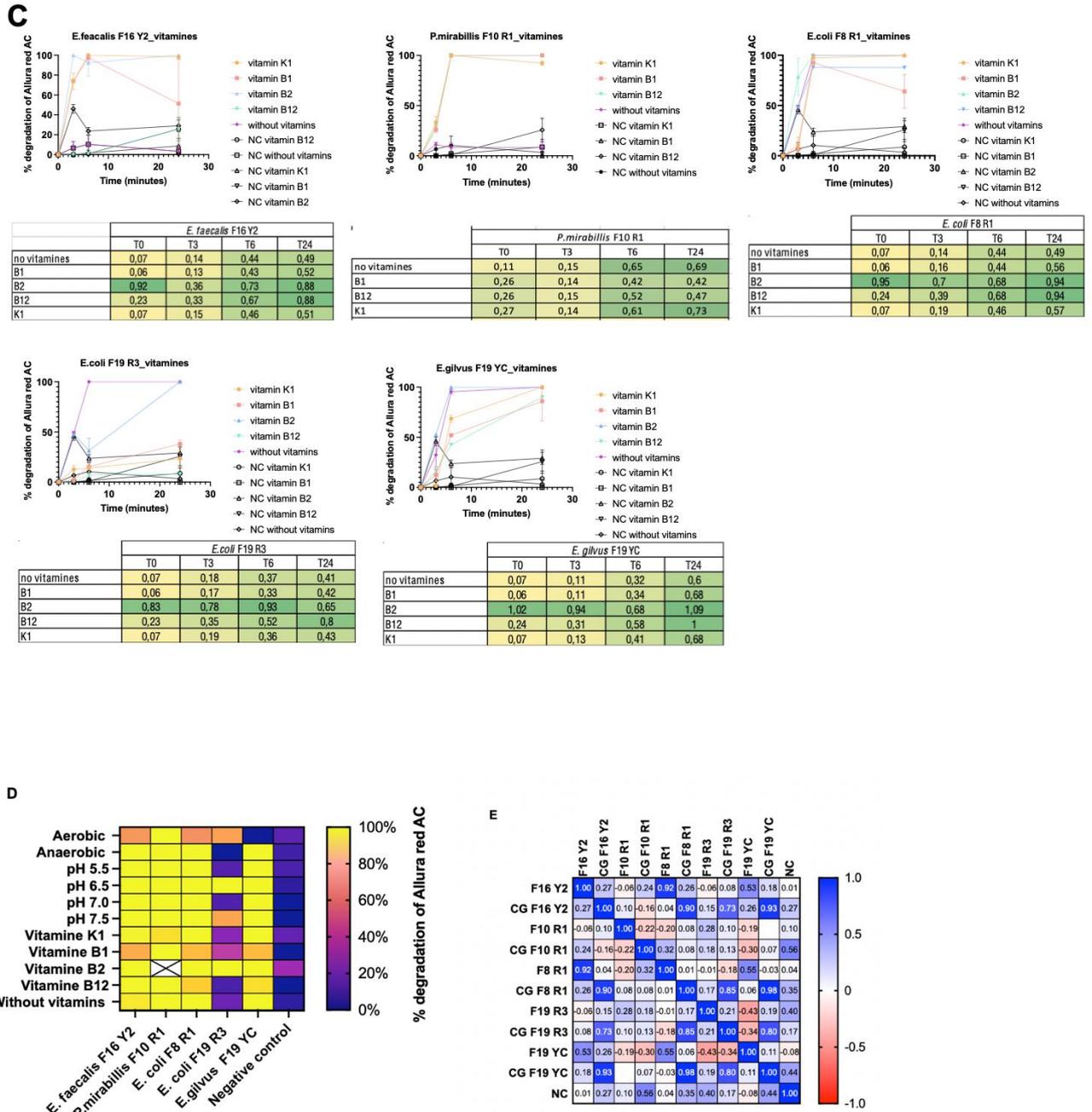


Figure 11. Impact of abiotic factors on bacterial metabolism in the presence of allura red AC. (A) impact of oxygen presence/absence on bacterial metabolism in the presence of allura red AC. (B) impact of pH on bacterial metabolism in the presence of allura red AC. (C) impact of vitamin supplementation on bacterial metabolism in the presence of allura red AC dye. (D) Impact of abiotic factors for the 5 isolates on the dye degradation 24 hours of incubation in presence of allura red AC. (E) Test of correlation between the bacteria growth and the dye degradation activity for all condition and all isolates after 24 hours of incubations.

## D. Effect of microbial azo dyes metabolism on viability of Hep62 and HT29 cells

I evaluated the influence of bacterial supernatants from five cultures (*E. faecalis* F16 Y2, *P. mirabilis* F10 R1, *E. coli* F8 R1, *E. coli* F19 R3 and *E. gilvus* F19 YC) treated with Allura Red (1.2 mM) or tartrazine (1.2 mM) and with bacterial supernatants with not azo dye on viability of the colon (HT29) and liver (HepG2) cells. Figure 12A and D show that the dye (tartrazine or allura red AC) or the mGAM medium alone or the combination of the two does not impact significantly the metabolic activity to reduce tetrazolium dye MTT of liver cells (HepG2) (Fig. 12A) or colon cells (HT29) (Fig. 12D).

For liver cells (HepG2), bacterial supernatant combined with allura red AC dye (Fig.12B) significantly reduced the viability (MTS assay,  $p = 0,0001$ ) in comparison to bacterial supernatant alone. But for the azo dye tartrazine, there was no significant difference (with  $p$  value less than  $p = 0,0797$ ) in the viability of liver cells between the condition with bacterial supernatant alone and that with bacterial supernatant associated to tartrazine (Fig. 12C).

For liver cells (HT29), there was no significant difference ( $p = 0,8699$ ) in the viability of liver cells between the condition with bacterial supernatant alone and that with bacterial supernatant combined with allura red AC (Fig. 12E). or tartrazine (Fig. 12F).

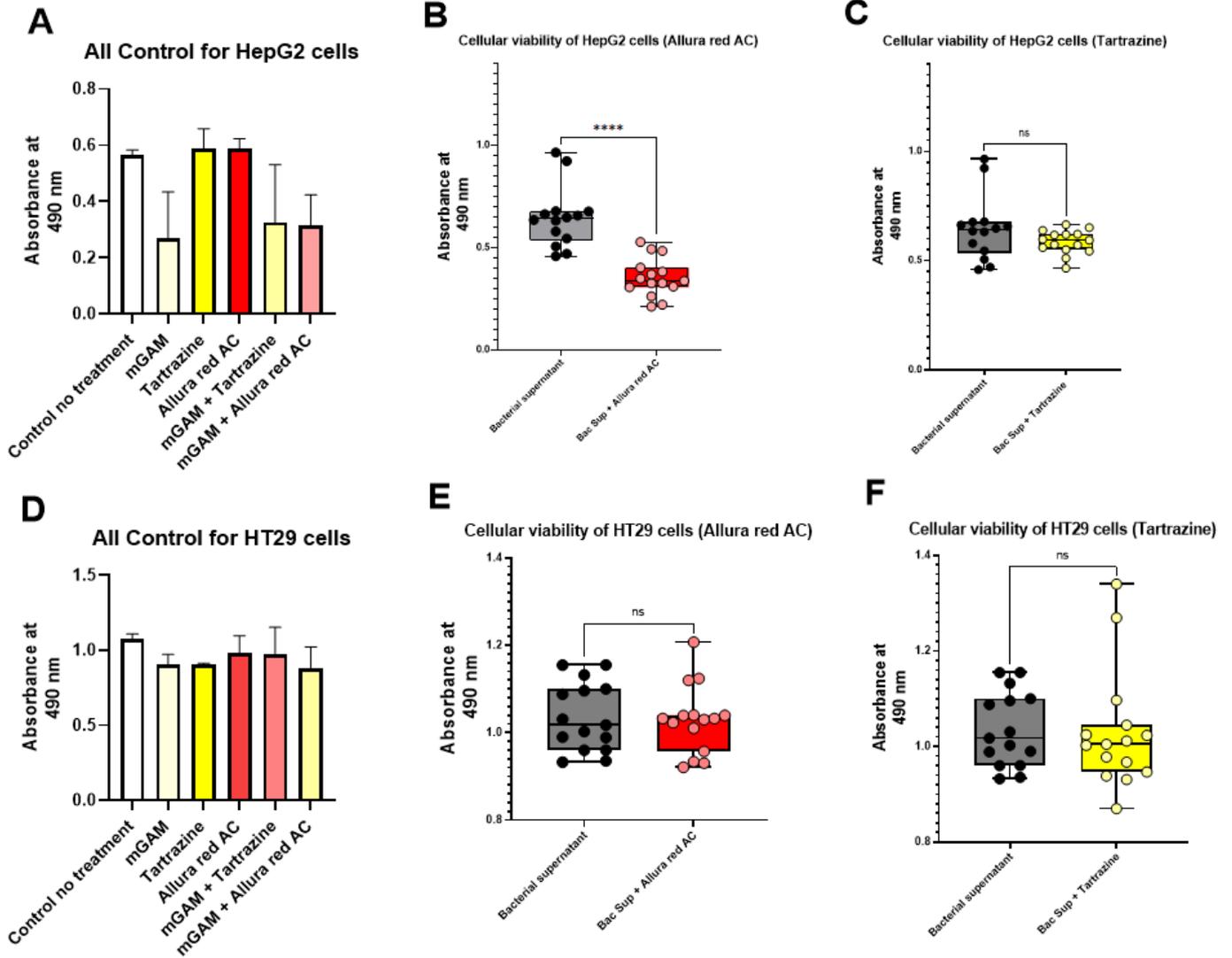


Figure 12. MTS assay to test the effect of bacterial supernatant with the presence of allura red AC or tartrazine on liver and colon cells. (A) Set of controls for liver cells. (B) viability of liver cells HepG2 in the presence of allura red AC. (B) Viability of liver cells HepG2 in the presence of allura red AC + bacterial overnegat VS in presence of bacterial supernatant only. (C) Viability of liver cells HepG2 in the presence of tartrazine + supernatant bacterium VS only in the presence of supernatant bacterium.

## VI. Discussion

The human gut microbiota is composed of approximately 4,300 bacterial species (Lecerf, 2021). This ecosystem will change throughout our lives and will be affected by various factors such as the presence of additives in food (i.e., azo dyes) or food containing vitamins, sugars and other elements (Lecerf, 2021). Previous studies have shown that members of the human gut microbiota are capable of degrading the azo dyes. Zahran's team identified three bacteria of human gut microbiota with azoreductase enzyme : *E. coli* (AZo-ec), *E. faecalis* (AZo-ef) and *E. avium* (Zahran *et al.* 2019). Other studies demonstrated that *Clostridium perfringens* and *Bacteroides uniformis* (Nakamura *et al.* 2002) or *P. mirabillis* was also capable of degrading capacity to degrade azo dyes (Olukanni *et al.* 2010). My results on isolation of azo dyes degraders were consistent with the previously identified azo dye degrading microorganisms because all the strains isolated have already been identified in previous research work.

The abiotic factors can have an impact on the metabolic activities mediated by the intestinal microbiota and act directly on the enzymes involved in bacterial metabolism (Zahran *et al.* 2019). For example the azoreductase produced by the organism *E.coli* are more efficient in aerobic condition in comparison of microaerophilic condition with percentages of 66.67 % and 26.06 % respectively after 5 hours in contact with the dye (Zahran *et al.* 2019). For the *Enterococcus* strains used in the study, the degradation of the dye was less significant in basic conditions (at pH 8) than in conditions with an acidic or neutral pH (Zahran *et al.* 2019). A pH greater than 6 but lower than 8 is favorable to the degradation activity of *Enterococcus* strains. However, for the metabolic activity of the strain I isolated, *Enterococcus* F16 Y2 was not impacted by the pH variations. Vitamins may also impact the azoreductase activity as they serve as coenzymes for azoreductases and other oxidoreductases that might be involved in the metabolism (Rishavy *et al.* 2011), (Kräutler, 2012). The addition of vitamin B2 promotes the bacterial activity that breaks down allura red AC. In contrast, a condition without vitamin supplementation is unfavourable to degradation activity. The B2 vitamin plays an essential role for azoreductases because it is the coenzymes FMN and FAD. To promote the microbial metabolism of the azo dyes, it will be necessary to supplement our diet with vitamin B2. Vitamin B2 can be found in eggs, salmon or milk (Avenue *et al.* 2020). These results highlight the importance of considering abiotic factors in the evaluation of azo dye metabolism.

Azo dye metabolism can also have negative health implications. A study has shown that this metabolism is toxic to our (Borzelleca, Depukat, et Hallagan 1990). I was able to show that bacterial supernatant in the presence of the red azo dye allura AC reduces the viability of HepG2 liver cells. The cleavage of the azo bonds (-N=N-) of the azo dyes are maybe responsible for the production of colourless aromatic amines that are potentially harmful to human health (Chung *et al.* 1992) (Zahran *et al.* 2019). A recent study proved that chronic exposition to textile azo dyes is linked to bladder cancers in textile industry workers (Singh and Chadha, 2016). Many studies denounced the genotoxic effect of the chemical molecules such as azo dyes and their implication on different types of cancers (Singh and Chadha, 2016). Our results showed that bacterial degradation of azo dye may produce adverse intestinal health effects. Further studies testing the impact of azo dyes degradation on animals model would provide insights into organisms-wide health effects.

My study has some limitations: the degradation profiles were limited to only a few strains that were culturable. Future studies that expand on isolation and characterization of various azo dyes are needed to better understand capacity of the gut microbiota to transfer azo dyes. The identification of the species and bacteria phyla was based on the 16S rRNA gene. It is possible to use more comprehensive methods such as Shotgun sequencing or MALDI-TOF to identify the bacteria with greater reliability during my internship.

I tested three abiotic factors but in reality, the human gut microbiota is affected by more than three abiotic factors. Different carbon sources such as glucose, fructose, lactose and sucrose might influence bacterial growth and hence azo dye metabolism. Moreover, it should not be forgotten that in reality the azo dyes used in the food industry are found in a food matrix (lollipop, cake, etc.), therefore, more complex food matrix effects needs to be further considered.

## VII. Conclusion

Bacteria isolated from children's intestinal microbiota were able to degrade the allura red AC dye between 5 and 100 % and tartrazine dye between 11 and 100 % after 24 hours of incubation. The strains of *Enterococcus faecalis* were rapid degraders are part of the rapid dissipators in contrast to the *E. coli* strains were slow degraders. Some of the abiotic factors I tested including vitamin B2, pH at 6.5 positively impacted the degradation of the allura red AC dye. The presence of oxygen or the addition of vitamin B1 had the greatest negative impact on the dissipation of the allura red AC azo dye because they reduced the azo dye metabolism of all five isolates. The presence of oxygen had the greatest negative impact on the dissipation of the azo dye allura red AC. Finally, bacterial supernatant in the presence of allura red dye decreased liver cell viability. However, we did not see any effect on the viability of liver cells in the presence of tartrazine dye, nor for colon cells, regardless of the dye to which the bacterial supernatant was exposed.

These dyes have been shown to be potentially harmful to human health, with risks of developing attention disorders such as ADHD in young children (WARD 1997). the cleavage of the azo bond by bacteria is toxic for us. It would therefore be interesting to understand how to avoid the production of metabolites that are toxic to our bodies, or how to avoid the production of azoreductases within our microbiota. It is therefore crucial to better define the bacteria involved in the degradation of azo dyes. We need to define the impact of abiotic factors on metabolism.

## VIII. References

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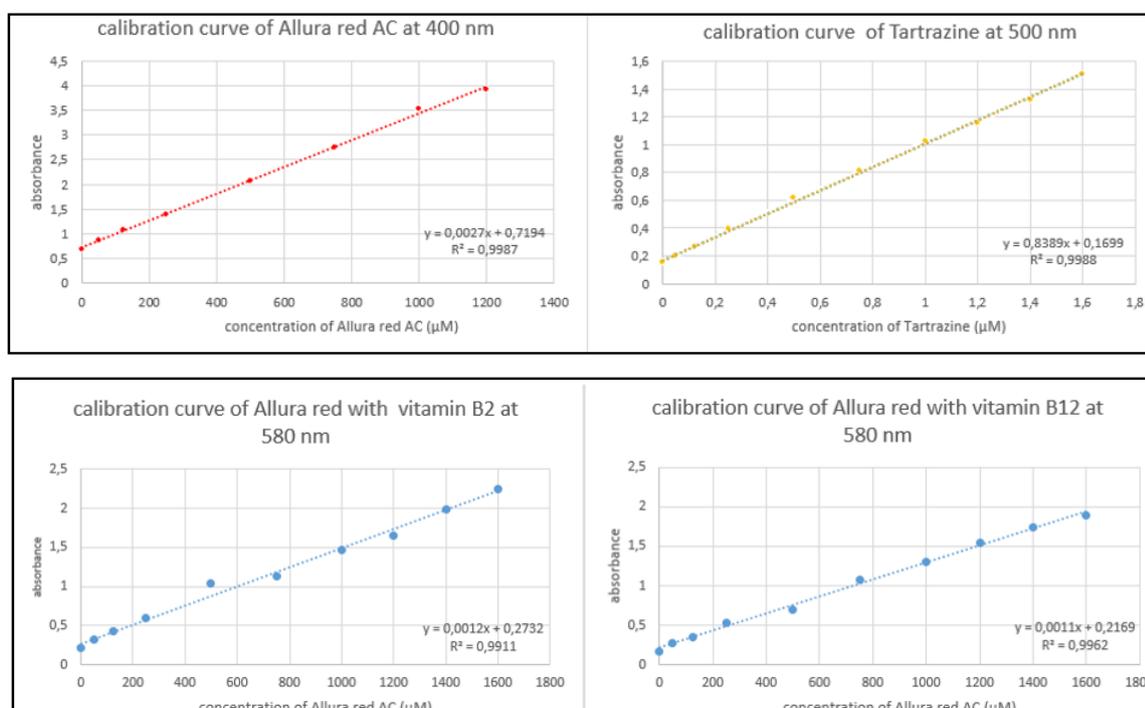
## VI. Annexes

### Annexe 1. Demographic characteristics of the faecal samples' donors.

Name of faecal extract	Age (in months)	Gender
F8	42	Male
F9	48	Male
F10	30	Female
F16	36	Female
F19	11	Male

### Annexe 2. Description (color, molecular weight, formula and solubility in water) of the four azo dyes tartrazine, allura red AC, Brilliant blue FCF and Brilliant black BN.

Azo dyes	Color	molecular weight	Formula	Solubility in water
tartrazine	Yellow	534.36	C <sub>16</sub> H <sub>9</sub> N <sub>4</sub> Na <sub>3</sub> O <sub>9</sub> S <sub>2</sub>	20 g/100 mL
allura red AC	Red	496.42	C <sub>18</sub> H <sub>14</sub> N <sub>2</sub> Na <sub>2</sub> O <sub>8</sub> S <sub>2</sub>	22 g/100 mL
brilliant blue FCF	Blue	789.85	C <sub>37</sub> H <sub>34</sub> N <sub>2</sub> Na <sub>2</sub> O <sub>9</sub> S <sub>3</sub>	22 g/100 mL
brilliant black BN	Black	867.68	C <sub>28</sub> H <sub>17</sub> N <sub>5</sub> Na <sub>4</sub> O <sub>14</sub> S <sub>4</sub>	100 mg/100 mL



### Annexe 3. Calibration curve (A) for allura red AC and tartrazine dye and (B) for allura red AC with vitamin B2 or vitamin B12.

Annexe 4. Student's t-test results for control growth under aerobic and anaerobic conditions.

Isolates	Result	P value
F16 Y2 anaerobic VS aerobic	ns	0,91
F10 R1 anaerobic VS aerobic	ns	0,91
F8 R1 anaerobic VS aerobic	ns	0,86
F19 R3 anaerobic VS aerobic	ns	0,82
F19 YC anaerobic VS aerobic	ns	0,99

ns = non-significant

Annexe 5. Student's t-test results for control growth in different conditions of pH (5.5; 6.5; 7.0 and 7.5)

Isolates	Result	P value
F16 Y2 pH5.5 VS pH7.0	*	0,31
F10 R1 pH5.5 VS pH7.0	ns	0,62
F8 R1 pH5.5 VS pH7.0	ns	0,06
F19 R3 pH5.5 VS pH7.0	*	0,05
F19 YC pH5.5 VS pH7.0	ns	0,07
F16 Y2 pH6.5 VS pH7.0	ns	0,12
F10 R1 pH6.5 VS pH7.0	ns	0,83
F8 R1 pH6.5 VS pH7.0	ns	0,23
F19 R3 pH6.5 VS pH7.0	**	0,005
F19 YC pH6.5 VS pH7.0	ns	0,05
F16 Y2 pH7.5 VS pH7.0	**	0,006
F10 R1 pH7.5 VS pH7.0	ns	0,94
F8 R1 pH7.5 VS pH7.0	ns	0,08
F19 R3 pH7.5 VS pH7.0	ns	0,22
F19 YC pH7.5 VS pH7.0	ns	0,19

ns = non-significant

Annexe 6. Student's t-test results for control growth with the supplementation of vitamins (B1, B2, B12 and K1).

Isolates	Result	P value
F16 Y2 B1 VS no vitamins	ns	0,73
F16 Y2 B2 VS no vitamins	ns	0,97
F16 Y2 B12 VS no vitamins	ns	0,97
F16 Y2 K1 VS no vitamins	ns	0,98
F10 R1 B1 VS no vitamins	ns	0,97
F10 R1 B12 VS no vitamins	ns	0,98
F10 R1 K1 VS no vitamins	ns	0,94
F8 R1 B1 VS no vitamins	ns	0,55
F8 R1 B2 VS no vitamins	ns	0,90
F8 R1 B12 VS no vitamins	ns	0,58
F8 R1 K1 VS no vitamins	ns	0,56
F19 R3 B1 VS no vitamins	ns	0,06
F19 R3 B2 VS no vitamins	ns	0,24
F19 R3 B12 VS no vitamins	*	0,02
F19 R3 K1 VS no vitamins	ns	0,05
F19 YC B1 VS no vitamins	ns	0,40
F19 YC B2 VS no vitamins	ns	0,76
F19 YC B12 VS no vitamins	ns	0,46
F19 YC K1 VS no vitamins	ns	0,56

ns = non-significant

## Convention de stage n° 104372 entre

*Nota : pour faciliter la lecture du document, les mots "stagiaire", "enseignant référent", "tuteur de stage", "représentant légal", et "étudiant" sont utilisés au masculin.*

<b>1 - L'ÉTABLISSEMENT D'ENSEIGNEMENT ou DE FORMATION</b>	<b>2 - L'ORGANISME D'ACCUEIL</b>
<p><b>Nom :</b> Université de Lorraine  <b>Adresse :</b> CS 25233 54052 Nancy  <b>Tél :</b>  <b>Représenté par (signataire de la convention) :</b>            Frédéric HAMELIN  <b>Qualité du représentant :</b> Administrateur provisoire de la Faculté des Sciences et Technologies  <b>Composante/UFR :</b> MASTER MICROBIOLOGIE - M2  <b>Adresse (si différente de celle de l'établissement) :</b> Campus Aiguillettes - BP 70239 54506 VANDOEUVRE-LES-NANCY CEDEX  <b>Tél :</b> 03 72 74 51 39  <b>Mél :</b></p>	<p><b>Nom :</b> INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE  <b>Adresse :</b> Inra 147 rue de l'Université 75007 PARIS 07EME FRANCE  <b>Représenté par (nom du signataire de la convention) :</b>            Mme TOUZE Nathalie  <b>Qualité du représentant :</b> Présidente du centre de recherche INRAE de Jouy-en-Josas-Antony  <b>Service dans lequel le stage sera effectué :</b>            UNITÉ MICALIS – BÂTIMENT 442  <b>Tél :</b> 04 73 62 40 00  <b>Mél :</b>  <b>Lieu du stage (si différent de l'adresse de l'organisme) :</b> Domaine de Vilvert 78352 JOUY-EN-JOSAS FRANCE</p>

<b>3 - LE STAGIAIRE</b>
<p><b>Nom :</b> REZER    <b>Prénom :</b> Emilie    <b>Sexe :</b> F    <b>Né(e) le :</b> 13/04/2000    <b>Numéro d'étudiant :</b> 31802034  <b>Adresse :</b> 11 RUE DU CHATEAU 88700 JEANMENIL FRANCE  <b>Tél :</b> 0750490463    <b>Portable :</b> +33750490463    <b>Mél :</b> emilie.rezer1@etu.univ-lorraine.fr  <b>INTITULÉ DE LA FORMATION OU CURSUS SUIVI DANS L'ÉTABLISSEMENT D'ENSEIGNEMENT SUPÉRIEUR ET VOLUME HORAIRE (ANNUEL OU SEMESTRIEL) :</b>            M2-Microbiologie (NANCY) (FI)  <b>Nombre d'heures de formation :</b> Plus de 200 heures.</p>

<p><b>SUJET DE STAGE :</b> Impact des facteurs biotiques et abiotiques sur des communautés microbiennes synthétiques.  <b>Dates :</b> du 09/01/2023 au 30/06/2023  <b>Correspondant à 840 heures de présence effective dans l'organisme d'accueil et représentant une durée totale de 5 mois 10 jour(s) et 0 heure(s)</b>  <b>Commentaire :</b></p>
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<b>Encadrement du stagiaire par l'établissement d'enseignement</b>	<b>Encadrement du stagiaire par l'organisme d'accueil</b>
<p><b>Nom et prénom de l'enseignant référent :</b>            JORAND Frédéric  <b>Tél :</b> +33 3 72 74 72 14  <b>Mél :</b> frederic.jorand@univ-lorraine.fr</p>	<p><b>Nom et prénom du tuteur de stage :</b>            LEPAGE Patricia  <b>Fonction :</b> Directrice de recherche, Responsable de l'équipe Phylogénie et Physiologie du Microbiome Humain  <b>Tél :</b> 0134652009  <b>Mél :</b> patricia.lepage@inrae.fr</p>

<p>Caisse Primaire d'Assurance Maladie à contacter en cas d'accident (lieu de domicile de l'étudiant sauf exception) :</p>
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Vu le code de la santé publique notamment ses articles L. 3131-1 et suivants ;  
Vu le code de l'éducation, notamment ses articles L. 124-1 à L. 124-20, L. 612-11 et D. 124-1 à D. 124-9 ; D714-21 et suivants ;  
Vu le code de la sécurité sociale et notamment les articles L. 242-4-1, L. 412-8 et D. 242-2-1 ;  
Vu le code du travail et notamment ses articles L. 1221-13 et D. 1221-23 et suivants ;  
Vu le Décret n° 2020-1257 du 14 octobre 2020 déclarant l'état d'urgence sanitaire  
Vu le PROTOCOLE NATIONAL POUR ASSURER LA SANTÉ ET LA SÉCURITÉ DES SALARIÉS FACE À L'ÉPIDÉMIE DE COVID-19  
Vu la consultation de la Commission de la Formation et de la Vie Universitaire et l'avis du Conseil d'administration de l'établissement ;

Préalable :

**Stages en France :** Compte-tenu des circonstances exceptionnelles dues à la pandémie de Covid 19, il est entendu entre les parties qu'elles auront vérifié au préalable que les missions confiées au stagiaire se prêtent à un travail en présentiel et/ou à distance et qu'il dispose du matériel adéquat.

Les tuteurs vérifient la possibilité d'utilisation par les parties d'outils de communication adéquats.

Le stage ne pourra être réalisé en présentiel que dans le strict respect du protocole national pour assurer la santé et la sécurité des salariés en entreprise face à l'épidémie de Covid-19 et de toute disposition hygiène, sécurité et santé applicable à l'organisme d'accueil.

Il est entendu entre les parties qu'en cas de confinement total, le stage basculera automatiquement en stage à distance ou fera l'objet d'une suspension par avenant en cas d'impossibilité de stage à distance.

Les stages des étudiants des filières médicales et paramédicales font l'objet de dispositions spécifiques.

Une visite médicale sera automatiquement organisée pour tous les étudiants exposés à un risque pendant leur stage.

Contact de la médecine préventive : \_\_\_\_\_

**Stages à l'étranger :** Compte-tenu des circonstances exceptionnelles dues à la pandémie de Covid 19, il est entendu entre les parties qu'elles auront vérifié au préalable que :

- Si le stage a lieu en présentiel, une assurance spécifique rapatriement est contractée par le stagiaire pour le retour, en cas de confinement ou d'autres circonstances rendant impossible la poursuite du stage.
- Les missions confiées au stagiaire se prêtent à un travail en présentiel et/ou à distance et qu'il dispose du matériel adéquat.

Les tuteurs vérifient la possibilité d'utilisation par les parties d'outils de communication adéquats.

Le stage ne pourra être réalisé en présentiel que dans le strict respect de toute disposition hygiène, sécurité et santé applicable à l'organisme d'accueil.

Il est entendu entre les parties qu'en cas de confinement, le stage basculera automatiquement en stage à distance ou fera l'objet d'une suspension par avenant en cas d'impossibilité de stage à distance, en complément des modalités de rapatriement.

Le stagiaire s'engage sur l'honneur par la présente à se signaler avant son départ sur ARIANE : <https://pastel.diplomatie.gouv.fr/fildariane/dyn/public/login.html>

Le stagiaire atteint du Covid19 pendant son stage devra respecter les conditions prescrites dans le pays d'accueil, notamment en termes de quarantaine. L'établissement d'enseignement ne pourra pas être tenu de rapatrier le stagiaire.

Une visite médicale sera automatiquement organisée pour tous les étudiants exposés à un risque pendant leur stage.

Contact de la médecine préventive : \_\_\_\_\_

Contact en cas d'urgence : (autre que le stagiaire) : \_\_\_\_\_

## Article 1 - Objet de la convention

La présente convention règle les rapports de l'organisme d'accueil avec l'établissement d'enseignement et le stagiaire.

## Article 2 - Objectif du stage

Le stage correspond à une période temporaire de mise en situation en milieu professionnel au cours de laquelle le stagiaire acquiert des compétences professionnelles et met en œuvre les acquis de sa formation en vue de l'obtention d'un diplôme ou d'une certification et de favoriser son insertion professionnelle. Le stagiaire se voit confier une ou des missions conformes au projet pédagogique défini par son établissement d'enseignement et approuvées par l'organisme d'accueil.

Le programme est établi par l'établissement d'enseignement et l'organisme d'accueil en fonction du programme général de la formation dispensée.

### ACTIVITÉS CONFIEES :

Evaluer la stabilité de communautés synthétiques microbiennes constituées in vitro et de déterminer les perturbations induites par l'ajout de différents agents biotiques ou abiotiques.

### Compétences à acquérir ou à développer :

Les compétences acquises feront appel à la microbiologie anaérobie et au suivi de populations microbiennes par des approches de biologie moléculaires et de métaboliques.

## Article 3 - Modalités du stage

La durée hebdomadaire de présence du stagiaire dans le lieu désigné dans l'entête sera de **35.00 heures** sur la base d'un **Temps Plein**.

Il est interdit de confier au stagiaire des tâches dangereuses pour sa santé ou sa sécurité.

Si le stagiaire doit être présent dans l'organisme d'accueil ou dans le lieu désigné dans l'entête la nuit, le dimanche ou un jour férié, préciser les cas particuliers :

## Article 4 - Accueil et encadrement du stagiaire

Le stagiaire est suivi par l'enseignant référent désigné dans la présente convention ainsi que par le service de l'établissement en charge des stages.

Le tuteur de stage désigné par l'organisme d'accueil dans la présente convention est chargé d'assurer le suivi du stagiaire et d'optimiser les conditions de réalisation du stage conformément aux stipulations pédagogiques définies.

MODALITÉS D'ENCADREMENT (visites, rendez-vous téléphoniques, etc..)

Si les conditions sanitaires le permettent : d'une part, le stagiaire est autorisé à revenir dans son établissement d'enseignement pendant la durée du stage pour y suivre des cours demandés explicitement par le programme, ou pour participer à des réunions (les dates sont portées à la connaissance de l'organisme d'accueil par l'établissement), d'autre part, l'organisme d'accueil peut autoriser le stagiaire à se déplacer, sauf pour les stages à l'étranger.

Toute difficulté survenue dans la réalisation et le déroulement du stage, qu'elle soit constatée par le stagiaire ou par le tuteur de stage, doit être portée à la connaissance de l'enseignant-référent et de l'établissement d'enseignement afin d'être résolue au plus vite.

## Article 5 - Gratification - Avantages

En France, lorsque la durée du stage est supérieure à 308 heures consécutives ou non, celui-ci fait obligatoirement l'objet d'une gratification, sauf en cas de règles particulières applicables dans certaines collectivités d'outre-mer françaises et pour les stages relevant de l'article L4381-1 du code de la santé publique.

Le montant horaire de la gratification est fixé à 15% du plafond horaire de la sécurité sociale défini en application de l'article L.241-3 du code de la sécurité sociale. Une convention de branche ou un accord professionnel peut définir un montant supérieur à ce taux.

La gratification due par un organisme de droit public ne peut être cumulée avec une rémunération versée par ce même organisme au cours de la période concernée.

La gratification est due sans préjudice du remboursement des frais engagés par le stagiaire pour effectuer son stage et des avantages offerts, le cas échéant, pour la restauration, l'hébergement, et le transport.

L'organisme peut décider de verser une gratification pour les stages dont la durée est inférieure ou égale à deux mois.

En cas de suspension ou de résiliation de la présente convention, le montant de la gratification due au stagiaire est proratisé en fonction de la durée du stage effectué.

La durée donnant droit à gratification s'apprécie compte tenu de la présente convention et de ses avenants éventuels, ainsi que du nombre de jours de présence effective du stagiaire dans le lieu indiqué.

LE MONTANT DE LA GRATIFICATION est fixé à ~~4,05~~ euros Net par heure. *4,05 euros net par heure.*

Modalités de versement de la gratification : **Inconnue**

### Article 5 bis - STAGES EN FRANCE Accès aux droits des salariés - Avantages

(Organisme de droit privé en France sauf en cas de règles particulières applicables dans certaines collectivités d'outre-mer françaises) :

Le stagiaire bénéficie des protections et droits mentionnés aux articles L.1121-1, L.1152-1 et L.1153-1 du code du travail, dans les mêmes conditions que les salariés.

Le stagiaire a accès au restaurant d'entreprise ou aux titres-restaurants prévus à l'article L.3262-1 du code du travail, dans les mêmes conditions que les salariés de l'organisme d'accueil. Il bénéficie également de la prise en charge des frais de transport prévue à l'article L.3261-2 du même code.

Le stagiaire accède aux activités sociales et culturelles mentionnées à l'article L.2323-83 du code du travail dans les mêmes conditions que les salariés.

L'organisme d'accueil s'engage à respecter toute directive sanitaire nationale ou sectorielle.

### Article 5ter - STAGES EN FRANCE Accès aux droits des agents - Avantages

(Organisme de droit public en France sauf en cas de règles particulières applicables dans certaines collectivités d'outre-mer françaises) :

Les trajets effectués par le stagiaire d'un organisme de droit public entre son domicile et son lieu de stage sont pris en charge dans les conditions fixées par le décret n°2010-676 du 21 juin 2010 instituant une prise en charge partielle du prix des titres d'abonnement correspondant aux déplacements effectués par les agents publics entre leur résidence habituelle et leur lieu de travail.

Le stagiaire accueilli dans un organisme de droit public et qui effectue une mission dans ce cadre bénéficie de la prise en charge de ses frais de déplacement temporaire selon la réglementation en vigueur.

Est considéré comme sa résidence administrative le lieu du stage indiqué dans la présente convention.

### Article 6 - Régime de protection sociale (maladie et accidents)

Pendant la durée du stage, le stagiaire reste affilié à son régime de Sécurité sociale antérieur. Il doit impérativement vérifier ses conditions de couverture maladie et fournir une attestation de couverture à son établissement d'enseignement au moment de la signature de la convention de stage et dans tous les cas avant le départ.

#### 6.1 - Gratification d'un montant maximum de 15% du plafond horaire de la Sécurité sociale - protection par la France (voir conditions article 6.4):

La gratification n'est pas soumise à cotisation sociale conformément aux termes de l'article R412-4 du code de la Sécurité sociale.

Le stagiaire bénéficie de la législation sur les accidents de travail au titre de l'article L.412-8 2° du code de la Sécurité sociale.

En cas d'accident ou de maladie professionnelle survenant au stagiaire soit au cours d'activités dans l'organisme, soit au cours du trajet, soit sur les lieux rendus utiles pour les besoins du stage et pour les étudiants en médecine, en chirurgie dentaire ou en pharmacie qui n'ont pas un statut hospitalier pendant le stage effectué dans les conditions prévues au b du 2c de l'article L.412-8 du code de la sécurité sociale, l'organisme d'accueil envoie la déclaration à la Caisse Primaire d'Assurance Maladie ou la caisse compétente (voir adresse en page 1) en mentionnant l'établissement d'enseignement comme employeur, avec copie à l'établissement d'enseignement.

#### 6.2 - Gratification supérieure à 15 % du plafond horaire de la Sécurité sociale : LE STAGIAIRE N'EST PAS COUVERT PAR LA FRANCE POUR LES STAGES A L'ÉTRANGER AVEC GRATIFICATION SUPÉRIEURE AU PLAFOND LEGAL.

Les cotisations sociales sont calculées sur le différentiel entre le montant de la gratification et 15 % du plafond horaire de la Sécurité sociale.

L'étudiant bénéficie de la couverture légale en application des dispositions des articles L.411-1 et suivants et de l'article R412-4 du code de la Sécurité sociale.

En cas d'accident survenant au stagiaire soit au cours des activités dans l'organisme, soit au cours du trajet, soit sur des lieux rendus utiles pour les besoins de son stage, l'organisme d'accueil effectue toutes les démarches nécessaires auprès de la Caisse Primaire d'Assurance Maladie et informe l'établissement dans les meilleurs délais.

#### 6.3 - Protection maladie du stagiaire à l'étranger :

##### 1) Protection issue du régime français

- pour les stages au sein de l'Espace Economique Européen (EEE) effectués par les étudiant(e)s de nationalité d'un pays membre de l'Union Européenne, l'étudiant doit demander la Carte Européenne d'Assurance Maladie (CEAM).

- pour les stages effectués au Québec par les étudiants de nationalité française, l'étudiant doit demander le formulaire SE401Q (104 pour les stages en entreprise, 106 pour les stages en université) ;

Dans tous les autres cas de figure:

Les stagiaires qui engagent des frais de santé à l'étranger peuvent être remboursés auprès de la mutuelle qui leur tient lieu de Caisse de Sécurité Sociale Étudiante, au retour et sur présentation des justificatifs : le remboursement s'effectue alors sur la base des tarifs de soins français, des écarts importants peuvent exister. Il est donc fortement recommandé au stagiaire de souscrire une assurance maladie complémentaire spécifique, valable pour le pays et la durée du stage, auprès de l'organisme d'assurance de son choix (mutuelle étudiante, mutuelle des parents, compagnie privée ad hoc...). Exception : si l'organisme d'accueil fournit au stagiaire une couverture Maladie en vertu des dispositions du droit local (voir 2 ci-dessous), alors le stagiaire peut choisir de bénéficier de cette protection Maladie locale. Avant d'effectuer un tel choix, il vérifiera l'étendue des garanties proposées.

##### 2) Protection issue de l'organisme d'accueil

En cochant la case appropriée, l'organisme d'accueil indique ci-après s'il fournit une protection Maladie au stagiaire, en vertu du droit local :

OUI (celle-ci s'ajoute au maintien, à l'étranger, des droits issus du régime français)

NON (la protection découle alors exclusivement du maintien, à l'étranger, des droits issus du régime français)

Si aucune case n'est cochée, le 6.3-1 s'applique.

#### **6.4 - Protection Accident du Travail du stagiaire à l'étranger**

1) Pour pouvoir bénéficier de la législation française sur la couverture accident de travail, le présent stage doit :

- être d'une durée au plus égale à 12 mois
- ne donner lieu à aucune rémunération susceptible d'ouvrir des droits à une protection accident de travail dans le pays étranger
- se dérouler exclusivement dans l'organisme d'accueil partie à la présente convention
- se dérouler exclusivement dans le pays étranger cité.

Lorsque ces conditions ne sont pas remplies, l'organisme d'accueil s'engage à cotiser pour la protection du stagiaire et à faire les déclarations nécessaires en cas d'accident de travail.

2) La déclaration des accidents de travail incombe à l'établissement d'enseignement qui doit en être informé par l'organisme d'accueil par écrit dans un délai de 48 heures.

3) La couverture concerne les accidents survenus :

- dans l'enceinte du lieu du stage et aux heures du stage,
- sur le trajet aller-retour habituel entre la résidence du stagiaire sur le territoire étranger et le lieu du stage,
- sur le trajet aller-retour (début et fin de stage) du domicile du stagiaire situé sur le territoire français et le lieu de résidence à l'étranger,
- dans le cadre d'une mission confiée par l'organisme d'accueil et obligatoirement sur ordre de mission,

4) Pour le cas où l'une seule des conditions prévues au point 6.4-1) n'est pas remplie, l'organisme d'accueil s'engage par la présente convention à couvrir le stagiaire contre le risque d'accident de travail, de trajet et les maladies professionnelles et à en assurer toutes les déclarations nécessaires.

5) Dans tous les cas :

- si l'étudiant est victime d'un accident de travail durant le stage, l'organisme d'accueil doit impérativement signaler immédiatement cet accident à l'établissement d'enseignement ;
- si l'étudiant remplit des missions limitées en dehors de l'organisme d'accueil ou en dehors du pays du stage, l'organisme d'accueil doit prendre toutes les dispositions nécessaires pour lui fournir les assurances appropriées.

#### **Article 7 - Responsabilité et assurance**

L'organisme d'accueil et le stagiaire déclarent être garantis au titre de la responsabilité civile.

Lorsque l'organisme d'accueil met un véhicule à la disposition du stagiaire, il lui incombe de vérifier préalablement que la police d'assurance du véhicule couvre son utilisation par un étudiant.

Lorsque dans le cadre de son stage, l'étudiant utilise son propre véhicule ou un véhicule prêté par un tiers, il déclare expressément à l'assureur dudit véhicule et, le cas échéant, s'acquitte de la prime y afférente. Dans le cadre d'un stage à son domicile, l'étudiant qui utilise son propre matériel le déclare à son assureur et, le cas échéant, s'acquitte de la prime afférente.

#### **Article 8 - Discipline**

Le stagiaire est soumis à la discipline et aux clauses du règlement intérieur qui lui sont applicables et qui sont portées à sa connaissance avant le début du stage, notamment en ce qui concerne les horaires et les règles d'hygiène et de sécurité en vigueur dans l'organisme d'accueil. Le stagiaire s'engage à signaler tout dysfonctionnement en termes d'hygiène et de sécurité à son organisme d'accueil ET à son établissement d'enseignement supérieur.

Toute sanction disciplinaire ne peut être décidée que par l'établissement d'enseignement. Dans ce cas, l'organisme d'accueil informe l'enseignant référent et l'établissement des manquements et fournit éventuellement les éléments constitutifs.

En cas de manquement particulièrement grave à la discipline, l'organisme d'accueil se réserve le droit de mettre fin au stage tout en respectant les dispositions fixées à l'article 9 de la présente convention.

#### **Article 9 - Congés - Interruption du stage**

En France (sauf en cas de règles particulières applicables dans certaines collectivités d'outre-mer françaises ou dans les organismes de droit public), en cas de grossesse, de paternité ou d'adoption, le stagiaire bénéficie de congés et d'autorisations d'absence d'une durée équivalente à celle prévues pour les salariés aux articles L.1225-16 à L.1225-28, L.1225-35, L.1225-37, L.1225-46 du code du travail.

Pour les stages dont la durée est supérieure à deux mois et dans la limite de la durée maximale de 6 mois, des congés ou autorisations d'absence sont possibles.

Pour toute autre interruption temporaire du stage (maladie, absence injustifiée...) l'organisme d'accueil avertit l'établissement d'enseignement par courriel.

Toute interruption du stage, est signalée aux autres parties à la convention et à l'enseignant référent. Une modalité de validation est mise en place le cas échéant par l'établissement. En cas d'accord des parties à la convention, un report de la fin du stage est possible afin de permettre la réalisation de la durée totale du stage prévue initialement. Ce report fera l'objet d'un avenant à la convention de stage. Un avenant à la convention pourra être établi en cas de prolongation du stage sur demande conjointe de l'organisme d'accueil et du stagiaire, dans le respect de la durée maximale du stage fixée par la loi (924 heures).

#### Article 10 - Devoir de réserve et confidentialité

Le devoir de réserve est de rigueur absolue et apprécié par l'organisme d'accueil compte-tenu de ses spécificités. Le stagiaire prend donc l'engagement de n'utiliser en aucun cas les informations recueillies ou obtenues pour en faire publication, communication à des tiers sans accord préalable de l'organisme d'accueil, y compris le rapport de stage. Cet engagement vaut non seulement pour la durée du stage mais également après son expiration. Le stagiaire s'engage à ne conserver, emporter, ou prendre copie d'aucun document ou logiciel, de quelque nature que ce soit, appartenant à l'organisme d'accueil, sauf accord de ce dernier.

Dans le cadre de la confidentialité des informations contenues dans le rapport de stage, l'organisme d'accueil peut demander une restriction de la diffusion du rapport, voire le retrait de certains éléments confidentiels.

Les personnes amenées à en connaître sont contraintes par le secret professionnel à n'utiliser ni ne divulguer les informations du rapport.

#### Article 11 - Propriété intellectuelle

En France, conformément au code de la propriété intellectuelle, dans le cas où les activités du stagiaire donnent lieu à la création d'une œuvre protégée par le droit d'auteur ou la propriété industrielle (y compris un logiciel), si l'organisme d'accueil souhaite l'utiliser et que le stagiaire en est d'accord, un contrat devra être signé entre le stagiaire (auteur) et l'organisme d'accueil.

Le contrat devra alors notamment préciser l'étendue des droits cédés, l'éventuelle exclusivité, la destination, les supports utilisés et la durée de la cession, ainsi que, le cas échéant, le montant de la rémunération due au stagiaire au titre de la cession. Cette clause s'applique quel que soit le statut de l'organisme d'accueil.

#### Article 12 - Fin de stage - Rapport - Evaluation

1) Attestation de stage : à l'issue du stage, l'organisme d'accueil délivre une attestation dont le modèle figure en annexe, mentionnant au minimum la durée effective du stage et, le cas échéant, le montant de la gratification perçue. Le stagiaire devra produire cette attestation à l'appui de sa demande éventuelle d'ouverture de droits au régime général d'assurance vieillesse prévue à l'art. L.351-17 du code de la sécurité sociale.

2) Qualité du stage : à l'issue du stage, les parties à la présente convention sont invitées à formuler une appréciation sur la qualité du stage. Le stagiaire transmet au service compétent de l'établissement d'enseignement un document dans lequel il évalue la qualité de l'accueil dont il a bénéficié au sein de l'organisme d'accueil. Ce document n'est pas pris en compte dans son évaluation ou dans l'obtention du diplôme ou de la certification.

3) Évaluation de l'activité du stagiaire : à l'issue du stage, l'organisme d'accueil renseigne une fiche d'évaluation de l'activité du stagiaire qu'il retourne à l'enseignant référent (ou préciser si fiche annexe ou modalités d'évaluation préalablement définis en accord avec l'enseignant référent).

4) Modalités d'évaluation pédagogiques : Rapport de Stage, Rapport et soutenance

NOMBRE D'ECTS (le cas échéant) : 30.00

5) Le tuteur de l'organisme d'accueil ou tout membre de l'organisme d'accueil appelé à se rendre dans l'établissement d'enseignement dans le cadre de la préparation, du déroulement et de la validation du stage ne peut prétendre à une quelconque prise en charge ou indemnisation de la part de l'établissement d'enseignement.

#### Article 13 - Droit applicable - Tribunaux compétents

La présente convention est régie exclusivement par le droit français.

Tout litige non résolu par voie amiable sera soumis à la compétence de la juridiction française compétente.

FAIT À Vandœuvre le 16/12/2022

#### POUR L'ÉTABLISSEMENT D'ENSEIGNEMENT

Viceur du centre, par délégation, Frédéric HAMELIN  
L'ADMINISTRATEUR PROVISOIRE,

STAGIAIRE (ou son représentant légal le cas échéant)

Emilie REZER

L'enseignant référent du stagiaire

Frédéric JORAND

#### POUR L'ORGANISME D'ACCUEIL

Nathalie TOUZE

P/O

Sylvie INIZAN

Directrice des Services d'Appui  
du Centre INRAE IdF - Jouy en Josas - Antony

Le tuteur de stage de l'organisme d'accueil

Patricia LEPAGE



Fiches à annexer à la convention : 1) Attestation de stage / 2) Fiche de stage à l'étranger (pour informations sécurité sociale voir site cleiss.fr - pour fiche pays voir site diplomatie.gouv.fr) / 3) Autres annexes (le cas échéant)

## Abstract

Durant mon stage de Master 2, je me suis intéressée aux colorants azoïques qui sont retrouvés très largement dans l'industrie agroalimentaire mais également dans l'industrie pharmaceutique et cosmétique. Dans un premier temps, le but était d'isoler et d'identifier les bactéries du microbiote intestinal capables de dissiper les colorants azoïques (allura red AC et tartrazine). Le deuxième objectif était d'étudier l'impact de différents facteurs abiotiques sur cette activité métabolique des bactéries en présence de colorants azoïques. Pour finir nous voulions évaluer l'impact du surnageant bactérien exposé aux colorants azoïques sur la viabilité des cellules hépatiques et intestinales. Il a été montré que certains facteurs abiotiques ont des effets positifs et d'autres négatifs. Enfin il a été montré que le surnageant bactérien en présence du colorant allura red AC impacte négativement la viabilité des cellules du foie.

During my Master 2 course, I became interested in azo dyes, which are widely used in the food industry, but also in the pharmaceutical and cosmetics industries. The first aim was to isolate and identify the bacteria in the intestinal microbiota capable of dissipating azo dyes (allura red AC and tartrazine). The second objective was to study the impact of various abiotic factors on this metabolic activity of bacteria in the presence of azo dyes. Finally, we wanted to assess the impact of the bacterial supernatant exposed to azo dyes on the viability of liver and intestinal cells. It was shown that certain abiotic factors have positive effects and others negative. Finally, it was shown that bacterial supernatant in the presence of the allura red AC dye had a negative impact on liver cell viability.